DESIGN, FORMULATION AND EVALUATION OF SOLENOSTEMMA ARGEL TABLETS (“ALHARGAL”)

A Thesis Submitted By
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Dedication

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To the Soul of our Prophet Sayedna Mohammad,
The Majestic (Peace be upon him),
Who leads and guides us to all good deeds,
Lightens our way with valuable knowledge,
And encourages us to search for it from birth to death;

To the Souls of my father and brother Mahgoub,
Who were the cause and support of
my studying and learning in this field;

To the Souls of all those who sacrificed themselves
for the welfare of this country under the banner of Islam,
especially Dr. Ahmed Bashier Elhasan and
Ahmed Hassan Mohamed Ahmed;

To the Soul of my friend Prof Ahmed Mahmoud Elga’ali,
Head Department of Pathology, Bahr Elgazal University,
who did all the histopathological investigations
in his own Khartoum Modern Laboratory, and
passed this world before seeing that this work reached its target;

May Allah Almighty make my effort good deeds
in their scales on The Doomsday;

To all my masters and teachers,
To my merciful mother and sisters, And
To my dear wife and children,
for their encouragement, support and patience
which made this work possible.

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Solenostemma argel: Whole Plant, Leaves and Tablets
خلاصة الأطروحة

يستخدم السودانون النباتات الطبية والادوية البينانية في حياتهم اليومية بكثره وذلك لتواجدها وتوفرها بمختلف أنواعها نسبة لتبني المناخ في مناطق السودان المختلفة بما يناسب قرص النمو الطبيعي. وقد كان لهذه النباتات الأثر الوثيق وكبير في المعالجة الشعبيه منذ القدم، ونما” عدد تورف الاعلام الصحية في العديد من المناطق الريفية وارتفاع تكلفة العلاج ساعداً على استعمال النباتات الدوبان. ولمعرفة التي اتشار استعمال النباتات الدوبان الأثر والأثر الرئيسي الذي يستخدم لعلاج المرض المغذي (أذى بسب الجلوب الهواسي) والمغذي الكولي (أذى بسب falsely والجاه البولي) والأمراض الفطرية والطفيلية، وهناك الوصفات البدنية. وقد يكون هذه النباتات مستخدمة في هذه المنطقة الريفية في علاجات مختلفة، واستعمال النباتات الدوبان أو الأثر (48%) في جملة أنواع النباتات البينانية مقارنة بترنيم العلاج، والعلاجات الأخرى المستخدمة (وهى الجرل) العلاجات، بذرة الخلة، الحيد، واليدوتوس) وفعالية الجرل حيث أكد 80% من الذين استعملوا الجرل أنهم مشعر بزوال الألم، وفقاً لقرص من قاسية.

لذلك تم عمل حصة لدراسة نبات الجرل وتحديده المواد الفعالة الرئيسية، وذلك باستخدام مختلف المداهمات مع التركيز على الاستخلاص بالماء الذي يمثل الاستعمال الرئيسي للنبات، والثاني، وماضياً، استعمال النبات الطبي البائي النوعي للون (الكروموتوغراوي) مع استخدام الشراج الطبيعية بجزيئات النبات (الكروموتوغراوي) متعدد، مع استعمال الكواشف التي تحدد وجود الفคะونات والفلاقونات مثل كاشف درافندر أو المستقبل الطبيعية على التوالي. ثم استعمال المجوهرات الرئيسي التي يمكن أن تكون لها الأثر الفعال في إزالة وتشكيك الأثر الفعال. حيث اتضح أن لها أثر تخدير موضعي وذلك باستخدام جريئة سحب الجرل الصدع الانقراضي وعمل مقارنة للمعالجات الجرل مع المجدر المقصدي اللونجوكيكي وأدوات الفلاقونات من الكنمرطور وكورسوت وروبران ذات التأثير الخصمني المعروف فجود أن المستخلصات الفموي من الجرل له أثر ساوى مع النو في الكنمرطور ومساوة” لتصبح مفعول اللونجوكاكي والذي أثير مرجعاً معماريًا في أثر التخدير الموضعي، وهذا المفعول ربما يكون مؤسراً على الأثر العلاجي للجرل كمزيج للالم ومسكن للالم. عملي دراسات أفرادية على عملة أماء الأرب من المتقلصات المستفادة وعملة أماء حرب غراب غربا ووجد أن مستخلصات الجرل لها أثر متزايد منفعلاً عملة أماء الأرب وأمامة خنزير غربا حتى عند أثارها الاستناد كولين أو كلونيو. كذلك كانت مستخلصات الجرل المتقلصة أثر متزايد لجملة العلاجات الدوبان والجلوب، النبات، الكلي، الطحال والأمعاء، وذلك تم رسم الخط البياني للعملية كأساس المركبات المترتبة للجرل الدوبان والتي كانت في القدرة 6.35 جرام لكل كيلوجرام بينما وجدت في الغار الكديائي الأبيض 5.49 جراماً/كيلوجرام. كما رصدت الأثار الجانبية الناجمة عن استعمال ثلاثة جرعات مزدوجة على الصغيرة المحمرة للدم (الهيموغلوبين) والكمية الجينية لمكونات الدم، وظهر أنه يؤثر على الكلي والكيراتين والكاسينوم، ويساوبرف والنيوكلاز وكمياً على الأعراض الكبدية، مما يتوجب الحذر عند استعماله للذين يعانون من أعراض الكلي والكلد، وفقاً لقرص من قاسية.

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التي أعطت جرعة سامة (5 أجرام/كيلوغرام يوميًا) لمدة 45 يومًا، لم يحدث أي تغيير في الأوزان لهذه الأنسجة مقارنة مع رضيعاتها في الحيوانات المرجعية التي لم تعط الأدوية. كما أخذت عينة من الأعضاء الحساسة لإدارة السببية السامية للآثار غير العادية في رصد توضيح الأثر الإندمي على هذه الأعضاء الحساسة من قبلي وكدف وهجر وطحال وكلي، وعلى الرغم من أن معظمها لم يؤثر لكن رصد بعض الأنسجة المتبقية التي أتت تأثر الكبد والكلي، مما عزز التحذير بوجود الحشر المدمج في الأعراف الذين يكونون من أعراض في الكبد أو الكلى، كذلك لم يظهر أي أثر للحشرات المحتملة على الفئران الجوامع ولا على الأحياء، ولكنه يجب "أيضا" الانتباه عند استخدام الحشرات لمواجهة أي الحشرات في الشهر الأول من الحمل.

وقد أكدت كل هذه التجارب سلامة وآمنة خلال الحشرات في الجرعات العالجة الإعدادية وأنها بعض الأثار السامة لم تحدث إلا عند استعمالها بجرعات كبيرة للغاية، وفقاً على نتائج التجارب الإقراضية والسمعية تم تحديد الجرعات الناجحة والعملية، وكانت هذه الجرعات الناجحة تتعلق في فئات الحشر تمثيل مايكروجراهام "الجرعة الناجحة" من مجمل الخلايا المائية لأوراق الحشرة للفتاحة وخمسة وسبعين مايكروجراهام" بينما بلغت الجرعات الناجحة من مساحيق أوراق الحشر نسبياً مايكروجراهام. وقد استكشفت هذه الجرعات من مختلف الأثر الإقراضي والسمعية وثابتة من استعمالات الشعوب.

بعد التأكد من الأثر اللامع لأول استخدامات الحشر وخلوها من أي أثار سامة في حدود الجرعات العالية، تم اختيار الأفراد المضمنين (المصطلحات) في مكان صباعة الحشر ومستخلصاتها فيها، وذلك للمزايا المتعددة للأفراد ومن أهمها الجرعة الإقراضية المحددة، والشكل الصيدلي المقبول، ونسل الاستعمال، والأقل تكلفة، والآثر أكبر نباً، وقد تم استخدام مختلف مصادر الصيدليات السامية والتي استنسلت على الطبي البالغات والاستخبارات والبحوث والدراسات والتجارب الصيغية المختلفة. تم ضغطها في أفراد ذات قطرتين أو 12 ميليمتر. وقد استخدم الطبي البالغ والبحوث من أجل معرفة حجم الجسيمات في المساحيق والتصنيف المستحيل حسب حجم الجزيئات وآثارها على فك وتبني الأفراد دوائية المواد القصوى. فوجد أن حجم الجزيئات ≥ 160 ميكرون هو أسباب الاحتياج بعد تكثيفه بواسطة عملية التخزين والمضادات مساهمة مختصرة للإيحاءات والمضادات ومساعدات الإحلال والمضادات وتفسيرات وتفسيرات ضغط ومرئيات.

العمليات الصيدلانية والتقنية المستعملة استعملت أيضاً على مختلف الخطوات التي تؤديها صباعة الأفراد (الورز،الشرايين،التجعية، تحصين الجزيئات، ضغط الجزيئات في أفراد، الاختبارات الطبية والكيميائية) ، وعليها تم صباعة عدد كبير من تركيبات الأفراد التي تحتوي على مختلف الجزيئات من مواد رابطة ومفيدة بأنواعها المختلفة وتركيزاتها المتبقي، بالإضافة إلى دراسة أثر حجم جزيئات مساحيق أوراق الحشر الموظفة لتصبح أفراد الحشر الموظفة والتي تم مقارنة صناعة الفيزيائية والكيميائية والتي شملت مغلفة قطر الفريش، سمك الفريش، صلابة الصيدلي، معدل ذوبانية محتوى الفريش ومحتوى الفريش من الفيرويديت. كذلك تم تقوم الأطر الإقراضي والذين الأفريقيين، وتركز الفيرويديت ذاتية، في الوسط البدني في اختبارات النوادي، بواسطة جهاز دماغ الإشعاعي 294.5nm عند موجات خطية مستقيمة ذات الطول (0.250-0.350) في الجهاز وعمل المعالج لعلا مع كميات معرفة التركيز ومن رسم الخط المباني المشابه، وتم الحصول على صبغة للشيء المادي عند اختبار ضبط الحدود، وتبني احتراف تقييم الفيرويديت الذي يتشابه في الهند وتشابه هذه الأنواع المهذبة من الأفراد المتساوي والمفعول الواحد عند اختبار ضبط الحدود وتبني احتراف تقييم الفيرويديت لتصنيف تلك الجزيئات وطلبات الفيرويديت المتفقة أو بدرة أوراق نبات الحشر.
وقد اعتمدت أقراس الهالوسين كمعيار قياسي للأثر العلاجي المقارن مع ذلك الذي تحدثه أقراس أدمنتات الجريل، فوجد أن 15 ملغًا من الهالوسين تعادل في مفعولها 40 ملغًا من الأدوية المستخلصة من الجريل.

كانت تلك الدراسات تستعين بمضارب الهالوسين والفلوريدات والفلوريدات. وتشمل اختبار نقاء الفسفور والشفاء، اختبار قابلية الفرق للفركس، اختبار زمن تفتت الفرق، اختبار معدل نحررة ودوانية المكونات الدوائية عند وضع الفرق في جهاز يستحب على الوسط المعوي، وكل نتائج التحليل وضبط الجودة المستخلصة أكدت مطابقة الأقراس للمواد المنصوص عليها في دستور الأدوية الرسمية البريطاني والأمريكي.

كانت استخدمت تقنية الحاسوب للتحليل الاحصائيا المناسب وعمل الصور المستنسخة من تلك المأخوذة بواسطة الكاميرا لتوضيح الصور الحقيقية بالألوان، والدراسات التحليلية كمكونات النبات بعد كشفها بالكواشف، واختبار التعبية المفردة في الدراسات الإكلينيكية ومقارنة فعالية أقراس الجريل الثلاثة مع أقراس دواء جنسي مستخدم لعلاج المرض وهمان المعدقات العصبية وأقراس فعال وهمية خالية من الفعالية، ورصد نتائج هذه الفعاليات المقارنة بعد تحليلها إحصائيًا.

نمت مناقشة هذه النتائج والملاحظات والاستنتاجات جميعها ومقارتها مع نتائج وجود استنتاجات في تجارب مماثلة أو ذات علاقة، وقد أثبتت كل نتائج هذه الدراسة سلامة وفعالية استخدام الجريل كدواء مزيج للمعان المعوي وتقليل العضلات المسمكة وقليل السمية وميين الأثر الجانبية وذلك للكثير من المؤثرات العلاجية للجريل ومستحضراته، كما أكدت الدراسة على امكانية وفاعلية ودودي صباعة الجريل في أقراس مضبوطة كشكل صيدلياني مناسب ومتفع والذي يناسب الفعالة والمفيد.

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Abstract

Challenges facing pharmaceutical efforts aimed at utilization of medicinal plants as formulated dosage forms are: formulation of an effective, safe, elegant and stable dosage form to be used by an appropriate route of administration at a suitable dose and frequency of administration to give the required therapeutic activity for the proper period of time, without or with minimal side or toxic effects. A questionnaire was used to investigate the practice of house-hold usage of medicinal herbs, and the data collected indicated that the herb most commonly used as an antispasmodic remedy was *Solenostemma argel* (48%) among another four medicinal plants.

Botanical and phytochemical experiments were carried out for the identification and classification of the herb as well as extraction, separation and identification of the main constituents of *S. argel* leaves. They included general identity tests of *S. argel* leaves, e.g. the macroscopical, microscopical, macro-chemical analysis, thin layer chromatography and column analysis for the presence of characteristic alkaloids and flavonoids. Detection, separation, and isolation of flavonoids involved the use of silica gel columns eluted with different solvent systems. Alkaloids were extracted from *S. argel* homogenised leaves in a soxlet with different solvent systems. The identification tests confirmed the presence of both alkaloids and flavonoids in these extracts.

Phytochemical experiments also included detection, separation and identification of flavonoid compounds in water or methanol extracts of *S. argel* by using paper chromatography or thin layer chromatography, silica gel, run in B:A:W system. The chromatograms were sprayed with natural substances reagent or ninhydrin. Separation of different flavonoid compounds was carried out by using an accelerated eluted solvent passage through a polyamide chromatographic column using different solvent systems, and these portions were examined for their pharmacological effects.

Microbiological quality control of *Solenostemma argel* leaves powders and preparations showed that these products were negative for *Escherichia coli* and *Salmonella* spp., and their levels of contamination did not exceed 200 cfu/g.

Pharmacological and toxicological experiments were conducted for verification and confirmation of the biological activities of the powder, extracts, and alkaloids of *S. argel* on different experimental models, and authentication of the effectiveness of *S. argel* preparations. They included using different experimental animal models, and this was followed by other experiments for further investigation of the possible toxic effects of *S. argel* preparations, in different dosage regimens, to ascertain the safety of these *S. argel* products.

*Solenostemma argel* extract showed inhibitory effects upon the spontaneous as well as induced contractions produced by acetylcholine (1µg/ml) or barium chloride (0.002mg/ml) in contracting rabbit small intestine model or guinea pig small intestine model, and the degrees of relaxation were compared with those produced by reference drugs such as hyoscine or papaverine. Other experimental models including isolated rabbit heart and intact African toad heart showed the depressant effects on the myocardium of different *S. argel* extracts, perfused continuously, and reduction of the heart rate and stroke. The local anaesthetic effects of *S. argel* extracts were studied on African toad’s foot withdrawal reflex and compared with those of lignocaine, kaempferol, quercetin and rutin. The relative potency of *S. argel* alkaloids was 50% of that of lignocaine and equivalent to that of kaempferol.

*Solenostemma argel* alkaloids diffusion across rabbit small intestine was studied before and after the addition of 0.01g of sodium fluoride. This revealed the inhibitory effects (70-90%) of sodium fluoride on *S. argel* alkaloids diffusion rate across the rabbit small intestine, and indicated involvement of an active transport mechanism.
Median effective doses (ED$\text{50}$) of *S. argel* alkaloids (0.275mg) and hyoscine (0.035mg) were calculated by the MedUSA system used to compare their antispasmodic effects by recording the inhibitory effects of these two drugs on acetylcholine-induced contraction of guinea pig ileum model. The activity of hyoscine was eight times that of *S. argel* alkaloids.

Toxicological investigations included evaluation of acute toxicity of *S. argel* by determination of lethal doses of *S. argel* in four Albino Canadian rats and four local species rabbits. The mean lethal dose was 6.35g/kg for the rabbits and 5.49g/kg for the rats (n=4). The median lethal dose, (LD$_{50}$), of *S. argel* alkaloids was determined in sixty Albino rats assigned to six groups of ten animals each. Starting from the lethal dose of 5.49g/kg, each group was given a different intra-peritoneal injection of: 25%, 37.5%, 50%, 75%, 87.5%, or 100% of the lethal dose, and the log dose was plotted versus the mean deaths; the LD$_{50}$ obtained from the graph was 5.0g/kg in Albino rats.

Evaluation of acute toxicity of *S. argel* was carried out in a group of seven young Nubian goats and compared with the control group. Individual animals in the test group were given a 5g/kg dose of *S. argel* syrup once daily for 45 days, and the animals were sacrificed and investigations carried out revealed: no significant (P<0.05) changes in the weight of the vital organs; but their histopathological examination revealed some tissue abnormalities including: congested heart; hyperaemia of the intestinal tissues and catarrhal inflammation with lymphocyte infiltration; liver tissue necrosis of centrilobular hepatocytes and fatty cytoplasm vacuolation; kidney tissue necrosis of renal tubules and interstitial mononuclear cells infiltration. There were changes in serum concentrations of urea, inorganic phosphorus, creatinine, calcium, total protein and albumin; alkaline phosphatase and aspartate aminotransferase had high levels. RBCs count, MCV and PCV were higher in treated animals compared to normal goats.

Similar studies of toxic effects of *S. argel* alkaloids were conducted in white Albino Canadian rats, without significant changes occurring in their vital organs, but there were significant increases in urea, creatinine, calcium, phosphorous, alkaline phosphatase and aspartate aminotransferase. Phosphorus, creatinine, calcium, total protein, albumin concentrations, as well as alkaline phosphatase and aspartate aminotransferase activities were at high levels in animals given high dose of 0.63gm/kg of *S. argel* alkaloids. Changes in haematological parameters included decreases in haemoglobin and packed cell volume with the maximum dose of 640mg/kg and the effective dose of 160 mg/kg, while there was an increase with the median effective dose of 320 mg/kg.

*Solenostemma argel* leaves preparations were designed and formulated in tablets as an oral dosage form similar to its oral use in folkloric medicine. Tablets were selected as a dosage form because of their several advantages including: improved dosage accuracy and reliable delivery of the drug; ease of their preparation, evaluation, control, storage, and dispensing; convenience of use and compliance of patients; their inherently higher chemical stability and their rapid mass production, and are the least expensive of the solid dosage forms.

Pharmaceutical experiments included optimization of physicochemical characters of *S. argel* tablets prepared from the powdered leaves, extracts or alkaloids in a wide range of pharmaceutical formulations with different types and concentrations of diluents, binders and disintegrants, in addition to optimization of drug particle size and process conditions, such as the state and time of binder or disintegrant additions. The optimized formulations were subjected to a wide range of quality control tests including physical and chemical parameters, particularly hardness, friability, disintegration and dissolution, and hence affecting drug release, bioavailability, absorption and therapeutic activity.

*Solenostemma argel* leaves were powdered using a hammer mill to a particle size and distribution that would flow freely. Sieve analysis and separation of *S. argel* leaves powder, applicable to particles in the size range of $\leq$160 to $\geq$ 250 microns, was used
both as a method of particle size analysis and separation of powder into various sizes ranges, in order to produce materials within a specified range, and screen out very fine particles or over-size materials. The amount of powder that passed through the 160-micron mesh was found to be 63%, and this fraction of powdered *S. argel* leaves was mainly used in the formulation of optimized *S. argel* tablets.

Preparation of the granules of different formulae involved preparation and use of various binders. The wet masses of different formulations were forced through a No. 12-mesh screen manually in the case of small batches, or by using Erweka granulating machine with large batches. The prepared granules were dried in a hot air oven at 50°C overnight, and the dried granules were sized by passing through a No. 14-mesh screen, to facilitate the granules flow and compressibility. Following granules sizing, the granules were mixed with the other formulation ingredients, such disintegrants, lubricants and glidants, and compressed into tablets using a single-punch tableting machine. Different formulae of *S. argel* tablets were prepared by compressing unit volumes equivalent to 500mg of the granulation or leaves powders and 250mg of the granulation in the case of the dried extracts or alkaloids of *S. argel*. From these optimization trials, it was concluded that: a) a combination of binding agents was needed and a reduction of binder concentration was a must to optimize these formulae; and b) the use of a disintegrating agent, before and after granulation, in *S. argel* leaves tablets also optimized these formulae.

Stability experiments were included investigation and determination of the possible effects of different storage conditions, including on-going stability conditions and accelerated stability conditions, on the physiochemical properties of optimized *S. argel* tablets. In quality assurance studies, stability is defined as the extent to which a product retains, within specified limits, and through its period of storage and use, i.e. its shelf-life, the same properties and characteristics that it possessed at the time of its manufacture. Accelerated stability testing are studies designed to increase the rate of chemical degradation and physical change of drug by using exaggerated storage conditions; they should be used consistently throughout a particular stability study.

These stability tests were carried out by placing 100 tablets of each formula at room temperature at 30°C±15°C and RH 60%±15%; in an incubator at 37°C±2°C and RH 70%±5%; and in another incubator at 50°C±5°C and RH 70%±5%, for intervals extending from 6 months to 48 months. Evaluation of the physiochemical properties was done at zero time, and at the specified time intervals. The results were tabulated for each formula. Physicochemical characters and properties changes evaluated included: uniformity of weight, diameter, thickness, microbial growth, tablet hardness, friability, disintegration time, dissolution rate of tablet constituents, powder contents estimation with time, and mottling, capping, and lamination. In certain formulae of the tested tablets, there were changes in the hardness, odour, friability and powder contents.

Questionnaire (II) was used to evaluate the practice of using herbal medicines as antispasmodic remedy, and revealed the effectiveness of the medicinal herbs used, with 60% of the participants feeling completely relieved of pain after a single administration of the medicinal herb, and 26% used the drug several times due to repeated attacks of pain. With regard to the types of herbal preparations used, 45% used decoctions, 38% used powder type, and 17% used macerated plants preparations.

Clinical investigations included evaluation of safety and efficacy of *Solenostemma argel* tablets, and determination of any possible side effects, adverse reactions or toxic manifestations at therapeutic dosage regimen in ten healthy male volunteers. This was followed by evaluation of therapeutic effectiveness and side effects of *S. argel* tablets in a group of hundred patients with different clinical complains mainly gastro-intestinal tract colics, or urinogenital tract spasms and menstrual pain. None of these subjects, healthy or patient, complained of any serious side effects or toxic reactions.
Ten healthy male subjects participated in these clinical trials after signing a written consent, and were subjected to a medical examination and follow up by a clinician. The trials were started with low doses, e.g. one tablet three times daily, and gradually increased till the highest dose of four tablets three times a day for each of the leaves, extracts or alkaloids tablets was attained. The volunteers were monitored for the occurrence of any side effects or adverse reactions. None of the volunteers complained of blurred vision, dryness of the mouth, tachycardia or bradycardia, palpitation, headache or vomiting during the period of the trial. Only one of these subjects complained of dryness of the mouth and another subject complained of stomach upset felt at the time of administration of *Solenostemma argel* leaves tablets.

The clinical trials were organized in three teaching hospitals under the supervision of four clinicians. Randomly selected 100 patients, 20-70 years old and 40 males and 60 females, were complaining of abdominal disturbances, and diagnosed as having gastritis, indigestion, heartburn, flatulence, irritable bowel syndrome, renal colics, or colics due to menstrual pain. These patients were treated by using two tablets of *Solenostemma argel* leaves or extracts administered when necessary. They were followed up for therapeutic effectiveness of the administered tablets as indicated by the relief of pain and colics, and monitored for occurrence of side effects or adverse reactions. When necessary, another two-tablet dose was administered to patients when there was recurrence of pain or colics.

The general observation in treatment of gastrointestinal disturbances with *Solenostemma argel* tablets was the quick suppression of symptoms and relief of pain occurring immediately following drug administration. However, in the case of renal colics, pain associated with menstrual disturbances, and irritable bowel syndrome, IBS, there might be a need for repetition of the treatment. In most cases, the pain was relieved without any pronounced side effects or adverse reactions. Different types of *Solenostemma argel* tablets showed a very good therapeutic effectiveness (71%-100%), and a great margin of safety (98%-100%).

Forty patients with diagnosed irritable bowel syndrome were treated with placebo tablets (5% pain relief), generic antispasmodic tablets (90%), and *Solenostemma argel* leaves (71%) or extract (88%) tablets. *Solenostemma argel* extract tablets had a quicker onset of action and were more effective than the equivalent leaves tablets. No side effects were recorded with extract tablets, but in two patients mild stomach upset was recorded with leaves tablets. Accordingly, *Solenostemma argel* tablets, especially those of the extract or alkaloid, would be useful in relieving IBS associated pain by administering two tablets half an hour before meals.

Success of the present project and implementation of its findings may pave the way for other projects to be designed and conducted for further utilization of other useful medicinal plants used in folkloric practices.

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1.1. Worldwide Use of Herbal Medicine

Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic or savoury qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. Herbal medicine is a form of treatment, which is as old as mankind itself. Most ancient forms of the medicine are coming back to use, but with more purified forms using new technologies of processing.

According to the World Health Organization (WHO, 2000), worldwide use of herbal medicines is three to four times higher than conventional medicines. The world of herbal medicine offers a wide range of applications and treatments on several different levels, ranging from everyday over-the-counter remedies for more symptomatic relief of minor ailments, to individualised prescriptions prepared specifically for patients after in depth consultation with a qualified medical herbalist. Certainly herbs have their place as self-help for minor infections, coughs, colds, catarrh, stomach upsets, indigestion, constipation and so on. The wealth of information that abounds today about herbs is enabling people to make increasingly informed decisions about the specific remedies they choose to self-administer (WHO, 2000; 2002; McIntyre, 2003).

Herbal medicine is the oldest form of healthcare known to mankind. Herbs had been used by all cultures throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine. Much of the medicinal use of plants seems to have been developed through observations of wild animals, and by trial and error. As time went on, each tribe added the medicinal power of herbs in their area to its knowledge-base. They methodically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 21st century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound (McIntyre, 2003).

The World Health Organization (WHO) estimates that four billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples’ traditional medicine and a common element in Ayurvedic, homeopathic, naturopathic, traditional oriental, and Native medicine (WHO, 2000).

The World Health Organization (WHO, 2000) noted that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value. There are already 100 well-known, biologically-active compounds from 1000 of the tropical species that are used in folk medicine (Duke, 1990).
Most of the drugs employed in folkloric or traditional medicine are obtained from plants, and are used in a wide range of simple preparation forms such as infusions, decoctions, extracts or poultices. They came into medical use by way of an accident, observation, or by herbal doctors (Swinyard, 1985a). Two hundred and fifty years ago, there were a few or no synthetic medicines, and 50,000 to 300,000 species of higher plants were the main sources of drugs in the world. During this period, the developed countries shifted largely from naturals to synthetics; life expenses and population doubled, the call “back to nature” became more urgent, and people began to argue that naturally-occurring substances are inherently safer and more healthful than synthetic compounds. Gradually interest in herbal medicines increased - especially over the past 20 years - as more and more people questioned the use of synthetic drugs and their prolonged and sometimes alarming side effects and toxicity.

Today, scientists are predicting that herbal remedies will lead a revolution in medical treatment within the next ten years, using ancient recipes with thousands of active compounds instead of pharmaceuticals with a single active element.

Herbal medicines have provided the world’s population with safe, effective and low cost medicines for centuries. They have a rich and extensive historical basis in use and study which can be referenced to ancient medical writings. More importantly, modern research has validated many of these traditional uses. When integrated into medical care with other medications, herbal medicines can provide consumers and patients with the best chance for maintaining a high quality of life and, in some cases, increase their chance of survival. They can also fill therapeutic niches that are not adequately addressed through conventional therapies (Le Grand and Wondergem, 1990; Gray, 1997).

The global market for herbal medications in 1996 was estimated at $14 Billion, distributed in the following manner: Europe; $7 Billion, Japan; $2.4 Billion; North America, $1.6 Billion; and the rest of the world, $0.3 Billion. Market performance in 2000 was lacklustre, with a gain of just 0.5% by value, measured in US dollars, to reach US$18.5 billion. This relatively slow growth comes after stronger gains (and slow growth), and evidence of strong growth in certain local markets. The market faced significant challenges in 2000. Among these was the effect of exchange rate fluctuations on sales in countries other than the US. European currencies tied to the euro declined significantly in relation to the US dollar during 2000, impacting sales in European countries when measured in US dollars (Research and Market, 2003).

1.1.1. Herbal Medicine Use in the Sudan

Traditional medicine in Sudan has roots in Islamic and West African medicine. People in many areas of the country depend on herbal medicines, which are an integral part of the health care system. There is wide experience with the use of herbs in medical treatment. Many families specialize in herbal medicines and this knowledge is passed on from one generation to another. Patients travel from the capital to rural regions to consult herbalists, especially for chronic diseases. Sudan has a rich tradition of plant use, an immense range of climates, cultures and species and has the human and natural resources to become an even greater producer of natural plant products. The pharmaceutical potentials of Sudanese medicinal plants are immense.

The Medicinal and Aromatic Herbs Research Institute was created 25 years ago and has trained a considerable number of specialists in different fields required for research in medicinal plants (ElTohami, 1996). The Sudan Atlas of Medicinal Plants records the scientific name of more than 2000 medicinal herbs collected from different parts of the country, many native to Sudan. All of these herbs are in current use in traditional medicine. With respect to regulatory situation of herbal medicines, there is legislation for the registration of herbal preparations and herbal products.
ElKheir and Salih (1980), in their investigation on medicinal plants of the Sudan, reported that the availability of medicinal plants, together with the lack of proper medical services in most rural parts of the country, compelled people to make use of indigenous medicinal plants in the treatment of diseases. Over 80% of the Sudanese population still depends on local medicinal herbs and plants to keep well.

1.1.2. The Necessity of Data Supply for Herbal Medicines Approval

Tyler (1996) has indicated that only a handful of herbs, mostly laxatives, e.g. senna, have actually been approved by Food and Drugs Administration (FDA) as drugs. This is attributed to the fact that the producers of herbal products have, in most cases, failed to supply the FDA, or other health authorities, with the data necessary for obtaining the official approval of such products.

Many countries do have procedures to register medicinal plant preparations which are widely used for the health care needs of the majority of the people. The regulations are very stringent requiring the same standards expected of modern medicines. WHO published guidelines for the assessment of herbal medicines taking into account their long and extensive usage. These guidelines should encourage developing countries to relax some of the current regulations to be realistic in recognizing the role of traditional medicines in the health care delivery systems (WHO, 1998; 2001).

The fast growing tendency on herbal medicine indicates the need for enforcing regulatory and salutary legislations for consumers’ protection. Therefore, policy and guidelines should be established to maintain the quality of herbal healthcare products and to promote the industry.

A stimulus consequently exists, in both developed and developing countries, to assess and rationalize practices, and to control commercial exploitation through over-the-counter sale of proprietary labelled herbal and other "natural" medicines. The legal situation regarding herbal preparations varies from country to country. In some, phytomedicines are well-established, whereas in others they are regarded as food and therapeutic claims are not allowed. Developing countries, however, often have a great number of traditionally used herbal medicines and much folk-knowledge about them, but have hardly any legislative criteria to establish these traditionally used herbal medicines as part of the drug legislation (Le Grand and Wondergem, 1990; Rukangira, 2001).

The many and various forms of traditional medicinal products have evolved against widely different ethnological, cultural, climatic, geographical, and even philosophical backgrounds. The evaluation of these products, ensuring their safety and efficacy through registration and regulation presents important challenges. These guidelines contain basic criteria for the assessment of quality, safety, and efficacy and important requirements for labeling and the package insert for consumers’ information.

The requirements for pharmaceutical assessment cover issues such as identification, analysis and stability. Safety assessment should at least cover the documented experience of safety and toxicological studies, where indicated. The assessment of efficacy and intended use includes evaluation of traditional use through appraisal of the literature and evidence to support the indication claims.

The WHO Guidelines are intended to facilitate the work of regulatory authorities, scientific bodies and industry in the development, assessment and registration of herbal medicines, reflecting scientific results which could be the basis for future classification of herbal medicines and would also accommodate cross-cultural transfer of traditional herbal medicinal knowledge between different parts of the world. The abstract is to share national experiences in formulating policies on traditional medicinal products and in introducing measures for their registration and regulation, and to facilitate information exchange on these subjects among different regions (WHO, 1998; 2000).

As botanical supplements are integrated into the health care programs of more and more people and countries, it becomes necessary that information regarding their
optimal use be made available. Similarly, quality control requirements for producing herbal products need to be established to ensure that the highest degree of safety and effectiveness is achieved. Information relative to their safe clinical use, toxicology, interactions with conventional drugs, etc., is especially important to safeguard the public health.

In conclusion, national policies are the basis for defining the role of traditional and complementary/alternative medicine in national health care programmes, ensuring that the necessary regulatory and legal mechanisms are created for promoting and maintaining good practice; assuring authenticity, safety and efficacy of traditional and complementary/alternative therapies; and providing equitable access to health care resources and information about those facilities. While herbal medicines are well integrated into the health care systems of many nations, authoritative information regarding proper use and manufacture of herbal medicines is mostly lacking.

1.1.3. The Importance of Scientific Studies of Herbal Medicines

The main problem facing the use of herbal medicines is the proof requirement that the active ingredients contained in medicinal plants are useful, safe and effective. This is a highly important requirement to get the approval of health authorities, and to assure the medical staff and the public with regard to the use of medicinal plants as drug alternatives. The proofs of pharmacological activity that are available at present are mostly based on empirical experience. The scientific and clinical proofs then become the most important priority in order to eliminate the concern of using medicinal plants as drugs for alternative treatment. Therefore, it is of vital importance to conduct research or provide scientific proof of pharmacology. International collaboration is important for utilization of these herbal medicines, as it would enhance the development of drugs obtained from medicinal plants to the benefit of all (Keller, 1998; WHO, 2000).

Therefore, conducting multi-disciplinary research is a must for formulation of efficacy and safety standards for herbal healthcare products. This will help to install a mechanism to screen all the herbal healthcare products for consumer protection and to keep the accountability on public health (Last and Chavundka, 1986; Rukangira, 2001).

A very large number of scientific studies were conducted on herbal medicines, and this reflected the importance of such studies of medicinal plants for their scientific utilization and uses. The scientific study of herbal medicines is a must, because it reflects the importance of the scientific use of these medicinal plants in diseases treatment and control. There is a world-wide recognition of the vital importance of these medicinal plants in health care, their importance in the eradication of diseases, and their expanding role in health care services. In addition, it has always been the directions of the WHO to the national health authorities in developing countries to pay attention to these medicinal plants and give them due care and role in national health care system (Le Grand and Wondergem, 1990; Rukangira, 2001).

Furthermore, folkloric medicines of pre-industrial cultures involved the use of active principles, derived mainly from plants and/or marine sources, and were used for the treatment of the most common physical or mental ailments. Plant products alone still provide the primary health care for the treatment of illnesses of a large sector of the world’s population (Last and Chavundka, 1986; McIntyre, 2003).

In the United States, it is estimated that eight billion dollars are spent annually on plant-derived medications alone. Plant and marine lives are being studied and recovered in particular to find new therapeutic agents for a wide range of medical uses and cure of a wide spectrum of ailments ranging from skin infections to cancer. For example, both Japan and USA have taken strong interest in the discoveries of ethno-pharmacology. Japanese herbal medicine, or kampo, originated from China and dated back to 3000 years ago (CNWWF, 1989).
Substantial sums of money are being poured into research into the world of plant medicines as scientists continue to search for remedies for devastating illness such as heart disease and cancer. For example, recently a substance derived from the bark of an African willow tree could revolutionise the treatment of cancer by initiating a new way to stop tumour growth.

Extracts from the bark of the African Bush Willow (*Combretum caffrum*) have been shown to shut down blood vessels supplying oxygen and nutrients to tumours, thereby inhibiting their growth. Similarly, research has shown that numerous other herbs look hopeful for cancer treatment, including the American yew tree, the Madagascan periwinkle, borage and a Chinese herb called camptotheca (Rukangira, 2001; McIntyre, 2003). Sudan has a huge and virgin herbal flora, and therefore attempts and efforts are made to allocate the necessary funds to conduct such vital studies to utilize these medicinal plants and achieve the green medicinal revolution expected to take place in this country (ElTohami, 1996).

Accordingly, all these findings and observations highlight the following:
- a) The vital importance of medicinal plants in provision of health care;
- b) The increasing and unacceptable loss of these medicinal plants, due to habitat destruction and unsustainable harvesting practices;
- c) The fact that plant resources in one country are often of critical importance to the other neighbouring countries;
- d) The significant economic value of the medicinal plants used today and the great potential of the plant kingdom to further provide new drugs;
- e) The continuing disruption and loss of indigenous cultures, which often hold plants that may be beneficial to the global community; and
- f) The urgent need for international co-operation and co-ordination to establish programs for conservation of medicinal plants and to ensure that adequate varieties and quantities are available for present and future generations.

1.2. *Solenostemma argel*: Origin, Distribution, Investigations, and Uses

1.2.1. Botanical Classification, Taxonomy and Distribution of *Solenostemma argel*

*Solenostemma argel* (Del) Hayne is known locally in Sudan as ‘hargal’, and belongs to the family Asclepiadaceae. Other members of the family include *S. oleifolium* (Nectoux) Bullocket Bruce, and *S. triste* (Nees) K. Muelli. It is an erect shrub reaching a height of 60-100cm, with many velvety, pubescent branches from the base. It is distributed in Saudi Arabia, Egypt, Libya, Chad and Palestine. In Sudan, it is indigenous in the northern regions between Barbar and Abu Hamad (ElKamali, 1991). *S. argel* leaves were, at one time, used to adulterate Khartoum Senna (Trease and Evans, 1989).

1.2.2. Some Uses of *Solenostemma argel* in Folkloric Medicine

*Solenostemma argel* is used in Sudanese folkloric medicine in four forms:
- a) Internally, in the form of hot water decoction or macerated water extract, as an antispasmodic and in the treatment of GIT disturbances, hypercholesterolemia and diabetes mellitus;
- b) Externally, in poultice form, as anti-inflammatory and anti-rheumatic, and
- c) Inhalation of its smoke in the treatment of measles and cold.

The stem is generally used as antispasmodic and to treat cough. ‘Hargal’ infusion is used to treat diabetes and jaundice; it is also used to treat measles (ElKamali and Khalid, 1996). *Solenostemma argel* (Del) Hayne has been considered as an effective remedy for cough. Infusion of the leaves is usually indicated for gastrointestinal cramps, cold and urinary tract infections. It is also used as a stomachic, anti-colic, and anti-syphilitic when used for prolonged period of 40 to 80 days (Boulos, 1983). The spectrum
of traditional uses of *S. argel* in Sudan is wide enough to include most of the common acute conditions encountered in daily ethno-medical practice. The most popular uses of ‘hargal’ include the treatment of GIT and menstrual disturbances. The smoke of the plant is also considered useful for measles and nasal congestion of common cold (ElKamali and Khalid, 1996).

1.3. Phytochemical Aspects

Traditional medicine practices, including herbal medicine, have developed within different cultures in different regions. There has been no parallel development of standards and methods — either national or international — for evaluating them.

Evaluation of traditional medicine products is also problematic. This is especially true of herbal medicines, the effectiveness and quality of which can be influenced by numerous factors. Unsurprisingly, research into traditional medicine has been inadequate, resulting in paucity of data and inadequate development of methodology. This in turn has slowed development of regulation and legislation for traditional medicine.

Phytopharmaceuticals available to physicians should be defined botanically as well as phytochemically. Therefore, the scientific advance of traditionally-proven methods as well as of pure and practical knowledge on medicinal and aromatic plants is fundamental to create a better understanding and utilization of medicinal and aromatic plants (Franz *et al.*, 1992).

Challenges to the safety, efficacy and quality of traditional medicines:

a) Lack of research methodology;

b) Inadequate evidence-base for TM/CAM therapies and products;

c) Lack of international and national standards for ensuring safety, efficacy and quality control of TM/CAM therapies and products;

d) Lack of adequate regulation and registration of herbal medicines;

e) Lack of registration of TM/CAM providers; and

f) Inadequate support for research.

Currently 1500 species of the estimated total of 300,000 higher plants are known to contain pharmaceutical active compounds. Only 95 species are used therapeutically worldwide. The production of these commercially handled medicinal plants is still largely gained by indiscriminately collecting from the wild, even when the crude drug will eventually be used for industrial purposes.

Domestication and breeding programmes are needed to assure the survival of many useful plants as well as to meet quality requirements based on pharmaceutical standards (Franz *et al.*, 1992)

Phytochemical evaluation of herbal medicine products is especially difficult. Accuracy of plant identification is essential, as is isolation of active ingredients. However, the latter is rather complex because medicinal plant properties are influenced by several factors, such as the time of plant collection and area of plant origin, and including environmental conditions.

At the same time, a single medicinal plant can contain a large number of natural constituents. Establishing which constituent is responsible for what effect can therefore be prohibitively expensive. Yet given the worldwide popularity of herbal medicines, a widely applicable, appropriate and effective means of evaluating herbal medicines with limited resources is urgently needed (WHO, 2000; 2002).

1.3.1. Previous Phytochemical Investigations on *Solenostemma argel*

Preliminary chemical investigations of the dried leaves of *S. argel* established the general constituents and proved the absence of anthraquinone derivatives either in free
or in the combined form (Maharani and Saber, 1964). In their chromatographic screening, they indicated the presence of at least nine substances, five of which were detected in the alcoholic extract.

Nevertheless, they did not isolate any of these substances, though they noted the presence of numerous crystals, both in the petroleum ether and the alcoholic extracts (Maharani and Saber, 1964).

Mahran et al. (1967) reported the isolation of two crystalline compounds from the alcoholic extracts of S. argel. These compounds were designated as argelin and argeloside. However, the exact chemical structures of these compounds have not been established.

Khalid and Harbone (1973) detected the presence of flavonoids, kaempferol, quercetin, rutin, flavonols, flavanones, chalcones and alkaloids in S. argel. Khalid et al. (1974) reported the isolation of kaempferol, its mono- and di-glycosides and quercetin from the leaves as well as the stems of S. argel of Sudanese origin. They confirmed the presence of pregnane ester glycosides in S. argel extracts. Phytochemical studies of the leaves, stems and flowers revealed the presence of α-amyrin and β-amyrin, β-sitosterol, 7-methoxy-3α,22α-dihydroxy-stigmastene, ethoxy derivative of vangurol acid, an unidentified sterol. Moreover, they detected the presence of flavonoids and saponins in the different organs, and alkaloids and/or nitrogenous bases in the leaves, stems and flowers.

Mahran et al. (1976) carried out a phytochemical study of S. argel and isolated two substances designated as argelin and argeloside.

In their report on S. argel, Khalid et al. (1974) showed the presence of kaempferol and steroidal glycosides, while Maharani and Saber (1964), Wahba and Saber (1967), ElFishawi (1977), and Markham (1982) separated α-amyrin, β-amyrin, β-sitosterol-containing rutin and quercetin from S. argel. Solenostemma argel contains an acidic resin, glycoside, choline, phytosterols and amyrins. It is used in indigenous medicine as an effective remedy for coughs. The infusion of its leaves is used for gastro-intestinal cramps and infections of the urinary tract (ElTohami, 1996).

Many previous studies have reported the presence of monoterpenes, pregnane glycosides and acylated phenolic glycosides in the leaves. In addition, there is occurrence of four new pregnane glycosides from the pericarps of S. argel (Plaza et al., 2003).

The structures of compounds were elucidated by extensive spectroscopic methods including 1D (1H and 13C) and 2D-NMR experiments (DQF-COSY, HSQC, HMBC, HOHAHA and ROESY) as well as ESIMS analysis. In particular the relative configuration of compound 1 has been defined combining the NMR data with ab initio conformational studies. Compounds 1 and 2 showed a novel unusual pregnane skeleton characterized by the opening of ring D between C-14 and C-15, and the presence of two hemiketalic functions in C - 14 and C - 20 generating two five member rings with alcoholic functions in position 16 and 15 respectively (Plaza et al., 2003).

1.3.2. Flavonols and Flavones
Flavonols are very widely distributed in plants as co-pigments to anthocyanins in petals, and also in leaves of higher plants. Like the anthocyanins, they occur most frequently in glycosidic combinations. Although there are over a hundred flavonol aglycones known, only three are at all common: kaempferol, corresponding in hydroxylation pattern to the anthocyanidin pelargonidin, quercetin (cf. cyanidin), and myricetin (cf. delphinidin) (Harborne, 1973). Like the corresponding anthocyanidins, these three flavonols can be clearly separated by simple paper chromatography. The known flavonols are mostly simple structural variants of the common flavonols, and are of limited natural occurrence.

In the case of quercetin, a number of O-methylated derivatives are known; the 3'-methyl ether, isorhamnetin, and the 5-methyl ether, azaleatin, being but two examples. Addition of a hydroxyl in the 8-position to the structure of quercetin gives gossypetin, one of a few flavonols which are pigments in their own right, providing yellow flower colour in the primrose, and in the cotton plant (Harborne, 1973).

There is a considerable range of flavonol glycosides present in plants; more than seventy different glycosides of quercetin alone have been described. By far, the commonest of these is quercetin-3-rutinoside, known as rutin, which is of pharmaceutical interest in relation to the treatment of capillary fragility in man.

Some of the best known glycosides of quercetin are quercetin-3-rutinoside, known as rutin, quercetin-3-glucoside, known as isoquercitrin, and quercetin-3-rhamnoside, known as quercitrin with Rf in butanol : acetic acid : water (BAW) system 45, 58 and 72, respectively (Harborne, 1973).

Flavones only differ from flavonols in lacking a 3-hydroxyl substitution. This affects their UV absorption, chromatographic mobility and colour reactions. There are only two common flavones apigenin and luteolin, corresponding in hydroxylation pattern to kaempferol and quercetin.

The five common substances: apigenin, luteolin, kaempferol, quercetin and myricetin, are readily separated and identified by paper chromatography in Forestal (hydrochloric acid conc., acetic acid and water in the ratio of 3:30:10, respectively) and other standard phenolic solvents (Harborne, 1976).

1.3.3. Flavonol Detection

Almost every higher plant contains a characteristic pattern of flavones and flavonol glycosides in leaves or flowers, and thus these substances are ideal taxonomic markers for use in studying problems of plant classification, hybridization or phytogeography.

Rutin, quercetin-3-rutinoside, was first isolated from buckwheat, *Fagopyrum esculentum*, and this plant is still used as the commercial source of rutin. Rutin is undoubtedly the most widespread of all quercetin glycosides, and probably occurs in up to 25% of any given local flora.

Easily available sources include flowers, leaves of different plants; the material of the plant is collected and extracted with either hot 95% ethanol for fresh tissue, or 70% ethanol for dried tissue, for 30 minutes, and the extract is concentrated to a small volume and separated by chromatography.

1.3.4. Flavones and Related Flavonoid Glycosides

The flavonoids, which occur both in the free state and as glycosides, are the largest group of naturally-occurring phenols. More than 2000 of these compounds are now known, with nearly 200 occurring in the free state. Of the many different types of flavonoid compounds, the coumarins, which are derivatives of α-pyrone, have already been described. The anthocyanins are sap-soluble and have an γ-pyrole ring.

The flavones and their closely-related compounds are often yellow (Latin flavus means yellow), and in young tissues, they occur in the cell sap. They have been
extensively used as chemotaxonomic markers. They are abundant in the *Polygonaceae*, *Rutaceae*, *Leguminosae*, *Umbelliferae* and *Compositae*.

They occur both in the free state and as glycosides; most are o-glycosides, but a considerable number of flavonoid C-glycosides are known. Dimeric compounds, involving for example a 5 to 8 C-C linkages, are also known as Bi-flavonyls.

The glycosides are generally soluble in water and alcohol, but insoluble in organic solvents. The genines are only sparingly soluble in water, but are soluble in ether. Flavonoids dissolve in alkalis giving yellow solutions which on addition of acid become colourless.

Although the high hopes for therapeutic usefulness of flavonoids have not been fully realized, the group is not without pharmaceutical interest and remains a current area of research. It is very probable that a number of herbal remedies, whose constituents are yet unknown, will be shown to contain active flavonoids. The group is known for its anti-inflammatory and anti-allergic effects, for anti-thrombotic and vasoprotective properties, for inhibition of tumour, and protective for the gastric mucosa (Alcaraz *et al.*, 1987).

These effects have been attributed to the influence of the flavonoids on arachidonic acid metabolism (Alcaraz and Fernandez, 1987). Many flavonoid-containing plants are diuretic or antispasmodic. Some flavonoids have antibacterial and antifungal properties, and others are also colouring and tannin materials.

Pure flavone, which is colourless, occurs on the surface of some species of *Primula*. Many flavones are phenolic or methoxy derivatives, and form sap-soluble glycosides. The intensity of their yellow colour increases with the number of hydroxy groups and with increase of pH. Isoflavones are found in the heartwood of species of *Prunus* and in species of *Iris*, or particularly abundant in the family *Leguminosae* (Harborne, 1973).

### 1.3.5. Alkaloids

The alkaloids, of which more than five thousands are known, comprise the largest single class of secondary plant substances. It is from those plants containing alkaloids that a large number of drugs are derived. The group, however, is a very varied one and it is only the chemical properties of the basic nitrogen that unify the many classes of alkaloids. For this reason, questions of the physiological role of alkaloids in the plant, their importance in taxonomy and biogenesis are often most satisfactorily discussed at the level of a particular class of alkaloids.

### 1.3.6. Definition of Alkaloids

A precise definition of the term “alkaloids” (alkali-like) is somewhat difficult because there is no clear-cut boundary between alkaloids and naturally occurring complex amines.

Typical alkaloids, derived from plant sources, are basic compounds that contain one or more nitrogen atoms (usually in a heterocyclic ring), and generally have a marked physiological action in man or other animals.

The name "proto-alkaloid" or “amino-alkaloid” is sometimes applied to compounds such as hordenine, ephedrine and colchicine, which lack one or more properties of typical alkaloids.

Other alkaloids, not conforming to the general definition, are those synthetic compounds not found in plants, but are very closely related to the natural alkaloids, e.g. homatropine (Evans, 1989).

In practice, those substances present in plants and giving the standard alkaloid qualitative tests are termed alkaloids. Frequently, in plant surveys, this evidence alone is used to classify a particular plant as alkaloid-containing.
Various nitrogen-containing compounds derived from bacterial, fungal and animal (including insects) sources are also regarded as alkaloids, and have recently received considerable attention.

They include the alkaloids of frogs of the genus *Phyllobates*, which constitute some of the most poisonous substances known to man. Some 200 alkaloids have been characterized from poisonous frogs and salamanders. Other alkaloids, such as some of the gliotoxins and chaetocins, are obtained from fungi and contain Sulphur (Evans, 1989).

1.3.7. The Nitrogen of Alkaloids

The alkaloids, taken in their broadest sense, may have a nitrogen atom which is primary (mescaline), secondary (ephedrine), tertiary (atropine), or quaternary (one of the nitrogen atoms of tubocurarine). This factor affects the derivatives of the alkaloid which can be prepared and their isolation procedures. In the plant, alkaloids may exist in the free state, or as salts, amines or alkaloid N-oxides.

1.3.8. Alkaloid N-Oxides

N-oxidation products of alkaloids, particularly the N-oxides of tertiary alkaloids, are well known laboratory products, which can easily be prepared from their original bases. As early as the 1920’s quite extensive pharmacological and toxicological comparisons have been made of common alkaloids, such as morphine, strychnine and hyoscyamine and their corresponding N-oxides. Some enthusiasm for the clinical use of N-oxides was engendered by their purported delayed release properties, low toxicities, and low addictive properties compared to the corresponding tertiary alkaloids.

Although the formation of N-oxides and other N-oxidation products of alkaloids in animal systems is well-known, forming part of the wider scheme for the metabolism of amines, the occurrence of such compounds in plants has, until relatively recently, received little attention. This is possibly due to the belief that such compounds represented artifacts arising during the extraction and work up of tertiary alkaloids, and because of the high polarity and water-solubility of alkaloid N-oxides, they were discarded by the normal alkaloid extraction procedures (Evans, 1989).

One of the two possible isomeric N-oxides of hyoscine has been isolated from species of the first four genera: *Datura*, *Hyoscyamus*, *Scopolia* and *Mandragora*. Morphine and Codeine are natural N-oxide constituents of the opium poppy latex and *Nicotiana* spp. They contain two isomeric nicotine N-oxides, based on the pyrrolidine nitrogen. Some N-oxides, for example, aspergillic acid and iodinin-1,6-dihydroxyphenazine dioxide, are isolated from microorganisms, and possess antibacterial activity.

1.3.9. Properties of Alkaloids

Most alkaloids are well-defined, crystalline substances which unite with acids to form salts. In the plant, they may exist in the free state, as salts or as N-oxides. In addition to the elements carbon, hydrogen and nitrogen, most alkaloids contain oxygen. A few, such as conine from hemlock and nicotine from tobacco, are oxygen-free and are liquids. Although coloured alkaloids are relatively rare, they are not unknown; for example berberine is yellow, and the salts of sanguinarine are copper-red.

Knowledge of the solubility of the alkaloids and their salts is of considerable pharmaceutical importance. Not only are alkaloidal substances often administered in solution forms, but also differences in solubility between the alkaloids and their salts provide methods for isolation of alkaloids from the plant and their separation from the non-alkaloidal substances also present. While the solubilities of different alkaloids and their salts show considerable variations as might be expected from their extremely varied structures, it is true to say that the free bases are frequently sparingly-soluble in water, but soluble in organic solvents; with the salts, the reverse is often the case as
these are usually soluble in water and sparingly-soluble in organic solvents. For example, strychnine hydrochloride is much more soluble in water than is strychnine base (Evans, 1989).

However, there are many exceptions to the previous generalization: caffeine base is readily extracted from tea with water, and colchicine is soluble either in acid, neutral or alkaline water. On the other hand, some alkaloidal salts are sparingly-soluble; for example quinine sulphate is only soluble to the extent of one part in 1000 parts of water, whereas one part of quinine hydrochloride is soluble in less than one part of water.

1.4. Pharmacological Aspects

In order for medicinal plants to be accepted in the medical field as alternative drugs, pharmacological research and the safety tests of active ingredients have to be carried out. Production of standardised phytomedicines requires specialised expertise and pilot plant facility.

Urgent action is needed for research that focuses on the generation of baseline information on the different pharmacological actions and possible toxic effects of these medicinal and aromatic plants, and for promoting value-added processing of herbal medicines from local materials for local industries with the formulation of simple, effective and safe dosage forms being standardised and packaged at low cost using appropriate technology (WHO, 2000).

The quantity and quality of the safety and efficacy data on traditional medicine are far from sufficient to meet the criteria needed to support its use worldwide. The reasons for the lack of research data are due not only to health care policies, but also to a lack of adequate or accepted research methodology for evaluating traditional medicine. It should also be noted that there are published and unpublished data on research in traditional medicine in various countries, but further research in safety and efficacy should be promoted, and the quality of the research improved (WHO, 2000).

A Traditional Medicine Strategy is relevant because traditional medicine continues to play an important role in health care. In many parts of the world, it is the preferred form of health care. Elsewhere, use of herbal medicines and so-called complementary and alternative therapies is increasing dramatically. There is no single determinant of popularity. But cultural acceptability of traditional practices, along with perceptions of affordability, safety and efficacy, and questioning of the approaches of allopathic medicine, all play a role. In view of this broad appeal, the general lack of research on the safety and efficacy of traditional medicines is therefore of great concern (WHO, 2002).

A traditional medicine strategy is urgently needed International, national and nongovernmental agencies continue to make great efforts to ensure that safe, effective and affordable treatments for a wide range of diseases are available where they are most needed. WHO estimates, however, that one-third of the world’s population still lacks regular access to essential drugs, with the figure rising to over 50% in the poorest parts of Africa and Asia.

Fortunately, in many developing countries, traditional medicine offers a major and accessible source of health care. Use of traditional medicine in primary health care, however, especially in the treatment of deadly diseases, is cause for concern. An evidence-base supporting its safe and efficacious use has yet to be developed. Therefore, a traditional medicine strategy has to be developed in response to these challenges. The world health organisation (WHO, 2002) has developed a strategy for traditional medicine to enable this form of health care to better contribute to health security. It focuses on working with WHO Member States to define the role of traditional medicine in national health care strategies, supporting the development of clinical
research into the safety and efficacy of traditional medicines, and advocating the rational use of traditional medicine.

Pharmacological and clinical trials should be conducted according to the directives outlined in the WHO’s "Good Laboratory Practice" and the "Good Clinical Practice" (WHO, 2002). The development of basic biological knowledge could better and better meet the requirements of man, and led to medicaments of plant origin as well as to new culture practices of medicinal plants. It is a pity that at the same time growing industrialization became a danger to both sides: mankind suffers from environmental problems, and plants, in turn, from loss of bio-diversity.

1.4.1. Previous Pharmacological Investigations on Solenostemma argel

Adam (1972) reviewed drugs hepatotoxicity in animals. Adam et al. (1973) studied the effect of Impomea cornea on the liver and serum enzymes in young ruminants. The antispasmodic activity of the various flavones isolated from S. argel has been investigated by Khalid (1974) who established the important pharmacological role of these compounds as antispasmodic agents.

ElTahir et al. (1987) studied the pharmacological activities of S. argel, including spasmolytic and uterine relaxant activities. They investigated the pharmacological activity of the chloroform / methanol extract of S. argel, and established that this extract has a biphasic effect on the rabbit jejunum, with an initial reversible inhibition which was not antagonized by haloperidol, propranolol or guanethidine. The extract suppressed the activity of the uterus, and the effect was not antagonized by cimetidine, haloperidol, or propranolol. Furthermore, this extract exhibited a local anaesthetic activity when tested using the foot withdrawal reflex of the frog. The chloroform extract stimulated the uterus, and this effect was antagonized by atropine or ciproheptadine. It was concluded that the inhibitory activity may by due to the presence of two saline-insoluble spasmolytic compounds in the chloroform extract of S. argel leaves having spasmolytic and uterine relaxant activities. The spasmyloytic activity of flavonols involved inhibition of peristalsis of guinea pig ileum in vitro, and it is mainly due to the aglycone quercetin (Mariana et al., 1994). Madani (2001) studied the toxicity and toxic doses of three Sudanese medicinal plants, including S. argel, on animals.

With respect to antimicrobial activity, Ross et al. (1980) reported antibacterial activities in S. argel. Likewise, Tharib et al. (1986) investigated the potential antimicrobial activity of S. argel. Furthermore, aerial parts of S. argel, extracted with methanol/water in four fractions and examined against eight bacteria, revealed the greatest effect against Streptococcus species, and a moderate action against Escherichia coli, Bacillus anthracis, Staphylococcus aureus, Klebsiella pneumoniae and Proteus vulgaris (ElHady et al., 1994).

1.4.2. Some Pharmacological Activities of Flavonoids

Although flavonoids are used empirically to treat a variety of diseases, until recently little was known about their biological activity. The growing interest of scientists in this class of natural products has led recently to a great deal of evidence on the effects of flavonoids, and contributed to a better understanding of their pharmacological and biochemical properties. Moreover, the interactions of flavonoids with the enzymes of the Arachidonic Acid (AA) metabolism can be used as a research tool to elucidate the functions of AA derivatives in processes that underlie certain pathological conditions.

Artuson and Jonsson (1975) reported the activity of several semisynthetic derivatives of rutin on the prostaglandin (PG) synthetase system. They found that 7-hydroxyethyl-rutoside and 7,4'-hydroxyethylrutoside stimulated PG biosynthesis, while 7,3',4'-hydroxyethylrutoside and 5,7,3',4'-hydroxyethylrutoside acted as inhibitors. Such observation led Baumann et al. (1978, 1979, 1980) to test a series of flavonoids and

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other phenolic compounds on the rat's renal medulla PG synthetase system. Some flavonoids and their physiological metabolites were able to replace adrenaline, acting as co-factors, while others behaved as enzyme inhibitors.

Most flavonoids were only tested at one concentration and thus there was no allowance for the biphasic effects to be observed. Moreover, the released AA can be converted to vaso-dilator factors (PGE and PGI₂), permeability-increasing leucotrienes (LTB₄, LTC₄ and LTD₄), vasoconstrictor factors thromboxanes (TXA₂, LTC₄ and LTD₄) and chemotactic activators for neutrophils hydroxyeicostetraenoic acids (HETEs, mainly LTB₄) (Williams, 1983). PGE₂ and PGI₂ produce erythema, oedema and pain and thus enhance the effects induced by other mediators (Higgs and Vane, 1983), LTB₄ is remarkably potent in increasing neutrophil adherence to vascular endothelium and thus stimulating the chemotactic migration of these cells into tissues. This mediator is also immunoregulatory and induces increased number of suppressor T lymphocytes from precursors. As a consequence, it inhibits mitogen-induced T cell proliferation (Rola-Plezczynski et al., 1982; Payan and Goetzl, 1983; Lewis, 1986).

Inhibition of PG biosynthesis is generally accepted as the mechanism of the anti-inflammatory action of aspirin-like drugs (Higgs and Vane, 1983). In a similar way, inhibition of cyclooxygenase (Cox) activity can explain the anti-inflammatory activity of flavonoids. Inhibition of PG biosynthesis in cells may be expected to affect excitatory activity since AA metabolites have been generally accepted as bioamplification messengers and PGs can influence cell Ca²⁺ availability and initiate or prolong the effects of Ca²⁺-dependent stimuli. PGs can affect membrane-bound enzymes, like ATPase or adenylate kinase. In addition, neutrophil activation can depend on peptide-mediated stimulation of phospholipase A (PLA) and subsequent LTB₄ synthesis, which could activate PLC with the release of intracellular Ca²⁺ for secretion. In a similar way, HETE₉ affects AA turnover in phospholipids and may alter membrane properties (Laychock, 1986).

Several flavonoids with anti-inflammatory activity inhibit neutrophil secretion in vitro and in vivo (Showell et al., 1981; Bennett et al., 1981; Alcaraz and Hoult, 1986; Villar et al., 1987). Quercetins were reported to inhibit PLA (Lee et al., 1982; Hope et al., 1983). They are widely distributed in plants and reported as one of the anti-inflammatory compounds of S. argel (Khalid, 1974). Quercetins may limit inflammation by reducing the availability of free AA. Besides, they inhibit lipoxygenase (LO), namely 5-LO (Baumann et al., 1980; Hope et al., 1983; Landolfi et al., 1984; Alcaraz and Hoult, 1986). Quercetins could suppress acute inflammation via inhibition of neutrophil migration and activation, along with inhibition of plasma exudation.

1.4.3. Flavonoids as Anti-Allergic Agents

Gallic acid, methyl gallate and quercetin were identified as biologically-active compounds. As anti-asthmatics, they prevent allergen- and platelet-activating factor-induced bronchial obstruction as well as bronchial hyper-reaction in guinea pigs (Dorsch et al., 1992). The role of dietary flavonoids is recognized in protection against cancer and coronary heart disease (Hollman et al., 1996). Lipoxygenase activity is involved both in the generation of anaphylactic mediators especially LTC, a potent bronchoconstrictor and mucus secretagogue, and in sensitization to their contractile actions (Lewis, 1986).

A selective LO inhibition is a goal in the development of new drugs to treat anaphylaxis. A dual inhibitor would have similar properties to the anti-inflammatory corticosteroids (Higgs and Vane, 1983). In this respect, it is noteworthy that certain flavonoids, which are dual inhibitors of Cox and Lo, can exert anti-inflammatory effects. Quercetin inhibits the release of mast cells and basophile mediators (Fewtrell and Gomperts, 1977; Bennett et al., 1981, Middleton et al., 1981; Middleton and Drzewiecki,
1982; Middleton and Drzewiecki, 1984; Middleton, 1986). Rutin, quercetin, baicalein and cirsiol, which are 5-Lo inhibitors, have anti-allergic activity in animals and suppress the release of LTs by sensitized guinea pig lung tissue challenged with an antigen. Consequently, studies on flavonoid inhibition of 5-Lo may lead to new approaches in the development of future anti-allergic therapy.

1.4.4. Antithrombotic and Vasoprotective Properties of Flavonoids

Many flavonoids are known for their anti-aggregating activity. The mechanisms involved include inhibition of phosphodiesterase activity (Beretz et al., 1982) and of Cox or Lo activities (Landolfi et al., 1984; Swies et al., 1984). Nevertheless, in the case of quercetin, a potent inhibitor of platelet aggregation, in a collagen strip super-fused with blood of anaesthetized cats, it was observed that such action may be due to inhibition of Cox activity because of the differences in the active concentrations (Swies et al., 1984).

Inhibition of Cox activity has been proposed as a mechanism of the anti-aggregating activity of certain flavones such as chrysin, apigenin and phloretin to human platelets (Mower et al., 1984; Landolfi et al., 1984).

Some flavonoids enhance the local generation of PGI and, by scavenging platelet-derived free radicals, are likely to protect endothelium-derived relaxing factor (EDRF) from destruction by superoxide anions in the vascular endothelium covered with platelet thrombi. These effects would result in the vaso-protective and anti-thrombotic properties of such flavonoids (Gryglewski et al., 1987).

1.4.5. The Role of Flavonoids in Inhibition of Tumors

Antioxidative flavonoids and flavonoid glycosides were isolated and measured by ferric thiocyanate method (Haraguchi et al., 1992). The role of dietary flavonoids in protection against cancer and coronary heart disease was reported by Hollman (1996). Both Cox and Lo products of AA metabolism have been related to the process of carcinogenesis in vitro and in vivo. There is increasing evidence that high concentrations of active oxygen, organic peroxides and radicals promote mutation, chromosomal aberrations, cytotoxicity, carcinogenesis, ageing and cellular degeneration (Cerutti, 1985). Inhibition of experimental tumor promotion has been demonstrated for Lo inhibitor quercetin in vitro and in vivo (Kato et al., 1983).

1.5. Toxicological Aspects

Long before the birth of modern pharmacology, Paracelsus (1495-1540) observed that ‘all’ substances are poisons; for there is nothing without poisonous qualities. It is only the dose which makes a substance poison. The first formal lectures that could be delivered to constitute a course in pharmacology were given at Paris in 1856 by Claude Bernard under the title “The Effects of Toxic and Medicinal Substances” (Ridgeway and Rupp, 1971; Goldstein et al., 1974).

There is a wide variety and availability of effective and potentially toxic herbal drugs in the Sudan, in addition to their increased uses, and this necessitates the scientific and clinical studies of these herbal medicines.

While mild modifications to use these drugs in modern medicine as remedies make it a must for comprehensive studies to select the most appropriate dosage forms, after determining the lethal, toxic, and potent effective doses.

All drugs are toxic in over-dosage, and people vary greatly in their sensitivity to drugs. What may be safe and appropriate dosage for one person can prove to be an overdose for another. Moreover, even in the therapeutic dose range, many drugs have unavoidable toxic side effects. As a consequence, a great deal of drug toxicity is inevitably produced by physicians, who must be constantly alert to recognizing such iatrogenic (i.e. physician–caused) diseases and treating them properly.
The widespread distribution of herbal drugs, like *Datura*, *Belladonna*, and *Opium*, is also associated with the increased hazards of accidental poisoning and misuse. Also, the hepatic, cardiac, renal, and haematological disorders are quite common, and therefore the adverse effects of such herbal medicines must be adequately known in order to advice and adjust the doses for such patients.

Broadly speaking, any adverse drug effect may be thought of as a manifestation of drug toxicity. It will be useful, however, to consider separately and in considerable detail, special categories of adverse drug effects such as idiosyncrasy, drug allergy, tolerance and physical dependence, mutagenesis, teratogenesis, and carcinogenesis.

Sometimes a toxic effect is simply an extension of the therapeutic effect at a higher dose level. Often, on the other hand, toxicity takes the form of a side effect more or less unrelated to the primary drug action; for example nausea and vomiting seen frequently with the cardiac glycosides, and the gastrointestinal distress that often accompanies the ingestion of ferrous sulfate.

If quantitative estimates of the efficacy and toxicity could be carried out routinely in humans, the decision whether a new drug should be adopted for clinical use would be a straightforward task. The drug would be administered at a low dosage, and increasing the dose cautiously, watching carefully for toxic effects. If the desired therapeutic effect could be obtained with little or no toxicity in a large number of patients, the drug would be accepted as efficacious and safe. In order to determine the margin of safety, the dose is increased beyond the therapeutically effective range, with the aim of determining by how much the therapeutic dose could be exceeded before toxicity ensues.

The initial evaluations have to be based upon experiments with lower animals, and these have to explore the qualitative as well as the quantitative aspects of toxicity. It is important at this stage to find out what kinds of harmful effects may be expected, and at what dosages they may be manifested. Several animal species have to be used because species differences are considerable (Ridgeway and Rupp, 1971).

A convenient measure of potency is the “median effective dose” (ED$_{50}$), which is the dose that would produce the specified effect in 50% of all subjects.

If the effect being measured is death, the corresponding measure is the “median lethal dose” (LD$_{50}$), and if the effect is a toxic one, the expression “median toxic dose” (TD$_{50}$) is appropriate.

Because of biological variations, it is meaningless to speak of “minimum lethal dose”, “minimum toxic dose” or “maximum tolerated dose”, as used to be customary at one time (Ridgeway and Rupp, 1971; Goldstein et al., 1974).

### 1.6. Pharmaceutical Aspects

Herbs have been used in a wide variety of dosage forms since they were first discovered to have medicinal qualities. These include the fresh or dried herb plant parts themselves including the leaves, stems, roots, flowers, seeds, or fruits. Many herbalists promote use of the fresh plant materials while herbal medicine suppliers offer the more convenient dried plant parts in bulk for making teas, or in the form of tablets or capsules.

There are many types of products including mashed herbs (pastes), juice squeezed from herbs, infusions or teas where the herb is steeped in hot water, decoctions or extracts of herbs made by boiling the herb in water to form a concentrate, cold teas where the herb is simply steeped in cold water, herbs pulverized into a powder and used as such or compressed into a tablet or filled into a capsule, herbal wines made by fermenting the herb with water and sugar, various tinctures made by extracting the herb with alcohol, glycerine or vinegar, liniments made with alcohol and vegetable oil and used externally, herbal ointments (salves) made with the herb mixed or...
dissolved in waxes or petroleum jelly, various syrups made with the herb or extract mixed into sugar, honey, or glycerine vehicles, poultices where the herb is moistened (cold or hot) and applied directly to a bruise or wound and held in place with gauze, and herbal oil usually formulated with a base oil (e.g., olive, sesame, almond seed oil); the herb is placed in any of these oils, allowed to stand for several days, strained and bottled (Complementary and Alternative Medicine Institute, CAMI, 2000).

Generally, the less processing the less decomposition of active principles will occur. Flower and leaf materials are best handled as delicate infusions because excess heat (boiling) will cause a loss or decomposition of volatile essential oils. As a rule, stems, bark, roots and seeds are best offered as decoctions.

Each manufacturer should fully define how his product is made because all the dosage forms noted above can be prepared by numerous means leading to great variations in product quality, shelf-life, bioavailability, and ultimate efficacy. Most reputable manufacturers use standard dosage forms as defined by the USP or a proprietary extract which has been tested for stability and activity.

Quality manufacturers are striving to produce "standardized" products. This means that the company has verified that the active ingredient (where known) or a biological "marker" compound(s) is present in the correct concentration so that the amount and activity of the "active(s)", is determined by quantitative analysis (GC, MS, HPLC, etc.).

Proponents of whole herbs, however, argue that focus on just a few "bioactives" may result in missing the total effect of a complex combination of constituents where the true "active" and possible synergistic materials are lost in processing. Nevertheless one should always attempt to determine which manufacturer can provide evidence that his product is stable and efficacious at a particular dosage level. Simply stated, this level of assurance has not been realized for the majority of herbal medicine products and much research remains to address this problem as these products move from nutritional supplements to OTC-type products (CAMI, 2000).

Clinical herbal therapy employs several different methods of presenting the herbal material to the body, and dosages are precisely calibrated according to individual need, all of these variables having significant impact on the efficacy of the remedial action (British Herbal Pharmacopoeia, PHB, 1983; Cabrera, 2000). Three main methods of extraction are in common usage: Water extraction, Solvent extraction and Fat extraction. Each method offers different extractive properties, applications and usefulness under various circumstances. An herbal extract made with water, a solvent or fat, could be incorporated as a remedy into various mediums and applied in various ways of administration. For example, water extractions may be employed in a number of ways: the tea may be drunk, or it may be used as a mouthwash, gargle, compress, skin-wash, eyewash, douche, enema, and hair-rinse and so on. Tinctures may be diluted as internal medicines or may be used in all the same ways as a water extraction. Acetacta (acetic acid extractions) and glycetracta (glycerol extraction) are almost exclusively used internally. Fat extractions are almost always applied externally in the form of skin oils or incorporated into ointments, lotions and creams. A cocoa butter extraction may also be used as the base for suppositories. Infusions, decoctions and solvent extractions may also be used as sprays, or dried and incorporated into tablets.

A final common method of preparing herbs for internal use is to make capsules. The herb is either air-dried or freeze-dried, ground and used to fill gelatin capsules. This has the advantages of being cheap and convenient but the shelf life tends to be fairly short, with the oxidation of the herb being a very real concern (Cabrera, 2000). These methods of herbal extraction are the traditional and time-honoured ways. They are still entirely viable in the modern world because they are simple, cheap and effective (Cabrera, 2000).
With regard to herbal medicines, the widely used routes of administration and common dosage forms are mainly:

a) The oral route, with the absorbing membrane is the mucus lining of the gastrointestinal tract, and popular dosage forms are infusion, decoction, tincture, acetracta, glycetracta, capsule, tablet, or taken in the form of food. Advantages include convenience and painless; the remedy passes first to the liver where potentially toxic agents may be deactivated; Disadvantages are mainly the absorption is slow, irregular or unpredictable and thus is not suitable when a fast or precise response is important; and because the remedy passes first to the liver, there is a possibility that useful constituents are metabolized and inactivated before reaching other sites of the body.

b) The sublingual route, having the mucus lining of the mouth as the absorbing membrane, and the commonly used dosage forms include lozenges, tablets and sprays. The uptake of active constituents into the blood-stream is very rapid, usually within few minutes, and they enter the systemic circulation directly without first passing through the liver. However, only a few remedies can be administered in this way, since it may be dangerous to avoid the first pass through the liver which acts as a filter or screen for many toxic substances.

c) The rectal route has the mucus lining of the rectum as the absorbing membrane, while the widely used dosage forms are suppositories, ointments or enemas. This route is particularly advantageous in the case of the vomiting, comatose or uncooperative patients and especially useful for remedies which would cause nausea or vomiting if given orally; furthermore the active constituents would bypass the liver and enter the systemic circulation directly. However, rectal route may be unacceptable to some sensitive patients.

d) The vaginal route, with the mucus lining of the vagina as the absorption membrane, and pessaries, creams or impregnated tampons as the commonly used dosage forms. Advantage of this route is that it permits local treatment with very little being absorbed into the blood-stream, but it may be messy or unacceptable to some patients, and is not suitable during pregnancy.

e) The nasal route has as the absorbing membrane the mucus lining of the nose and upper respiratory tract, in addition to the alveolar epithelium, whereas the widely used dosage forms are the sprays, aerosols, inhalations, and drops. Drug uptake is almost instantaneous, and a very local effect may be obtained, or it may be used to produce some drug absorption into the systemic circulation. The route may not be convenient for some patients.

f) The epidermal route of administration utilizes the skin as its absorbing membrane, and has a wide range of dosage forms including ointments, creams, liniments, powders, poultices and plasters. With the route, a very local effect can be obtained, or it may be possible to get better access to a poorly vascularized area from the outside (e.g. a capsicum plaster over an arthritic joint); some absorption may occur into the systemic circulation and this may be variable and unpredictable.

Another important aspect to be considered is the dosage and frequency, which must depend upon several factors: the strength or concentration of the remedy, the severity of the symptoms and the underlying physiological strength of the system or person. Thus, for example, a large, muscular man in generally good health but suffering from an acute head cold will tolerate and, indeed, need, a much higher dose of herbal medicine than would a frail old lady with chronic arthritis, or a 5 year old child with emotionally-based asthma (Cabrera, 2000; CAMI, 2000).

In general, acute conditions will require higher doses and/or stronger medicines than will chronic conditions. Likewise, a small body requires less than a large body. Those people with compromised liver function will metabolize their remedies differently and may need lower doses to prevent a cumulative action. The same is true for people
with impaired kidney function who may not be able to excrete remedies as fast as could otherwise be expected.

Children and the elderly are particularly at risk of liver or kidney insufficiency, but previous medical history, current complaint, other drugs or remedies being used and alcohol and drug abuse should all be taken into account when determining a dose of an herbal medicine.

Another factor to be considered when estimating the dose is whether there is any concomitant constipation or diarrhoea. If there is hyper-motility of the gastro-intestinal tract, then a remedy will pass rapidly through the system and not as much will be absorbed as in the case of hypo-motility.

Biochemical assays, clinical research and empirical evidence have been amalgamated to create a solid body of knowledge about the safety and efficacy of very many herbs. In the British Herbal Pharmacopoeia (BHP, 1983), dosages are given for over 200 herbs and they range from small doses (e.g. 0.5 mL., three times daily for a 1:5 tincture of poisonous herbs such as Deadly nightshade (*Atropa belladonna*) to large doses (e.g. up to 10 mL. three times daily for a 1:5 tincture of *Taraxacum off.* radix). For most herbs in the BHP, the typical dose is between 2 and 6 mL. three times daily for a 1:5 tincture (BHP, 1983; Cabrera, 2000; CAMI, 2000).

Although presented in a wide range of drug delivery systems, herbal medicines are widely formulated by different manufacturers as solid dosage forms including in particular different types of tablets and capsules (Cabrera, 2000; CAMI, 2000).

### 1.6.1. Tablet Dosage Forms

#### 1.6.1.1. Definition of Tablets

Tablets may be defined as solid dosage forms containing drug substances with or without suitable additives, and prepared either by compression or molding methods (Rudnic and Schwartz, 1990).

The vast majority of tablets are made by compression, and the compressed tablets are the most widely used dosage form. They are prepared by the application of high pressures utilizing steel punches and dies to powders or granules.

Although tablets can be defined as “circular discs with either flat or convex faces”, they can be produced in a wide variety of sizes, shapes and surface markings depending upon the design of punches and dies. Capsule-shaped tablets are commonly referred to as caplets, and boluses are large tablets for veterinary use (USP, 1995).

#### 1.6.1.2. Advantages of Tablet Dosage Forms

Solid dosage forms, most notably tablets, provide advantages to pharmacists in storage, dispensing, and control; to patients in convenience of use and compliance; and to physicians in ease of product identification, improved dosage accuracy and precision, and more reliable therapy.

For oral dosage forms, tablets and capsules forms represent the preferred drug delivery systems because they are unit dosage forms, and therefore, they deliver the dose accurately. Beside these, their inherently greater chemical stability, compared to liquid dosage forms. At any temperature, drugs in the dry state may be expected to be in their most stable form and have the greatest shelf life. Accordingly, prescriptions for drugs in tablet dosage forms out-number the sum total of prescriptions for all other dosage forms filled in the community pharmacy.

**Prevention of incompatibilities between constituents of tablet dosage forms**

is almost the best in all dosage forms. Likewise, chemical **incompatibilities between drugs and excipients are diminished in the dry state. In tablet systems,**
compression of the tablet components into dense, compact mass can bring reactants into intimate contact that physical incompatibilities, such as eutectic formulations, become a problem, whereas no such problem occurs in a loose powder-filled capsule. Often such reactions in tablets can be circumvented by employing layered tablets.

When comparing tablets with capsules, the cost of filling the capsules and other machines may be greater than that of fabricating the entire compressed tablets, particularly if a simple tablet granulation process is employed. Until recently, the full-automatic capsule machines were finding increasingly wide usage but they are much slower than modern high-speed tablet compression machines. Compressed tablets are easier to carry and are compact and easy for pharmacists to store. They are lightest in weight per dosage unit, and hence the cheapest to package and strip.

Another advantage of tablets is that every single dosage unit can be identified as to manufactures and absolute product identity. Coated tablets may be imprinted with edible ink for identification code using engraved tablets punches. Thus, identity of such coded products is never lost regardless of separation from the original containers. Rapid product identification, at best, can be a life and death matter in cases of accidental or purposeful over-dosing, or of therapeutic incompatibility or drug interaction.

At least, absolute product identity is helpful to the pharmacist and physician in verifying the identity of the products about which patients may have questions. Recently, most drug manufactures are identifying individual dose.

Pharmaceutical manufactures are always hopeful that new systemically-active drugs will be effective in oral dosage forms, because a drug that is effective only on parenteral administration will usually be restricted to use in clinics or hospitals. It also needs expensive sterilization process and care. Besides it induces pain at the site of administration, and the hazard at the site of injection is expected. Unless a drug has a unique advantage, it is not likely to have a wide or extensive use when formulated in a parenteral dosage form. In addition, pharmaceutical manufactures prefer conventional compressed tablet because it best lends itself to rapid mass production and is the least expensive of the solid dosage forms.

Furthermore, solid dosage form can readily be designed to provide controlled drug dissolution so that the rate of drug release, and not the inherent absorbability of the drug, determines the rate of absorption. The dosage form release may influence the blood level patterns generated, and consequently the safety of the drug product. Most drug products provide for immediate and uncontrolled drug absorption, with resultant sharp, high peak blood levels, which on overdosing may cause toxic reactions or death.

For some classes of drugs, including most if not all antidepressant drugs, such uncontrolled drug release can not be considered to be optimized drug delivery from a safety stand-point. Similarly control of drug absorption rate and duration alone in the GIT can not influence drug effectiveness and reliability. Such control of absorption following oral administration is best achieved with solid dosage forms.

Occasionally it may be desirable to prevent drug dissolution in the stomach for drugs that are nauseating or irritating if released there. Other drugs are chemically degraded in the stomach if allowed to dissolve there, and must be protected against dissolution in the stomach. Enteric-coated tablet is commonly employed to prevent gastric drug dissolution. Continuous sustained-drug release, over an 8-12 hours period following oral administration of a dosage unit, can be achieved with solid dosage forms.
It is easiest to fabricate for tablets, and is much more difficult to produce with liquid products, such continuous drug release which may be desirable to reduce the frequency of dosing (sustained-release), or to produce a more uniform therapeutic response.

1.6.1.3. Types of Tablets

The various types of tablets and abbreviations used in referring to them include:

1.6.1.3.1. Compressed Tablets (CT)

These are standard uncoated tablets prepared by compression of powder, crystalline or granular materials, alone or in combination with suitable additives. They can be prepared by any of the basic manufacturing methods such as wet granulation, direct compression, or double compression.

1.6.1.3.2. Sugar-Coated Tablets (SCT)

These are compressed tablets that are covered with a sugar coating, which may be coloured. This sugar coating is useful in covering up drug substances with objectionable tastes or odours, and in protecting formulations sensitive to oxidation (Rudnic and Schwartz, 1990).

1.6.1.3.3. Press-Coated Tablets (Dry-Coated Tablets)

These are prepared by compressing another granulation layer around previously compressed tablets which are fed into a special tableting machine. They have all the advantages of a compressed tablet, while retaining those of sugar-coated tablets (Rudnic and Kottle, 1996).

1.6.1.3.4. Film-Coated Tablets (FCT)

These are compressed tablets which are covered with a thin layer or film of a water-soluble material. A number of polymeric materials with film-forming properties are used.

Film-coated tablets have the same general characteristics as sugar-coated tablets, with the additional advantages of a greatly reduced time required for coating process, better mechanical strength, the ability to retain de-bossed markings on tablet, and the avoidance of sugar which is contra-indicated in the diets of diabetic patients (Banker and Anderson, 1996).

1.6.1.3.5. Enteric-Coated Tablets (ECT)

These are the most common example of delayed-action tablet product which is intended to release a drug after the tablet has passed through the stomach into the intestine. Enteric coating can be used for tablets containing drugs which are inactivated or destroyed in the stomach, for those which irritate the mucous, or as a means for a delayed-release of the drug (Rudnic and Schwartz, 1990).

1.6.1.3.6. Controlled-Release Tablets

These are intended to release the drug slowly over a prolonged period of time, and have been referred to as "prolonged-release" or "sustained-release" dosage form as well. These tablets can be categorized into three types:

a) Tablets which respond to some physiological condition to release the drug, such as enteric-coated tablets;

b) Tablets that release the drug in a relatively steady, controlled manner; and

c) Tablets that involve combination of mechanisms to release "pulses" of drug such as repeated-action tablets.

1.6.1.3.7. Layered Tablets (Multiple-Compressed Tablets)
These are prepared by feeding previously compressed tablets into special tableting machine and compressing another granulation layer around the preformed tablets. These are compressed tablets made by more than one compression cycle, and the process may be repeated to produce multi-layered tablets.

Tablets in this category are usually prepared to separate physically or chemically incompatible ingredients or to produce repeat-action or prolonged-action products (Rudnic and Schwartz, 1990).

1.6.1.3.8. Effervescent Tablets

These are designed to produce a solution rapidly. They contain, in addition to the drug, sodium bicarbonate and an organic acid such as tartaric acid or citric acid. In the presence of water, these substances react liberating carbon dioxide, which acts as a disintegrant and produces effervescence (Rudnic and Schwartz, 1990).

1.6.1.3.9. Buccal and Sublingual Tablets

These are small, flat, oval tablets intended for buccal administration by inserting into buccal pouch, where they dissolve or erode slowly. They are formulated and compressed to give a hard tablet. Sublingual tablets are placed under the tongue where they dissolve rapidly, and the drugs are absorbed readily by this route of administration.

1.6.1.3.10. Molded Tablets (Tablet Triturates)

These are usually prepared from moist materials using a suitable triturate mold which gives them the shape of cylinder cut-sections. They must be rapidly and completely soluble. The problem associated with the compression of these formulations is the failure to find a completely water-soluble lubricant.

1.6.1.3.11. Vaginal Tablets or Inserts

These are designed to undergo slow dissolution and drug release in the vaginal cavity. They are typically ovoid or pear-shaped to facilitate penetration in the vagina (Banker and Anderson, 1986). In this type, as well as for any tablets intended for administration other than by swallowing, the label must indicate the manner in which it is to be used (Rudnic and Schwartz, 1990).

1.6.1.3.12. Solution Tablets

These are compressed tablets designed to be used for preparing solutions or imparting given characteristics to solution. Therefore, they must be labeled to indicate that they are not to be swallowed.

1.6.1.3.13. Dispensing Tablet (DT)

These are supplied primarily as a convenience for extemporaneous compounding and should never be dispensed as dosage form. These tablets are intended to provide a convenient quantity of a potent drug that can be incorporated readily into powders and liquids, thus circumventing the necessity to weigh small quantities.

They may be added to a given volume of water by the pharmacist, or consumer, to produce a solution of a given drug concentration (Rudnic and Schwartz, 1990).

1.6.1.3.14. Implantation Tablets

These are sterile tablets designed for subcutaneous implantation in animals or man, with the purpose of providing prolonged drug effects ranging from one month to a year. There are two major safety problems with this form of administration: a) the need for surgical technique to start and discontinue therapy, and b) tissue irritation and
Toxicity. Therefore, this class has achieved little use in humans (Banker and Anderson, 1986).

1.6.1.3.15. Hypodermic Tablets

These are soft, readily-soluble tablets, which were originally used for preparation of solution to be injected. Since stable parenteral solution is now available for most drug substances, there is no justification for the continued use of hypodermic tablets for the injection, and nowadays they are not in use (Rudnic and Schwartz, 1990).

1.6.1.4. Tablet Ingredients

Regardless of how tablets are manufactured, conventional oral tablets usually contain, in addition to the active ingredient(s), one or more ingredients functioning as diluents, binders or adhesives, disintegrants, lubricants, and glidants. They also contain some optional components including colourants, flavours and sweeteners. All non-drug components of a tablet formula are termed excipients, which may also be classified, according to the role they play in the finished tablets, into two major groups:

a) Those materials which help to impart satisfactory processing and compression characteristics to the formulation. This group includes diluents, binders, lubricants and glidants; and b) Those materials which help to give additional desirable physical characteristics to the finished tablets. Included in this group are disintegrants, colourants, sweeteners and flavours, and in the case of controlled-release tablets, polymers, waxes or other solubility-retarding materials (Rubenstein, 1988).

1.6.1.4.1. Diluents

An increasing number of drugs are used in very low dosage, and to make tablets of reasonable size, it is necessary to dilute the drug with an inert material which is termed diluent, or sometimes referred to as filler (Marshall, 1979).

Diluents, and all other tablet excipients, must meet certain criteria in the formulation; these include that they must be non-toxic, not contra-indicated by themselves such as sucrose or because of component such as sodium, stable physically and chemically when combined with the drug or other tablet components, not produce any off-colour appearance, commercially available in an acceptable grade, and of low cost (Banker and Anderson, 1986).

Carbohydrates are widely used as diluents due to their general ability to improve tablet mechanical strength as well as to their acceptable taste and reasonable solubility profiles. Lactose and other carbohydrates based on o'-lactose are frequently used as diluents because they adequately meet most of the requirements of good diluent. Beside different size grades of normal, hydrous o'-lactose, there is spray-dried lactose, which is agglomerate of o'-lactose.

Spray-dried lactose has very good flow properties when mixed with amorphous materials, but its poor compression characteristics necessitate the addition of a binder. USP Fast-flo is a brand of spherical, crystalline, amorphous agglomerate of ‘o-lactose which possesses superior compressibility (Marshall, 1979). Lactose has been used for several years as diluent before its interaction with certain amine drugs was recognized. This classic drug-excipient incompatibility results in discoloration of tablets in presence metal stearate (Castillo and Mattocks, 1962).

Starch is also occasionally used as tablet diluent. It consists of polysaccharide granules obtained from the caryopsis of maize, Zea mays L., of rice, Oryza sativa L., of wheat, Triticum aestivum, or from the tubules of potato, Solanum tuberosum L. (BP, 1980). It can be obtained from sorghum, Sorghum bicolor Moench, which is a food crop widely grown in tropical Africa including Sudan. Starch is a fine, white powder which
creeps when pressed between the fingers. It is practically insoluble in cold water and in ethanol (96%) (BP, 1980).

Various grades of directly-compressible starches are available commercially and can be used as diluents, binders and/or disintegrants. Hydrolyzed starches, consisting basically of dextrose and maltose, can be used instead of mannitol in chewable tablets, because of their sweetness, smooth feeling in the mouth and lower cost than mannitol. However, mannitol is sometimes combined with its optical isomer sorbitol to minimize its cost. Sugar and different sugar-based diluents are employed in tablet manufacture, although some manufacturers avoid their use in concentrations that would be hazardous to diabetic patients. Some other sugars are now being produced in special granules by several manufacturers with different trade names. Most of these sugars involve the combination of sucrose with invert sugar or modified dextrins, and they are of particular value in the formulation of chewable tablets (Marshall, 1979).

Certain inorganic salts are frequently used as fillers. The commonly used salts are calcium carbonate, di-calcium phosphate, calcium triphosphate, magnesium carbonate and sodium chloride. The most popular salt is calcium phosphate di-hydrate, which is a comparatively cheap insoluble diluent with good flow and stability properties, but poor compression characteristics. It is slightly alkaline and must not be used with drugs that are sensitive to pH value above 7.3. The combination of calcium phosphate di-hydrate and calcium carbonate in the formulation of griseofulvin tablet gave superior results (Khan and Rhodes, 1971). On the other hand, such combination is not suitable with ascorbic acid and thiamine hydrochloride since it results in deterioration of hardness and disintegration properties of both vitamins and chemical deterioration of ascorbic acid (Marshall, 1979).

1.6.1.4.2. Binders and Adhesives

These are materials that are added to the tablet formulation when the powders themselves lack sufficient cohesion to form tablets of suitable hardness. They impart cohesiveness to the tablet formulation that improves the free-flowing characteristics of the granules of desired hardness and size, and ensures the tablets remaining intact after compression. The amount of binder added has a significant effect on the characteristics of the compressed tablets, and the use of excess amount of binder, or too strong binder, will result in too hard tablets that will not disintegrate easily and cause excessive wear of punches and dies (Rubenstein, 1988; Rudnic and Schwartz, 1990).

They are added either as dry powder to promote cohesion of directly compressed tablets, and often referred to as binders, or in liquid form during wet granulation, and generally termed as adhesives. Most granulating agents have adhesive properties such as gelatin, acacia, liquid glucose, sucrose syrup, starch paste, as well as aqueous solutions of cellulose polymers such as carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, and mucilages of colloidal clays.

Other materials used as binders include microcrystalline cellulose, powdered or spray-dried acacia, amylose, and polyvinylpyrrolidone (PVP) (Banker, 1974).

1.6.1.4.3. Disintegrants

These are materials which are added to the formulation to promote tablet break-up and disintegration after ingestion. This is an important step towards efficient drug release from the tablet material to allow rapid drug dissolution and absorption. Materials added as disintegrating agents include celluloses, starches, alginates, gums, clays, and cross-linked polymers. Super disintegrants are those materials which have gained popularity as disintegrating agents due to their high effectiveness at low concentrations (2-4%). They include cross-linked celluloses, such as croscarmelose; cross-linked
polymers, such as crospovidone; and cross-linked starches, such as sodium starchglycolate (Rubenstein, 1988; Rudnic and Kottke, 1996).

1.6.1.4.4. Lubricants

These are materials which are added to the tablet granulation to play the following roles in tablet manufacture: a) To prevent adhesion of tablet material to the surfaces of the punches and dies; b) To reduce the inter-particle friction and improve the rate of powder flow; and c) To promote tablet ejection from the die cavity.

The lubricants commonly used include: talc, magnesium stearate, calcium stearate, stearic acid, hydrogenated vegetable oils, and polyethylene glycol. Most lubricants are used in concentration below 1%, with the exception of talc which is used in concentrations as high as 5%. Most lubricants are hydrophobic materials, and poor selection or excessive amounts can result in water-proofing the tablets, resulting in poor tablet disintegration and/or delayed or failure of drug dissolution and absorption.

1.6.1.4.5. Glidants

These are substances which are added, in the dry state, to the tablet granulation just prior to compression, to improve the flow characteristics of the powder mixture. The most commonly used glidant added during the lubrication step is silicone dioxide (Cab-o-sil, Cabot) which is used in low concentrations of 1% or less. Talc (asbestos-free), when used, may serve the dual role of lubricant and glidant (Rubenstein, 1988).

1.6.1.4.6. Sweetening Agents

These are substances which are added to the tablet formulation to improve the organoleptic properties of the finished products, and mask unpleasant tastes of some drugs. They are either natural sweeteners, such as sugars and related compounds, or artificial sweeteners, such as cyclamates, saccharin, and aspartame. However, cyclamates were banned; saccharin has an indefinite status with regard to its health hazards, and aspartame has found application in pharmaceutical formulations. Since aspartame is used in low concentrations, it will not affect markedly the physical characteristics of the tablet granulation (Rudnic and Schwartz, 1990).

1.6.1.4.7. Colouring Agents

These are substances which are included in the tablet formulation to serve the following functions: a) To make the dosage form more aesthetic in appearance; b) To help to control the product during its preparation; and c) To serve as a means of identification to the users. All colourants used in pharmaceuticals must be approved and certified by the FDA. The most common method of adding colour to tablet formulation is to dissolve the dye in the binding solution prior to the granulation process. Another approach is to adsorb the dye from its aqueous solution on starch or calcium sulphate and the resultant powder is dried and blended with the formulation ingredients.

1.6.1.5. Processes of Tablet Manufacture

The selection of an appropriate method of tablet production is based on several criteria, the most important of which are the physico-chemical, biopharmaceutical and pharmacokinetic properties of the drug substances, the physico-chemical characteristics of the excipients, the method of administration, the site, onset and duration of action, and the therapeutic effect of the drugs involved.

Large-scale tablet production involves the use of one of the following processes: (Rudnic and Schwartz, 2000)

1.6.1.5.1. Wet Granulation Process
This is the most widely used method of tablet preparation or production. The principal steps in wet granulation method are weighing, mixing, granulation, screening the moist mass, drying, dry screening lubrication and compression. Wet granulation method has the advantages of improving the physical properties of the drug substances, such as flowability, compressibility, wettability, and solubility.

However, this process is not suitable for thermolabile or moisture-sensitive drug substances.

1.6.1.5.2. Double Compression Process (Dry Granulation Process)

This method of tablet production consists of two compression cycles, the first is intended to prepare slugs, which are crushed, and the resultant particles are further re-sized, lubricated, and prepared for the second compression process into the required tablets. This method is suitable for moisture-sensitive or thermolabile drugs since it involves neither moisture nor heat in the preparation of the granules or the final tablets.

1.6.1.5.3. Direct Compression Process

This method of tablet manufacture involves the direct compaction of the powdered material into tablets, without major modification of the physical characteristics of the drug substance or excipients.

1.6.1.5.4. Moist Granulation Process

This is a newly-developed technique which closely resembles the wet granulation method in that the powdered material is moistened, but using only an aqueous solution. However, the drying of the sized-granules is carried out by employing sodium carboxymethylcellulose as a water-withdrawing agent.

This granulation method is suitable for thermolabile drugs, but inappropriate for moisture-sensitive drug substances (Rudnic and Schwartz, 2000).

1.6.1.6. Problems Associated with Tablet Formulation and Compression

1.6.1.6.1. Processing Problems

Various problems occur in the normal process of developing a tablet formulation and in the routine manufacturing of tablets. The sources of the problems may be the formulation, compression equipment, or combination of the two.

1.6.1.6.1.1. Capping and Lamination

Capping is a term used to describe the partial or complete separation of the top or bottom crowns of the tablets from the main body of the tablet into two or more distinct layers. Usually these processing problems are readily apparent immediately after compression; however, they may occur hours or days later. They have been attributed to air entrapment. Hiestand et al. (1977) have shown that these problems are due to deformational properties of the formulation during and immediately after the compression stress relaxation, which may be defined as the ability of solids “that exhibit plastic flow” over a period of time to relieve the stress (Train, 1957). Therefore, this could be the explanation for some practical tableting problems...

Tablet lamination or capping is often eliminated by re-compression, slowing the tableting rate, and reducing the final compression (Banker and Anderson, 1986). Drugs such as paracetamol and ibuprofen, which cap or laminate during or after decompression, are modified by formulating them with polymeric substances which deform plastically under shear stress such as PVP and starch (Dwivedi et al., 1991).

1.6.1.6.1.2. Picking and Sticking
Picking is a term used to describe the surface material of a tablet that is sticking to and being removed from the tablet surface by a punch. Picking is a particular concern when punch tips have engraving or embossing.

1.6.1.6.1.3. Mottling

The appearance of tablets is one of the characteristics investigated during the stability and quality control studies carried out on pharmaceutical formulations (USP, 1985). Mottling is unequal distribution of colour on a tablet with light or dark spots standing out in an otherwise uniform surface. This problem may occur when the drug’s colour differs from that of other tablet excipients, or when the drug degradation products are coloured. To overcome this problem, the formulator may change the solvent system, binder system, reduce the drying temperature, or grind to small particle size.

Deviation from this property, i.e. “tablet appearance”, is one of the first things that a patient notices, and therefore it is important that this parameter is correctly qualified during the formulation and stability study. The judgment of appearance is often made by visual inspection of the tablets, but such a subjective judgment does not always yield unbiased information; it tends to be influenced to a great extent by external factors such as the location and time of the day. Wirth (1991) has replaced the visual judgment of colour difference with a photometric measurement using commercial instruments. Two of the measured parameters, brightness and yellowishness, were found to reflect the change in coloration observed in stability test on white metoprolol tablets.

1.6.1.6.1.4. Poor Flow

The die fill process during tableting is based on a continuous and uniform flow of granulation from the hopper through the feed frame. Incomplete die filling occurs when the granulation does not flow readily and uniformly, or when the machine speed is greater than the granulation flow capabilities. The addition of talc or colloidal silica may be helpful. Since most tablet granulations consist of materials with a range of particles, the use of devices to improve poor flow is often problematic because the variation or mixing action of such devices may induce segregation and stratification of particles.

1.6.1.6.1.5. Mechanical Faults

Unequal lengths of lower punches may result in different filling capacities of dies because the filling is volumetric. Punch tip geometry has some influence on the tablet friability. Depending on the concavity depth, extra-deep concave punches were found to produce tablets with low friability than when using standard or deep concave punches (Chowlan, 1992). To overcome all these processing problems, in order to have favourable physico-chemical properties, trials must be done in these fields by adding different additives to fulfill the requirements of stated properties identified by recent monographs like BP or USP. This can be defined as optimization of physico-chemical properties, which helps in avoidance of the processing problems and mechanical faults.

1.6.1.7. Optimization of the Physicochemical Properties

The basic philosophy of optimization in tablet formulation is that the production procedure is allowed to evolve to the optimum by careful planning and constant repetition (Schwartz, 1979). However, as statistical design is a separate field, the concept of interest to the pharmacist planning to utilize optimization techniques, is that there are methods available for selection which are considered. Furthermore, analysis of the results will allow separation of variables, i.e. statistical analysis can be performed which allows the experimenter to know which variable caused a specific result.

1.6.1.8. Tablet Evaluation
After optimization of the tablet formulation, by using different kinds of additives, (e.g. diluents, binders, disintegrants, glidants and lubricants) which will affect greatly its physiochemical properties, the tablet must be subjected to evaluation according to the BP or USP monograph specifications. These include general appearance, diameter, size, shape, thickness, weight, hardness, friability, disintegration and dissolution. These physicochemical properties are affected by a lot of variables each, like inheritance properties and nature of each additive, wettability, flocculation, agglomeration or variation of particle size of each of the granules (Bodwan et al., 1995).

Some of the important tests conducted for evaluation of tablets include:

1.6.1.8.1. Dissolution

This is one of the major methods of evaluation of solid dosage forms, especially the tablets, and which is stated by many recent monographs like BP and USP.

Dissolution is an important parameter which can influence drug absorption and activity, particularly in the case of poorly-soluble drugs. Furthermore, it is an important tablet characteristic because there are many factors likely to affect dissolution rate.

1.6.1.8.1.1. Factors Affecting in vitro Dissolution Rate

a) The characteristics of the drug itself

The characteristics of the drug itself, particularly its surface area and particle size characteristics (Tutadhar et al., 1983). Attempts to correlate the dissolution of spironolactone, from different formulations, with its bioavailability indicated a non-significant correlation (Clark et al., 1977). These discrepancies were attributed to drug’s particle size as a formulation variable (Reynolds, 1982).

For optimization of spironolactone tablets, the effect of particle size was minimized by inclusion of sodium dodecyl sulfate in the formula as a solubilizer. Such inclusion in the dissolution medium resulted in enhancement of dissolution and narrowing of the variations between particle size fractions, to an extent where variations due to particle size may be masked (Bodwan et al., 1995). Other physical properties such as viscosity, density and wettability contribute to general dissolution problems of flocculation and agglomeration (Wagner, 1961). Normally reduction in particle size results in increased dissolution rate. However, the reverse has been demonstrated for acetylsalicylic acid-phenacetin powders, and explanation of this behaviour is attributed to hydrophobic nature of the surface resulting in decreased wetting rate (Finholt, 1974).

b) Factors related to tablet formulation

i. Effect of the diluents and disintegrants

Finholt (1974) suggested that the hydrophobic crystals of aspirin-phenacetin acquire a surface layer of fine starch particles that impart hydrophilic property to the granular formulation, thereby increasing surface area, and enhancing dissolution rate.

ii. Effect of binders

It is generally accepted that wet granulation has been shown to improve the rate of dissolution of poorly-soluble drugs by imparting hydrophilic properties to the granules surface (Wagner, 1961).

However, Marlowe and Shangraw (1967) have shown that phenyl butazone tablets prepared with spray-dried lactose, by direct compression, exhibited higher dissolution rates than those prepared by wet granulation with starch. They have also shown that the dissolution rate increases as the starch concentration was increased.

The effect of various starches (corn, potato, arrowroot, rice and compressible starch) on dissolution rate of salicylic acid tablets was determined. It was concluded that
different types of starch did have different effects on dissolution rate, and compressible starch formulation had the fastest dissolution rate (Underwood and Cadwallader, 1972).

iii. Hardness of the Tablets

The general trend is that increasing the applied pressure on tablets formulations may greatly reduce the dissolution rate. However, a study of the effect of pressure on the dissolution rate of aspirin-phenacetin-caffeine tablet had shown that the dissolution rate increased as the applied pressure was increased; this was attributed to the nature of the formula more than to pressure (Knoechel et al., 1967).

iv. Effect of Lubricants

Levy and Guntow (1963) investigated the effect of tablet lubricants on the dissolution rate of salicylic acid tablets, and they have found that the use of hydrophobic tablets lubricants, e.g. magnesium stearate, stearic acid or talc, retarded the dissolution, while the water-soluble lubricant sodium lauryl sulfate enhanced it. Magnesium stearate decreased the effective drug-solvent interfacial area by changing the surface characteristic of the tablets which results in reducing wettability, prolonging the disintegration time, and decreasing the area of interface between the active ingredient and the solvent. On the other hand, sodium lauryl sulfate’s effect was suggested to be due, in part, to increase in the micro-environment pH surrounding the sparingly-soluble weak acid, and to increased wetting and better solvent penetration into tablets and granules as aerosil lowers the interfacial tension between the solvent and the solid.

Since 1970, The USPNF has provided procedures for dissolution testing. This test is provided to determine compliance with dissolution requirements where stated in the individual monograph for a tablet or capsule, except where the label states that the tablets are to be chewed. Unless otherwise directed in the monograph of the type of the appearance described, the one that is specified in the individual monograph is used (USP, 1992).

2. Aim, Objectives and
2. Aim, Objectives and Scope of the Project

2.1. GENERAL

Herbal medicine is a form of treatment, which is as old as mankind itself. Most ancient forms of the medicine are coming back to use but with more purified forms using new technologies of processing. Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations thereof.

The use of traditional medicines (TM) and complementary and alternative medicine (CAM), in particular the use of herbal medicinal products, continues to grow world-wide. In their recent report, WHO (2002) have highlighted that: ‘The use of TM/CAM is increasing rapidly in developing/developed countries.

In many parts of the world, policy-makers, health professionals and the public are wrestling with questions about the safety, quality, availability, preservation and further development of this type of health care.

Although many TM/CAM therapies have promising potential, and are increasingly used, many of them are untested and their use not monitored. As a result, knowledge of their potential therapeutic benefits and side-effects is limited. This makes identification of the safest and most effective therapies and promotion of their rational use more difficult. If TM/CAM is to be promoted as a source of healthcare, efforts must be made to promote its rational use, and identification of the safest and most effective therapies will be crucial.’

Furthermore, TM/CAM has many positive features including: diversity and flexibility; accessibility and affordability in many parts of the world; broad acceptance
among many populations in developing countries; increasing popularity in developed
countries; low level of technological input; comparatively low cost; and growing
economic importance. These can all be seen as opportunities to be maximized.

However, other features of this type of health care can be regarded as challenges
to be overcome. They include: the varying degree with which it is recognized by
governments; the lack of sound scientific evidence concerning the efficacy of many of
its therapies; difficulties relating to the protection of indigenous TM knowledge; and
problems in ensuring its proper use.

Sudan has an old and very rich tradition of herbal medicine with its roots extended
in the African, Arabic and Islamic cultures. However, this wealth of Sudanese herbal
medicine is not expanded in terms of commoditization and commercialization to
compete with global trends. Many Sudanese plants are used in Sudanese folk medicine
for their therapeutic values.

For the majority of people in Sudan, traditional medicine remains the main or even
the only source of health care especially in remote rural areas, but there is a lack of
sufficient scientific and ethno-medical data of most, if not all, of these traditional herbal
preparations. Therefore, Sudan needs a national policy on herbal healthcare products
industrialization, which focuses on product development and promotion of research.

There must a consolidated plan for promoting herbal healthcare products in a
commercial perspective. This will definitely help to generate alternative incomes for rural
people. Current situation is the ideal ground for implanting such a national concept,
which will be an enamoured venture for investors.

2.2. AIM

Therefore, considering all these challenges and needs, the AIM of the present
study is to plan and conduct an elaborative, multidisciplinary research project which is a
must for formulation of efficacy and safety standards for an herbal healthcare product.

This will help to install a system and mechanism to manipulate other herbal
healthcare products for consumer welfare, and to establish a knowledge data-base on
herbal medicines and their uses.

This is also aimed to be a very important step towards driving the progression of
the pharmaceutical industry towards new productive goal and coalition with
mainstreamed industrial interventions

2.3. Objectives

Addressing different needs and requirements of an effective and safe herbal
healthcare product, the main objectives enunciated in the current research project
include:

a) Selection, verification and authentication of a widely used medicinal herb or plant
species;

b) Qualitative and/or quantitatively determination of the main constituents in the
selected plant;

c) Examination of the biological actions of the selected plant on different isolated
tissues, organs or whole experimental animals;

d) Study the toxic effects of the selected plant in experimental animals;

e) Design, formulation and evaluation of a suitable, plant-based pharmaceutical
dosage form;

f) Evaluation of the therapeutic activity of the prepared pharmaceutical dosage form;

and

g) Investigation of the stability of the prepared pharmaceutical dosage form.

In conclusion, the ultimate objective of the present study is to formulate an
effective, safe, non-toxic, non-expensive, reasonable-sized, elegant, easy-to-administer,
stable drug delivery system based on the active constituents of a selected medicinal herb or plant.

2.4. Scope of the Work

The study highlights very significant areas related to the scope of the research project, and which can be grouped into the following disciplines:

1) Phytochemical Aspects covering all the studies on the chemical constituents of the selected medicinal herb;
2) Pharmacological Aspects dealing with all the investigations of the biological actions of the medicinal herb on different experimental settings;
3) Toxicological Aspects addressing all the possible toxic effects in different experimental models and determination of toxicity parameters;
4) Pharmaceutical Aspects embracing all the stages of production of an appropriate drug delivery system of the selected medicinal herb;
5) Clinical Aspects involving comparative evaluation of the therapeutic effect of the prepared pharmaceutical dosage form; and
6) Stability Aspects including studies of the effects of different storage conditions on the physicochemical characteristics of the prepared pharmaceutical dosage form.

3. Experimental
3. Experimental

3.1. Materials

3.1.1. Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid, Glacial</td>
<td>BDH Chemicals Ltd, England</td>
</tr>
<tr>
<td>Acetylcholine</td>
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<tr>
<td>Adrenaline</td>
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<td>Aluminium Oxide</td>
<td>E. Merck England</td>
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<td>Ammonia Solution</td>
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<tr>
<td>Bismuth subnitrate</td>
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<tr>
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<tr>
<td>Dragendorff’s Reagent</td>
<td>BDH Chemicals Ltd England</td>
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<tr>
<td>Ether</td>
<td>Gifer and Barbesat</td>
</tr>
<tr>
<td>Heparin</td>
<td>Sigma Co. Ltd., England</td>
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<tr>
<td>Hydrochloric acid</td>
<td>Riedel-Dehaen, AG</td>
</tr>
<tr>
<td>Kits</td>
<td>Biochemia, Spain</td>
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<tr>
<td>Lignocaine</td>
<td>Sigma Co. Ltd., England</td>
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<tr>
<td>Methanol</td>
<td>Riedel-Dehaen, AG</td>
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<td>Natural Products Reagent</td>
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<td>Worm company</td>
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<td>Polyethylene glycol 4000</td>
<td>BDH Chemicals Ltd, England</td>
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<tr>
<td>Potassium iodide</td>
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<td>Silica Gel</td>
<td>E. Merck England</td>
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<tr>
<td>Sodium fluoride</td>
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</tr>
<tr>
<td>Sodium Nitrate</td>
<td>Sigma Co. Ltd., England</td>
</tr>
<tr>
<td>Sulphuric Acid</td>
<td>Riedel-Dehaen, AG</td>
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</table>

3.1.2. Raw Materials

<table>
<thead>
<tr>
<th>Raw Material</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>Local Market, Khartoum</td>
</tr>
</tbody>
</table>
3.1.3. Apparatus, Equipment, Instruments

<table>
<thead>
<tr>
<th>Apparatus, Equipment, Instruments</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disintegrations tester. ZL3</td>
<td>Erweka GmbH, Germany.</td>
</tr>
<tr>
<td>Dissolution tester</td>
<td>Erweka GmbH, Germany.</td>
</tr>
<tr>
<td>Friability tester</td>
<td>Erweka GmbH, Germany.</td>
</tr>
<tr>
<td>Granulator Machine</td>
<td>Erweka (Korsch), West Germany</td>
</tr>
<tr>
<td>Hammer mill (size inch 8)</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Incubator</td>
<td>Baird and Tatlock, England.</td>
</tr>
<tr>
<td>Infra red.</td>
<td>Perkins Elmer, Germany.</td>
</tr>
<tr>
<td>Melting point apparatus</td>
<td>Gallenkamp-Gerrant</td>
</tr>
<tr>
<td>Oven</td>
<td>Memmert, Germany</td>
</tr>
<tr>
<td>Roto-vap</td>
<td>Perkins Elmer, Germany.</td>
</tr>
<tr>
<td>Sieves Mechanical Shaker</td>
<td>Podmores Engineers LTD. England</td>
</tr>
<tr>
<td>Sieves (Test Sieves)</td>
<td>Retsch 5657 HAAN, West Germany</td>
</tr>
<tr>
<td>Tableting Machine, Single Punch</td>
<td>Erweka GmbH, Germany.</td>
</tr>
<tr>
<td>UV Spectrophotometer (Spectronic 2000)</td>
<td>Bausch and Lomb, Germany.</td>
</tr>
<tr>
<td>Vernier caliper</td>
<td>Amnesty, England</td>
</tr>
</tbody>
</table>

3.1.4. Pharmacology Equipment and Instruments
Palmer Bioscience via an A100 coupler unit transducer compatible with
Recorder (Harvard) attached to graphic 1002 Lloyd device
Equipment for setting up isolated gut
Gas mixture (95% oxygen and 5% carbon dioxide
Surgical instruments scalped and blade
Dissection scissors thread, size 000, and S-shaped hooks
Petri dish (paraffin wax tissue mat)
Air pump

3.1.5. Animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chickens</td>
<td>Local Market, Khartoum</td>
</tr>
<tr>
<td>Frogs (African Toad)</td>
<td>Local Supplier, Khartoum</td>
</tr>
<tr>
<td>Goats (Nubian spp)</td>
<td>Local Supplier, Khartoum</td>
</tr>
<tr>
<td>Guinea Pigs</td>
<td>National Research Centre, Sudan</td>
</tr>
<tr>
<td>Rabbits (Local strain)</td>
<td>Local Supplier, Khartoum</td>
</tr>
<tr>
<td>Rats (White Albino Canadian)</td>
<td>Animals House, Faculty of Pharmacy</td>
</tr>
</tbody>
</table>
3.1.6. Culture Media

<table>
<thead>
<tr>
<th>Culture Medium</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deoxycholate Citrate Agar</td>
<td>Oxoid Ltd, Basingstoke Hants, England</td>
</tr>
<tr>
<td>McConkey’s broth</td>
<td>London Analytical and Bacteriological Media Ltd.</td>
</tr>
<tr>
<td>Nutrient Agar</td>
<td>Oxoid Ltd, Basingstoke Hants, England</td>
</tr>
<tr>
<td>Nutrient Agar, CFU</td>
<td>Plasmatec, UK</td>
</tr>
<tr>
<td>Nutrient Broth</td>
<td>Oxoid Ltd, Basingstoke Hants, England</td>
</tr>
</tbody>
</table>

3.1.7. Subjects

a) Healthy adult male students and staff members aged 18-50 years, Faculty of Pharmacy, University of Khartoum, volunteered in the clinical trials after giving their written informed consents; and

b) Patients, from Khartoum State Hospitals, regardless of gender, aged 25-60 years, complaining from different degrees of gastro-intestinal colic, (abdominal pains, colics, and irritable bowel syndrome) participated in the clinical part of the study.

3.2. Methods

The methods followed were pertinent to the following major subject areas:

3.2.1. Solenostemma argel Leaves: Selection, Identity, Quality and Purity

3.2.1.1. The Questionnaire of Herbal Medicine Usage

A questionnaire, concerning the practice of house-hold usage of herbal medicines for different minor ailments, was distributed to three hundred participants. The data collected in this questionnaire was recorded, tabulated and formed the basic criterion, among others, for the selection of the medicinal herb to be used in the present study.

3.2.1.2. General Identity Tests of Solenostemma argel Leaves

These included a macroscopic, microscopic, macro-chemical analysis, thin layer chromatography and column analysis for the presence of characteristic alkaloids, and flavonoids identification test. Different solvent systems were used for isolation of the active constituents using (ElGazali et al., 1998):

a) Silica Gel Column

The column was prepared by 100gm of silica gel; 200ml of different systems were eluted through, and started by n-hexane; n-hexane:ethylacetate as 1:1, 1:2, and 1:3; diethyl ether: ethyl acetate as 1:1; ethyl acetate; ethylacetate:methanol as 2:1, and 1:1; methanol; and water. This was used for detection, separation, and isolation of flavanoids. Other systems were used for the same purpose, and included: cyclohexane:ethylacetate as 3:1, 2:1, 1:1, 1:2, and 1:3; chloroform:methanol as 85:15; chloroform:acetic acid:water as 3:3.5:0.5; and chloroform:ether:alcohol as 30:20:20.

b) Alkaloids Extraction from Solenostemma argel Leaves

For Extraction of Alkaloids, the Following Steps were followed:

The fresh leaves and flowers were homogenized for complete extraction in a soxlet with methanol:water system (4:1). The filtrate was evaporated below 40°C till the volume was 1/10th the original volume; then acidified and extracted with chloroform (3 times) (chloroform extract) (i).

The aqueous layer was acidified, evaporated to 1/10th of its volume; then made alkaline (pH 10) with ammonia solution; the solution was extracted with chloroform: methanol (2:1) system, and then with chloroform to have the chloroform layer (ii).
The chloroform extract (ii) and aqueous extract were evaporated to dryness; then extracted with methanol; and taken for alkaloids detection.

3.2.1.3. Determination of Foreign Matter

Macroscopic examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable for powdered materials. Any soil, stones, sand, dust and other foreign inorganic matter must be removed before medicinal plant materials were cut or ground for testing. According to WHO specifications, foreign matter is material consisting of any or all of the following: a) Parts of the medicinal plant material or materials other than those named with the limits specified for the plant material concerned; b) Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned; and c) Mineral admixtures not adhering to the medicinal plant materials, such as soil, stones, sand, and dust.

Recommended Procedure

In the case of foreign matter, in whole or cut medicinal plant material, a 250g sample of the plant material was weighed and spread in a thin layer. The foreign matter was sorted into groups either by visual inspection, using a magnifying lens (6x or 10x), or with the help of a suitable sieve (according to the WHO requirements for the specific plant material). The remainder of the sample was sifted through a No. 250 sieve; dust was regarded as mineral admixture. The portions of this sorted foreign matter were weighed to within 0.05g. The contents of each group, in grams per 100g of air-dried sample, were calculated.

Foreign Matter should not be more than 2.0% of stems; and not more than 1.0% of other organic matter.

3.2.1.4. Determination of Ash

The ash remaining following ignition of medicinal plant materials was determined by three different methods which measured total ash, acid-insoluble ash and water-soluble ash.

The total ash method was designed to measure the total amount of material remaining after ignition. This included both "physiological ash", which was derived from the plant tissue itself, and "non-physiological" ash, which was the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

Acid-insoluble ash was the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measured the amount of silica present, especially as sand and siliceous earth.

Water-soluble ash was the difference in weight between the total ash and the residue after treatment of the total ash with water.

Recommended Procedures

Total Ash

About 2-4g of the ground, air-dried plant material, accurately weighed, was placed in a previously ignited and tared crucible (usually of platinum or silica). The material was spread in an even layer, and ignited by gradually increasing the heat to 500-600°C until it was white, indicating the absence of carbon. It was cooled in a desiccator, and weighed. If carbon-free ash could not be obtained in this manner, the residue was cooled and moistened with about 2 ml of water or a saturated solution of ammonium nitrate R. It was dried on a water-bath, then on a hot-plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 minutes; then weighed without delay. The content of total ash, in mg per g of air-dried material, was calculated.

Acid-insoluble Ash
To the crucible containing the total ash, 25 ml of hydrochloric acid, (~70g/l) TS, was added, covered with a watch-glass, and boiled gently for five minutes. The watch-glass was rinsed with 5ml of hot water, and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter-paper, and washed with hot water until the filtrate was neutral. The filter-paper, containing the insoluble matter, was transferred to the original crucible, dried on a hot-plate and ignited to constant weight.

The residue was allowed to cool in a suitable desiccator for 30 minutes, then weighed without delay. The content of acid-insoluble ash, in mg per g of air-dried material, was calculated.

**Water-soluble Ash**

To the crucible containing the total ash, 25 ml of water was added and boiled for five minutes. The insoluble matter was collected in a sintered-glass crucible or on an ashless filter-paper, washed with hot water, and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C.

The weight of this residue, in mg, was subtracted from the weight of the total ash. The content of water-soluble ash, in mg per g of air-dried material, was calculated.

Total Ash should not be more than 12%; Acid-insoluble Ash not more than 30%; and Water-soluble Ash not less than three percent.

## 3.2.1.5. Determination of Water and Volatile Matter

An excess of water in medicinal plant materials would encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content are therefore set for every given plant material.

This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water.

The test for loss on drying determines both water and volatile matter. Drying can be carried out either by heating to 100-105 °C or in a desiccator over phosphorus pentoxide R under atmospheric or reduced pressure at room temperature for a specified period of time.

The desiccation method is especially useful for materials that melt to a sticky mass at elevated temperatures.

### Recommended Procedures

#### Preparation of Material

A suitable quantity of the sample was prepared by cutting, granulating or shredding the unground or unpowdered material, so that the thickness of the parts did not exceed 3 mm.

The use of high-speed mill should be avoided in preparing the sample, and taking care that no appreciable amount of moisture was lost during preparation. It is important that the portion is large enough to be a representative sample.

#### Loss on Drying (Gravimetric Determination)

A sample of 2-5g of the prepared air-dried material was accurately weighed and placed in a previously dried and tared flat weighing bottle. The sample was dried by one of the following techniques:

a) In an oven at 100-105°C;

b) In a desiccator over phosphorus pentoxide R under atmospheric pressure, or

c) Under reduced pressure and at room temperature.

Drying was continued until two consecutive weighings did not differ by more than 5mg. The loss of weight, in mg per g of air-dried material, was calculated. Moisture Content should not be more than ten percent.

## 3.2.1.6. Determination of Extractable Matter

### Cold Maceration Method
Place about 4.0g of coarsely powdered air-dried material, accurately weighed, in a glass-stoppered conical flask. Macerate with 100ml of the solvent specified for the plant material concerned for 6 hours, shaking frequently, then allow standing for 18 hours.

Filter rapidly taking care not to lose any solvent, transfer 25 ml of the filtrate to a tared flat-bottomed dish and evaporate to dryness on a water-bath. Dry at 105°C for 6 hours, cool in a desiccator for 30 minutes and weigh without delay.

Calculate the content of extractable matter in mg per g of air-dried material.

3.2.1.7. Determination of Microbial Contamination of *Solenostemma argel* Leaves

**Tests for Specific Microorganisms**

3.2.1.7.1. Test for *Escherichia coli* Contamination of *Solenostemma argel* Leaves

A suitable quantity (0.5g) of the powdered *S. argel* leaves was placed in a sterile screw-capped container, 50ml of nutrient broth were added, and the mixture was incubated for 24 hours at 37°C (enrichment culture).

Then 0.1ml of the enrichment culture was transferred to a tube containing 5ml of MacConkey broth and incubated at 36-38°C for 48 hours. The contents of the tube were examined for the presence of acid and gas which indicated the possible presence of *Escherichia coli*.

3.2.1.7.2. Test for *Salmonella* spp. Contamination of *Solenostemma argel* Leaves

A suitable quantity (0.5g) of the powdered *S. argel* leaves was placed in a sterile screw-capped container, 100ml of nutrient broth were added, shaken and allowed to stand for one hour, shaken again, then the cap was loosened, and the container was incubated for 24 hours at 37°C (enrichment culture).

Then 0.1ml of the enrichment culture was transferred to each of two tubes containing either a) 10ml of Selinite broth, or b) 10ml of Tetrathionate broth and incubated at 36-38°C for 48 hours. Then from each of these cultures one plate was inoculated containing a layer of Deoxycholate citrate agar; the plates were incubated at 36-38°C for 24 hours, and the colonies, if any, were examined for the possible presence of *Salmonella* spp.

**Microbiological Quality**

*Solenostemma argel* preparations were tested for *Salmonella* species, and should be negative. According to the WHO specifications, the maximum acceptable limits of the different microorganisms for preparation of decoction were as follows: aerobic bacteria-\(10^7/g\); moulds and yeast-\(10^5/g\); *Escherichia coli*-\(10^2/g\); *Salmonella typhi*- 0/g; other Enterobacteria- \(10^4/g\). In the case of preparations for internal uses: aerobic bacteria-\(10^5/g\); moulds and yeast- \(10^4/g\); *Escherichia coli*-0/g; *Salmonella typhi*-0/g; and other Enterobacteria-\(10^3/g\).

3.2.2. Phytochemical Methods

3.2.2.1. Preparation of Powders of *Solenostemma argel* Leaves

Inspected and identified *S. argel* leaves were reduced to the required particle size by using a hammer mill for particle size reduction, followed by sieving through a series of sieves with an electrical shaker for powder separation and analysis. *Solenostemma argel* ground leaves were used for determination of identity, purity and quality of *S. argel*; for direct incorporation into tablet formulations, or for preparation of extracts which were incorporated into tablet formulations.

3.2.2.2. Preparation of Water Extracts of *Solenostemma argel* Leaves

**Stage I**
Inspected and weighed crude *S. argel* leaves were charged into the boiling pot, and allowed to boil for 90 minute with intermittent stirring. The decoction was collected by a 60-mesh filter and transferred to an open dish or tank.

**Stage II**

Boiling water was added to the residue of the first decoction and boiled for another 30 minute with intermittent stirring. The decoction was collected through a 60-mesh filter and transferred to the storage dish or tank. The residue after the second stage was pressed and the decoction obtained was also transferred to the storage dish.

### 3.2.2.2.1. Concentration of the Aqueous Extracts of *Solenostemma argel* Leaves

#### Evaporation, 1st Stage

The stored decoction was evaporated on a water-bath to remove about 80% of its water content. This aqueous extract was added in this form when a binding agent was not included in the tablet formulation.

#### Evaporation, 2nd Stage

The decoction from the evaporator was transferred to open pans for final processing in an aerated oven having a fan at 45°C; the extract was collected at this stage, and 0.01% methyl- and propyl- parabens were added as preservatives. Then it was cooled and kept in suitable well-closed plastic containers, kept ready for use in the formulation of tablets.

### 3.2.2.3. Preparation of Total Alkaloids of *Solenostemma argel* Leaves

The following stages were followed for the preparation of *S. argel* alkaloids:

#### Stage I

Boiling of *S. argel* leaves with hot methanol:water mixture (4:1) for 90 minutes on a boiling water-bath. The decoction was collected by a 60-mesh filter (sieve) and was transferred to an open storage dish or tank.

#### Stage II

The boiling methanol:water mixture was added to the marc or residue (from stage I), boiled on a water-bath for 30 minutes, and filtered by 60-mesh sieve; the filtrate was pooled with the stage I decoction in the storage dish or tank. The decoction was concentrated at 40°C to 1/10th of the original volume by a Roto-vap apparatus; then it was acidified with 2M sulphuric acid and extracted three times with chloroform.

#### Stage III

The aqueous solution was made alkaline to pH 10 with ammonia solution, and then extracted twice with chloroform:methanol mixture (3:1), followed by chloroform.

#### Stage IV

The alkaline aqueous layer was evaporated to dryness, and then extracted with methanol. This methanol extract was dried and stored in well-closed plastic containers till further used.

### 3.2.2.4. Identification of Some Constituents of *Solenostemma argel* Leaves

The identification methods of *S. argel* constituents consisted of using suitable reagents or tests for determination of the chemical identities of these active constituents. The main tests used for identification included: a) Ninhydrin, natural compound and concentrated hydrochloric acid, coupled with UV, IR, NMR and MS spectral analysis, were used for flavonoids identification. b) Dragendorff’s reagent, Mayer’s reagent and Mandelic acid for alkaloids Identification; and c) Foaming test for detection of the presence of saponins.

#### Preparation of Different Used Reagents

Natural Products Compound
1.0% methanol, diphenyl boric acid β-ethylamine-ester (diphenyl benzyl oxy-ethylamine), followed by 5% ethanol, polyethylene glycol 4000 (10ml and 8ml, respectively). For detection of flavonoids, a loin intense fluorescence was produced immediately or after 15 minutes in UV spectrophotometer at λ 365nm. Polyethylene glycol increased the sensitivity from (10mg to 2.5mg); the fluorescence behavior was structure-dependent.

**Dragendorff’s Reagent**

- a) Bismuth subnitrate, 0.8g; Glacial acetic acid, 10ml; and Distilled water, 40ml.
- b) Potassium iodide, 20g; and Distilled water 50ml

a) and b) were mixed overnight to form a stable mixture; mixed with 20ml glacial acetic acid, and then the volume was completed to 100ml distilled water. 5% sulphuric acid in alcohol is prepared for the fixation of the color.

**3.2.2.4.1. Detection, Separation and Identification of Flavonoid Compounds**

The presence of flavonoid compounds was tested in the water or methanol extracts of *S. argel* by using paper chromatography (PC) or thin layer chromatography (TLC, silica gel) run in B:A:W system (40:10:50). The chromatograms were sprayed with natural substances reagent or ninhydrin.

Separation of the different flavonoid compounds was carried out by using an accelerated eluted solvent passage through a polyamide chromatographic column. A vacuum pump was applied to the polyamide column from underneath, and a reservoir at the top to enhance the solvent movement and keep the column continuity intact. The method was carried out as follows:

Twenty grams of the powdered *S. argel* leaves were defatted with petroleum ether and dichloromethane several time, and extracted with 200ml of ethyl acetate for many times at room temperature (25°C), using a magnetic stirrer and a closed system to prevent evaporation. The extract was concentrated with a Roto-vap to about 10ml which became as a thick green substance. After the application of the extract at the top of the column, 200ml of water were passed together with increasing amounts of methanol to change polarity of the system (10%, 20%, 30%, 40%, 50%, 60%, 70%, 75% 80%, 85% 90%, 95% and, 100%). Systems with different polarities separate different materials in each eluted portion. The pharmacological activities for each of the separated portion were checked on rabbit’s contracting small intestine.

**3.2.2.4.2. Detection and Identification of Kaempferol-3-glucoside, Astragalin**

This was a preliminary study of flavonoids of *S. argel* extracts. The plant material was successively extracted with light petroleum, chloroform, ethyl acetate, methanol, methanol-water, and water. The extract was fractionated with flash chromatography; the fractions were further analyzed by thin layer chromatography (TLC); and finally the constituent compounds were purified by HPLC; UV, IR, NMR and MS spectra were used to characterize the flavonoids from the polar extracts.

**3.2.2.4.3. Detection and Identification of Solenostemma argel Alkaloids**

- a) Detection and identification of *S. argel* alkaloids was carried out after alkaloids extraction as previously described. The extract was applied to TLC silica gel eluted in B:A:W system, and detection after spraying with Dragendorff’s reagent. The color fixation by using 2M sulphuric acid ethanol solution.

- b) One gram of *S. argel* alkaloids extract was eluted by different solvents with increasing polarity (ether, benzene, chloroform, ethyl acetate, methanol, methanol:water, and water) using a neutral aluminum oxide column as a stationary phase. The different portions contents were subjected to TLC on aluminum oxide acidic plates using methanol:ammonia, 0.5%, system.

- c) Spectrophotometer scan was carried for the highest pick of the most available alkaloidal constituents in *S. argel* prepared extract.
3.2.3. Pharmacological Methods

3.2.3.1. Isolated Small Intestine Tissue Experiments

Isolated pieces of small intestine of adult male guinea pig (300-500g), or alternatively adult male rabbit (1.5-2kg) were used as a model. Small pieces of the isolated gut tissue, 2-3cm each, either of intestine or ileum, were obtained from a guinea pig or a rabbit. The test compound was a freshly prepared solution of acetylcholine chloride, at a concentration 1µg/ml, or substituted by barium chloride, 0.002mg/ml.

3.2.3.1.1. Experimental Procedure

Preparation of the rabbit intestinal strip from a rabbit fasted, from food but not water, and sacrificed by a blow on the back of the neck and head. A scalpel and blade were used to make a mid-line incision on the abdomen to expose the contents of the gut; scissors were used carefully to remove a substantial length of ileum into a Petri dish (with a paraffin wax tissue mat) or a beaker containing Tyrode’s solution bubbled with a mixture of oxygen (95%) and carbon dioxide (5%).

The residual food content was carefully washed out of this segment of the ileum by using a 30ml syringe filled with Tyrode’s solution. The omentum was trimmed off from this piece of ileum and several pieces of 2-3cm long were cut. These pieces of intestinal tissue were kept in ice-cold Tyrode’s solution to preserve viability until ready for use.

Setting up of the preparation was according to the method most commonly used, and which was originally described by Magnus (1904) and adopted by Boura (1954). In this method, 2-3cm ileum strip was tied with a piece of thread and attached to a fixed glass rod. Into the other end of the tissue, there was an S-shaped hook to which an ample length of thread was tied. The glass rod and the strip were placed into a Magnus tissue bath containing Tyrode’s solution warmed up to 37°C and aerated constantly with oxygen (95%) and carbon dioxide (5%) mixture (4bubbles/sec). The loose end of the thread, connected to the S-shaped hook and inserted into the movable end of the strip, was attached to a forced displacement transducer. This was adjusted to put only a tension of 0.5g on the tissue, or to a kymograph. The smooth muscle of the intestine tissue was allowed to equilibrate for 30-60 minutes. The preparation contracted and relaxed rhythmically in response to drugs added in the bath.

A specified amount of acetylcholine chloride (1µg/ml) or barium chloride (0.02mg/ml) was added to the bath and the activity was recorded for three minutes; after recording the sub-maximal dose and the tissue response, measured in centimeters, the full tissue bath, with Tyrode’s solution and acetylcholine (1µg/ml), was drained. The tissue was washed several times with fresh warm Tyrode’s solution. This procedure was repeated for all other reference compounds to be tested on the same tissue preparation. After the last drug has been tested, repeated washes were done, and the tissue was allowed to equilibrate. After the contractions returned to approximately pre-treatment levels, the same dose of acetylcholine was repeated. At the end of the three minutes period following the addition of acetylcholine, or barium chloride, at a sub-maximal dose, S. argel water extract, an authentic sample of S. argel alkaloids, or any other drug was added to the tissue bath, and the effects on contraction were recorded for ten minutes. The activity of S. argel extract was tested for its inhibitory effect upon the increased tone (contractility) produced by acetylcholine or barium chloride, and estimating the degree of relaxation that has occurred at their sum-maximum doses. The tissue bath was washed out several times with warm Tyrode’s solution. The tissue was allowed to recover to about the pre-drug state, and then the test was repeated with the same dose of acetylcholine and a higher dose of the S. argel extract or a reference drug (hyoscine, papaverine, or atropine).
3.2.3.1.2. Effects of *Solenostemma argel* Extract on Isolated Rabbit Heart

A local species rabbit (weighing 1.5 kg) was killed by dislocating its neck. The thorax cavity was opened, the heart plus one-cm intact aorta were removed, as quickly as possible, and transferred to a Petri dish containing ice-cold Ringer’s solution aerated with oxygen (95%) – carbon dioxide (5%) mixture. Any clots or blood were removed from the organ. About 0.5 cm of a cannula tip of the perfused system was located into the aorta and tied firmly with a thread and mounted to a heart-bath apparatus holder. The apex of the ventricles was attached to an isometric transducer with a button thread. Exclusion of air bubbles in the perfusate was important to prevent any air emboli in the system. The Ringer and perfusate chamber temperature was 37°C. A three-arm cannula was used with a rubber diaphragm inserted over the upper arm through which gas was invented and drugs were also given. The effects of different *S. argel* extracts, perfused continuously, on the heart stroke were recorded.

3.2.3.1.3. Effects of *Solenostemma argel* Extract on Intact African Toad Heart

An African toad was decapitated and the upper part of the spinal cord was damaged. It was fixed to a board, and the heart was exposed and attached to an isometric transducer. The heart stroke was recorded, and the effects of different *S. argel* extracts perfused continuously were recorded.

3.2.3.1.4. Effects of *S. argel* Extract on African Toad’s Foot Withdrawal Reflex

African toads (*Rana temporiana*) were used, irrespective of their weight or sex. Each frog was decapitated and the upper part of the spinal cord was damaged down to the level of the third vertebra. A transverse incision was made in the abdominal wall just below the sternum. The viscera were removed through this opening, carefully exposing the lumbar plexus without damaging it. The frog was pinned to a vertical board. The local anesthetic lignocaine was used, as a reference standard, at a concentration of 4mg/ml, which was arbitrarily chosen. This same concentration was used for other authentic samples of flavonoids (kaempferol, rutin, and quercetin), and extracted tested solutions of *Solenostemma argel* extract; each was placed in the pocket formed by the lower abdomen. The amount of solution used was irrelevant as long as the plexus was submerged. A record was made of the time required to abolish the reflex contraction to a sensory stimulus, which was the immersion of both feet of the frog once every minute into dilute HCl (0.05N; 0.1N; and 0.2N) for not longer than ten seconds, after which the frog’s feet were immersed in saline and washed several times. Before the addition of the tested drug in the abdominal pocket, and to avoid sensory nerve damage, the sensitivity of the nerve preparation was checked by immersion into the first solution of 0.05N HCl, and when the frog failed to respond, a stronger solution of 0.1N HCl was used; if it was ineffective, the tested frog was excluded from the experiment. The experiment is started with the tested drug to determine the reflex response time by a stop-clock to the lowest acid concentration for ten seconds and at one minute intervals. If it failed at this point, the stronger acid solution (0.1N; and 0.2N) was used to elicit foot withdrawal reflex. The end point was taken as failure to withdraw the feet from 0.2N HCl after ten seconds contact.

Six frogs were tested simultaneously for the sensory nerve response, and for each the observation was made of the time taken by a given concentration of the local anesthetic to abolish the reaction to 0.2N HCl. The experiment was carried out with lignocaine, kaempferol, quercetin, rutin, an alcoholic extract, alkaloid and rutin from *S. argel* leaves. Lignocaine was taken as a reference local anaesthetic drug used to calculate the relative potencies of other tested drug substances.

3.2.3.1.5. *Solenostemma argel* Alkaloids Diffusion across Rabbit Small Intestine
The diffusion of *S. argel* alkaloids was studied by using a rabbit small intestine preparation in 50ml of Tyrode’s solution in a 100ml-beaker with controlled-temperature at 37°C; a controlled water-bath was used for that purpose. The aeration was carried by an air-pump. A 10cm segment of rabbit’s small intestine was used as a model, which was tightly tied at each end with a thread, after addition of 2ml of *S. argel* alkaloid extract, 20mg/ml concentration, in its lumen and was suspended in the Tyrode’s solution in the beaker. Each end of the small intestine segment was firmly tied by a thread, and thus not allowing any leakage of the inside content to the outside.

After specific time intervals half a milliliter samples of the Tyrode’s solution in the beaker were withdrawn using a pipette, and replaced by an equal volume of fresh Tyrode’s solution. The alkaloid concentrations in the withdrawn samples were determined by using a UV spectrophotometer at λ 294.5nm. The mean readings of five determinations were calculated. Graphs were obtained by plotting alkaloid concentration readings at λ 294nm versus time.

The same experiment was repeated after the addition of 0.01g of sodium fluoride to the Tyrode’s solution in the beaker. The alkaloid concentration analysis was done using the same procedure as above. Graphs were obtained by plotting alkaloid concentration readings at λ 294nm versus time. The two sets of graphs were used to reveal the effect of sodium fluoride on the *S. argel* alkaloids diffusion rate across the rabbit small intestine preparation.

3.2.3.1.6. Calculation of Median Effective Doses (ED₅₀) of *Solenostemma argel* Alkaloids and Hyoscine using MedUSA System

The median effective doses (ED₅₀) of *S. argel* alkaloids and hyoscine were calculated using the MedUSA System by recording the abilities of these two drugs to reduce the contraction of guinea pig ileum model induced by 1 µg/ml of acetylcholine, recording their inhibitory effects at sub-maximum doses.

The reductions of the stimulated contractions were recorded for each concentration on six preparations; means of the seven readings were calculated and used to draw the relevant graphs.

The median effective doses, ED₅₀, were calculated for both hyoscine and *S. argel* alkaloids to compare their antispasmodic effects.

3.2.4. Toxicological Methods

3.2.4.1. Determination of Acute Toxicity of *Solenostemma argel* in Albino Rats and Rabbits: Determination of Lethal Dose of *Solenostemma argel*

Four animals of each of Albino Canadian rats (200g–250g) and local species rabbits (1.250-1.500kg) were used for determination of *S. argel* acute toxicity.

Intra-peritoneal doses of *S. argel* were carefully given every five minutes, without damaging a vein or a nerve.

Starting from 0.025mg/kg dose and doubling it successively till the animal death occurred. The toxic signs were observed closely and recorded, if any.

The experiment was carried out on a group of four rats and a group of four rabbits, and the median lethal dose was calculated for each group.

3.2.4.2. Determination of Median Lethal Dose (LD₅₀) of *Solenostemma argel* Alkaloids in Albino Rats

Sixty Albino rats were used. A serial number was designed to each animal, table ranking number, and assigning animals to six groups of ten animals each. Starting from the recorded lethal dose of 5.49g/kg (dose1) in the pervious experiment, each group was given a different dose.
Each of the following doses was given by intra-peritoneal injection to an assigned group of ten animals: 25%, 37.5%, 50%, 75%, 87.5%, and 100% of the lethal dose. The animals were observed for twenty four hours.

The number of deaths in each group was recorded as percentage of the whole number (sixty). The log dose was plotted versus the mean deaths; the log LD$_{50}$ was obtained from the graph.

3.2.4.3. Determination of Acute Toxicity of *Solenostemma argel* in Young Nubian Goats:

**Determination of Toxic Effects of *Solenostemma argel***

Fourteen young Nubian goats, aged 4-6 months and weighing 7-9kg, were used in the present experiment aimed at the determination of acute toxicity of *S. argel* alkaloids extracts and the toxic effects in these animals.

The young goats were randomly assigned to two groups, 7 animals each; group I was used as the control being fed with the same food (sorghum and clover) and drinking water freely from the same source as the test group.

The individual animals in the test group were given a decoction of *S. argel*, 5g/kg, orally by using a feeding bottle once daily for 45 days. Ten-milliliter blood samples were withdrawn for haematological and serum analysis, and the animals were sacrificed, vital organs isolated, weighed and examined for any abnormalities, and subjected to histopathological examination.

3.2.5. Pharmaceutical Methods

3.2.5.1. Preparation of *Solenostemma argel* Leaves

3.2.5.1.1. Size Reduction of *Solenostemma argel* Leaves

After being identified and inspected for quality and purity, *S. argel* leaves were reduced to the required particle size by using a hammer mill, for particle size reduction, followed by sieving through a set of sieves with an electrical shaker for powder separation and analysis. *Solenostemma argel* ground leaves were used for direct incorporation into tablet formulations, or for preparation of extracts to be incorporated into other tablet formulations.

3.2.5.1.2. Sieve Analysis of Ground *Solenostemma argel* Leaves

Sieve analysis of the ground *S. argel* leaves was carried to determination the particle size distribution of the powdered *S. argel* leaves. A 150g-sample of the powdered leaves was placed in the upper sieve of a series of successively smaller sieves, and the sieve set was then placed in the mechanical sieve shaker which was operated for 15 minutes. The portions of the powder retained in each sieve were weighed. For separation of the powder into different sieve fractions, the above mentioned process was carried out several times until sufficient amount of each fraction was obtained and stored in well-closed plastic containers for utilization in this work.

3.2.5.2. Design of the Different Formulae of *Solenostemma argel* Tablets

Different formulae were designed to attain the optimized physico-chemical tablet properties. These formulae included different types of additives and excipients in various ratios. The formulae were designed for 250g whole powdered leaves, or for active ingredients of different particle sizes, after particle size analysis.

3.2.5.2.1. Optimization of Physicochemical Properties of *S. argel* Tablets

Various trials were required for the complete experimental design in Optimization of *S. argel* tablets; and these trials were based on:

a) Particle size of *S. argel* powdered, ground leaves, extracts or alkaloids;
b) Type and quantity of diluents (Stanleywood et al., 1979);
c) Type and quantity of binder (Doelker, 1977);
d) Type and quantity of disintegrating agent, and the time of its addition - before or after granulation (Esezobo et al., 1976); and
e) Type and quantity of lubricating and glidant agent.

These optimization studies included investigation of the:
a) Effects of particle size of S. argel powders on physicochemical properties;
b) Effects of type of starches on the powder passing through mesh 80 (≤ 160 µ) and on some physicochemical properties of ground leaves S. argel tablets.
c) Effects of type and quantity of binding agents on hardness, disintegration time, Dissolution rate of the tablets (Abd Alalim et al., 1987);
d) Effects of microcrystalline cellulose addition, before or after granulation, on Tablet physico-chemical properties;

3.2.5.3. Preparatory Methods of Solenostemma argel Tablets

3.2.5.3.1. Wet Granulation Method

For preparation of granules of powdered S. argel leaves, a 250g-samples of each of the sieve fractions 250,160 or less than 160 micron, or the whole powdered leaves, were wet granulated alone or mixed with starch, or lactose, and microcrystalline cellulose. The method adopted was as follows:

A Porcelain mortar and pestle were used in mixing the powders for five to ten minutes, and then moistened with the proper binding agent. Appropriate amounts of aqueous solutions or muclages of one or more of the following binders were used: polyvinylpyrrolidone, starches from guar, sorghum, and maize, and gum.

Starch paste used as a granulating agent was prepared by first making a slurry with specified amounts of starch and water, then an appropriate amount of boiling purified water was added with continuous stirring till a translucent gel was formed (Parrot and Saski, 1971; Banker and Anderson, 1986). The guar starch paste was made in hot boiling water, and then milled with a homogenizing mill, or by mixing 2% polyvinylpyrrolidone thoroughly with guar before adding the hot water. They were used to moisten and bind the different powder formulations, and kneaded to a consistency that produced granules containing different amounts of different granulating agents expressed in terms of w/w % of the specified formula.

The wet masses were then forced manually through a Number 12-mesh screen, or using a granulating machine in the case of large batches. The resultant granules were dried in a hot air oven, at 50°C for overnight. The dried granules were passed through Number 14-mesh screen. The dried and sized granules were mixed with disintegrating, lubricating and glidant agents, and compressed into the required S. argel tablets.

For preparation of Solenostemma argel extract or alkaloid tablets, the same methods were followed.

3.2.5.3.2. Direct Compression Method

The cross-linked sodium carboxymethyl cellulose (AC Di-Sol), 10%, with 10% of the different binding agents: polyvinylpyrrolidone, microcrystalline cellulose, starches (maize, sorghum and guar). Five percent of these starches were used in combination with 2% of polyvinylpyrrolidone, 5% of microcrystalline cellulose, 1.5% of Talc powder and 0.5% of magnesium stearate. The powder mix was subjected to slugging with a 12mm-die, and the slugs were crushed, sized and directly compressed using 9mm-die.

3.2.5.3.3. Tablets Compression
Compression of the prepared granules of different formulas was done by using a single- punch tableting machine (Korch, Erweka). Formulae of *Solenostemma argel* tablets were prepared by compressing appropriate amounts of 400mg of ground leaf formulae, or 200mg of granulated or non-granulated powdered leaves, or 250mg of water extract and alkaloids using 12mm- or 9mm- stainless steel dies fitted with normal flat-face or convex punches of the single-punch tableting machine (Korsch, Erweka) (Armstrong, 1988)

### 3.2.5.4. Production of *Solenostemma argel* Tablets

The basic general method adopted for production of different types of *S. argel* tablets consisted of the following main steps:

a) receipt, inspection and evaluation of ingredients/input materials, which must comply with the WHO or other official requirements and specifications;

b) storage of active ingredients and other raw materials and chemicals;

c) milling of *S. argel* leaves, using hammer mill to have the particle size needed, i.e. passing through mesh-80 or having a particle size $\leq 160\mu$;

d) sieving and collecting powdered *S. argel* leaves in particle size range of $\leq 160\mu$;

e) Weighing the ingredients of the formulation including the active ingredients of *S. argel*, whether powdered leaves, extracts or alkaloids, and the different additives or excipients;

f) mixing the *S. argel* active ingredients and additives together thoroughly for ten minutes;

g) preparation of the binder mucilage and, addition of sufficient quantity to the powders till the needed moistening degree is attained;

h) granulation using mesh-12, drying and sizing;

i) addition of lubrication and disintegration agents;

j) tablet compression cycle;

k) tablet de-dusting and inspection;

l) packing of tablets;

m) quality control and evaluation of tablets; and

n) Stability testing of *S. argel* leaf tablets.

Two different basic methods were followed for the preparation of *S. argel* tablets; in the first method, ground *S. argel* leaves were directly used, while in the second method, different *S. argel* extracts were incorporated in the prepared *S. argel* tablets.

### 3.2.5.4.1. Preparation of *Solenostemma argel* Ground Leaf Tablets

The following major steps were followed:

a) Receipt of ingredients/input materials which complied on inspection to the WHO Requirements;

b) Storage of active ingredient;

c) Weighing leaves and additives;

d) Comminution of *S. argel* leaves, using hammer mill;

e) sieving using mesh 80, to have suitable particle size $\leq 160\mu$;

f) conducting particle size analysis for mesh 80, size $\leq 160\mu$;

g) mixing together plant material (active ingredients) with additives for 10 minutes;

h) preparation of the binder mucilage and addition of sufficient quantity to the powders till the needed moistening degree were attained;

i) granulation using mesh 12 or Erweka granulator;

j) drying and sizing of the prepared granules;

k) addition of disintTEGRANT and lubricant;

l) mixing of the powders;

m) tablet compression;
n) de-dusting and Inspection of tablets;
o) packing of tablets;
p) quality control and evaluation of *S. argel* leaf tablets; and
q) stability testing of *S. argel* leaf tablets.

### 3.2.5.4.2. Preparation of *Solenostemma argel* Extract Tablets

The following main steps were followed:

a) receipt of *S. argel* crude plant material; inspection and separation of foreign materials and plant parts other than leaves; and sieving the leaves to free them from any dust;
b) weighing the right quantity; and quick washing with water for clearance from dust, if any;
c) extraction with boiling water in a pan or dish; collection of decoction after boiling for 90 minutes; and separation by filtration (1st stage);
d) addition of boiling water for second decoction and boiling for 30 minutes; decoction collection (2nd stage); and pressing the macerated residue;
e) collection of decoctions together; and evaporation on a water-bath to reduce the volume to 1/10th the original volume (stage 1);
f) final evaporation in pan at 45°C using an oven with a fan under reduced pressure; at this stage, 0.01% methyl- and propyl-parabens were added as preservatives; and the percentage of the dried extract to the used quantity of plant material was calculated;
g) particles size reduction and sieving of the dried extract to have it in a powder form;
h) weighing the right weight; and mixing together the extract (active ingredients) with the additives for 10 minutes;
i) preparation of the binder mucilage and addition of sufficient quantity to the powders till the needed moistening degree was attained; granulation using mesh 12, or Erweka granulator;
j) drying and sizing of the prepared granules; addition of disintegrant and lubricant; and mixing the powders together;
k) tablet compression; de-dusting and Inspection of tablets; and packing of tablets;
l) quality control and evaluation of *S. argel* leaf tablets; and
m) stability testing of *S. argel* leaf tablets.

### 3.2.5.4.2. Preparation of *Solenostemma argel* Alkaloids Tablets

The following main steps were followed:

a) receipt of *S. argel* crude plant material; inspection and separation of foreign materials and plant parts other than the leaves; and sieving the leaves to free them from any dust;
b) weighing the right quantity; and quick washing with water for clearance from dust, if any;
c) extraction of *S. argel* leaves with methanol:water mixture (4:1) for 90 minutes; collection of decoction by a 60-mesh filter (sieve), transference to an open storage dish or tank (Stage I); and separation by filtration (1st stage);
d) addition of methanol:water mixture to the marc or residue from stage I, boiling for 30 minutes, and filtering by 60-mesh sieve; the filtrate was pooled with the stage I decoction in the storage dish or tank; and decoction was collected (2nd stage);
e) pressing the macerated residue; collection of decoctions together and concentration at 40°C to 1/10th of the original volume by a Roto-vap apparatus; then acidified with 2M sulphuric acid and extracted three times with chloroform (Stage II).
f) aqueous solution was made alkaline to pH 10 with ammonia solution, and extracted twice with chloroform:methanol mixture (3:1), followed by chloroform (Stage III)
g) alkaline aqueous layer was evaporated to dryness, and then extracted with methanol. (Stage IV).
h) methanolic extract was dried and stored in well-closed plastic containers till further used.
i) the percentage of the dried extract to the used quantity of plant material was calculated;
j) particles size reduction of the dried extract to have it in a powder form;
k) weighing the right weight; and mixing together the alkaloidal extract (active ingredients) with the additives for 10 minutes;
l) preparation of the binder mucilage and addition of sufficient quantity to the powders till the needed moistening degree was attained; granulation using mesh 12, or Erweka granulator;
m) drying and sizing of the prepared granules; addition of disintegrant and lubricant; and mixing the powders together;

3.2.5.5. Quality Control of Solenostemma argel Tablets of Different Formulations

Different physico-chemical tests were conducted on the various types of Solenostemma argel tablets prepared during the course of the present study to check on the quality of these tablets and their conformity to the good manufacturing requirements of tablets according to the BP or USP monographs. These physicochemical tests included:

3.2.5.5.1. Determination of Diameters of S. argel Tablets of Different Formulae

According to the BP (1988), ten tablets from each formula were taken and the diameters of the individual tablets were measure using a Vernier caliber. The mean diameters ± deviations from standard were calculated for tablets of each formula.

3.2.5.5.2. Determination of Thickness of S. argel Tablets of Different Formulae

Thickness of each tablet was determined by screw gauge/vernier caliper, and the tablet mean thickness ± standard deviation was calculated for tablets of each formula.

3.2.5.5.3. Determination of Mean Weight of S. argel Tablets of Different Formulae

As described in the US P (1995), ten tablets, from each type of Solenostemma argel tablets of the different formulae, were taken and weighed together. Thus the total weight of the ten tablets was determined, and the mean weight of the tablet of each type was calculated.

3.2.5.5.4. Determination of Weight Variation of S. argel Tablets of Different Formulae

Twenty tablets from each type of Solenostemma argel tablets, of the different formulae, were weighed individually using a sensitive balance, Mettler type. The mean tablet weight, the standard deviation and percent deviation were calculated. Not more than two tablets should be different from the average by more than 5%, according to the BP(1988) monograph.

3.2.5.5.5. Determination of Hardness of S. argel Tablets of Different Formulae

Using Monsanto Hardness Tester
As described in BP (1993), ten tablets from each formula were tested for their hardness, i.e. the resistance to crushing of *Solenostemma argel* tablets, using Monsanto hardness tester and the mean values were calculated and recorded.

**Using Erweka Hardness Tester**

This is another method for determination of hardness of *Solenostemma argel* tablets. This test was done by using an Erweka TDH30 hardness tester as per BP 1993. Ten tablets from each of *Solenostemma argel* tablet formulations were tested for their hardness. The test was repeated three times for each type of tablets, and the average values were calculated.

### 3.2.5.5.6. Determination of Friability of *S. argel* Tablets of Different Formulae

According to the BP (1993), twenty tablets from each formula were taken, brushed with a soft brush to remove any adhering particles, weighed accurately and placed in the drum of the friabilator (Erweka type). The drum was then rotated at the rate of 20 revolutions per minute for 5 minutes, i.e. a total of 100 revolutions. The tablets were removed from the drum, brushed again to remove any loose dust, and then weighed.

The loss in weight of tablets was determined and calculated as percentages of the initial weight of tablets.

### 3.2.5.5.7. Determination of Drug Content of *S. argel* Tablets of Different Formulae

#### Determination of Tablet Content Uniformity

A calibration curve was constructed and used for determination of content of active constituents in the tablets of the different formulation of *Solenostemma argel* tablets.

**Calibration Curve from a Reference Standard**

Ten milligrams of *Solenostemma argel* alkaloids were accurately weighed, placed in a volumetric flask and dissolved in 100ml of methanol; from this stock solution, different dilutions were prepared, in volumetric flasks, at low concentrations (20, 15, 8, 5, 3 ug/ml). The absorbance of each diluted solution was measured in a UV Spectrophotometer at $\lambda$ 294.5nm, using methanol as a blank. From these spectrophotometry results, a calibration curve was plotted. The different spectrophotometry readings of the tablet solutions were read from the calibration curve to determine the drug content in the *S. argel* tablets of the different formulations.

**Determination of Drug Content of *S. argel* Extract Tablets**

The same procedure was followed with *Solenostemma argel* tablets of the different formulae incorporating the extracts and powdered *S. argel* leaves.

The different tablets were dissolved in 900ml of water with continuous stirring and the resultant solutions were assayed to determine the mean alkaloidal content of each type of tablets.

**Determination of Drug Content of Powdered *S. argel* Leaf Tablets**

The powders of *Solenostemma argel* leaves were standardized in reference to the pure alkaloids extracts. Different concentrations of *Solenostemma argel* powder in solutions were prepared by dissolving accurately weighed quantities of the powder in one liter quantities of water, with continuous stirring for 45 minutes. Then two milliliters were withdrawn from each solution and read in a UV spectrophotometer at $\lambda$ 294.5nm.

### 3.2.5.5.8. Determination of Disintegration Time of *Solenostemma argel* Tablets of the Different Formulae

According to BP (1993) and USP. (1995) monographs, six tablets from each formula were randomly selected and introduced one into each tube of the disintegration tester; disc was added to each tube to avoid floating of tablets when immersed into disintegration medium. The medium used was distilled water at 37°C ± (0.5-1). At 30 minutes from the operation of the tester, the tablets in the tubes were checked for...
complete disintegration. The test was repeated three times for each type of tablets, and the mean values of disintegration time were recorded.

3.2.5.9. Determination of Dissolution of S. argel Tablets of Different Formulae

Dissolution test was done as per USP (1995), using 900ml of freshly prepared distilled water as a dissolution medium in the vessel at a temperature of 37°C ± 0.5, and the rotation was adjusted at 100 rpm for 45 minutes. The absorbance of aliquots samples of the dissolution medium was read in UV spectrophotometer at λ 294.5 nm.

The absorbance of 0.2ml samples of each Solenostemma argel tablets were compared with the absorbance of a known concentration of a pure material of S. argel powder, without any additives. The percentages of dissolved amounts were calculated at different time intervals in the dissolution test to determine the rate and extent of dissolution at (15, 30 and 45 minutes).

3.2.6. Stability Studies

Stability studies of S. argel tablets, from the different formulations, were carried out by the following two principal testing procedures:

3.2.6.1. Accelerated Stability Testing

In this method of stability testing, 100 tablets of each formula were packed in glass containers and placed in an oven, at 50–55°C and relative humidity of 75% for six months. All the quality control tests were carried out at zero time, and at the end of each month till the sixth month.

3.2.6.2. On-going Stability Testing

In this stability testing method, 100 tablets of each formula were stored at room temperature for 24 months. The tablets were subjected to all the quality control tests at the following time intervals: zero time, 6th, 12th, and 24th months.

The results were tabulated for each formula, and the evaluation of the relative importance of formulation and process variables was followed.

3.2.7. Clinical Studies

The clinical studies were carried out at University of Khartoum Clinic, Khartoum Teaching Hospital and Khartoum North Teaching Hospital.

3.2.7.1. The Questionnaires of Antispasmodic Herbal Medicine Usage

Two questionnaires, concerning the practice of herbal medicines usage as antispasmodics, were distributed to three hundred participants. The data collected in these two questionnaires were recorded and tabulated.

3.2.7.2. Observation of Adverse Reactions to Solenostemma argel in Healthy Subjects

Ten healthy adult male volunteers, from the faculty of pharmacy staff, participated in this part of the study after being informed about the study, and giving their written consent. They were examined by an experienced clinician, and then given a two-tablet dose of each of S. argel alkaloid, extract or leaf tablets. The participants were kept under medical supervision, and observed for any adverse reactions or complaints.

3.2.7.3. Comparison of Irritable Bowel Syndrome (IBS) Treatment Using Different Preparations of Solenostemma argel Tablets, Placebo and Generic Antispasmodic Tablets.
Two hundred and seventy patients with different types of GIT colics or pains, and forty of these patients having irritable bowel syndrome (IBS), were treated with a placebo, generic antispasmodic tablets, S. argel extract tablets and S. argel leaf tablets.

Comparison of the pain-relieving effects in the four groups, each of ten participants, in a week-rotation for one month to each preparation, was carried out at four different clinics and hospitals in Khartoum State...

3.2.8. Data Analysis

Experimental data and results were computerized and statistically analyzed using SPSS, version 3-1, (Statistical Package for Social Sciences; Norusis, 1993).

Frequency tables were prepared to show the patterns of variables; and cross-tabulation tables were obtained to show the association between determinants of different parameters to fulfill the requirements of the study objectives.

Chi-square test was used to determine the significance of association at 95% confidence level (p ≤ 0.5). Some of the data analysis was done manually using a scientific calculator.
4. RESULTS AND DISCUSSION

4.1. Solenostemma argel Leaves: Selection, Identification, Quality and Purity

4.1.1. Selection of A Medicinal Herb

The selection of a medicinal herb, for the purpose of the present study, was based on the findings of questionnaire (I) which was randomly distributed to three hundred citizens from ElDroshab area, Khartoum North, Khartoum State. The questionnaire was designed to collect data with regard to the practice of herbal medicines usage for treatment of minor ailments, particularly for GIT upset, spasm and colics.

According to the data of the questionnaire, the five Sudanese traditional medicines mostly used were “AlHargal” (Solenostemma argel) herb, “AlHilba” Fenugreek, (Trieonilla foenum-graetum) seeds, “AlKhilla” (Ammi visnaga) seeds, “AlNa’ana” (Mentha piperita) herb and “AlYanson” Anise, (Pimpinella anisum) fruits. The number of participants preferring the use of medicinal plants, rather than the modern medicines, was 230 (77%); 90% were from the elderly subjects (above 40 years), while 70% were young subjects (below 40 years).

The personal assessment of treatment success was found to be about 70%; 60% of these successful treatments were observed immediately after a single dosage, without the need for medication repetition; 15% had repeated for a second dosage, while 10% needed a third administration. Twenty five percent of the traditional medicines users had the practice of using a particular medicine frequently on the occasion of pain attack due to GIT discomfort, or as a beverage tea.

The most commonly used traditional medicines were “AlHargal”, (47%); “AlNa’ana”, 29%; “AlKhilla”, 10%; “AlHilba”, 8%; and “AlYanson”, 6%. From these data, it was evident that “alhargal” was one of the most popular antispasmodic Sudanese herbal medicines. Several investigations were conducted to evaluate effectiveness, tolerability, adverse actions and safety of Solenostemma argel, but no efforts were traced with respect to the formulation of S. argel leaves in an appropriate dosage form.

4.1.2. Definition of the Selected Medicinal Plant, Solenostemma argel

According to the WHO monograph requirements, the selected medicinal plant S. argel consists of the dried leaves of Solenostemma argel, Family Ascelapediaeae (WHO, 1997).

4.1.3. Documentation and Identification of Solenostemma argel

(Based on the WHO, 1997, Monograph on Selected Medicinal Plants)

The selected medicinal plant, known locally in Sudan as ‘alhargal’ or ‘hargal’, consisted of the dried leaves of Solenostemma argel (Del) Hayne, Family Asclepiadaceae, and conformed to the WHO specifications.

4.1.4. Synonyms of Solenostemma argel

Solenostemma argel synonymous to Asclepiadaceae family is considered botanically of the single species, Hayne.

4.1.5. Selected Vernacular Names of Solenostemma argel
Solenostemma argel species (Del) Hayne; family Asclepiadaceae, is known “AlHargal or “Hargal”

4.1.6. Geographical Distribution of Solenostemma argel

It is commonly found in Libya, Egypt, Palestine, Saudi Arabia, and Northern Sudan, at Abu Hamad - Rubatab area.

4.1.7. Macroscopic Description of Solenostemma argel

Solenostemma argel is an erect perennial shrub, 1.5m high, with many velvety pubescent branches from the base. The leaves are weak yellow – to pale green (olive), and lancesolate. Apex is acute to subacute mucronate, fleshy, subsessile, ovate-oblong to elliptic and has a velvet-like pubescent. Inflorescence amble is axillary with short peduncle. The calyx consists of five separate sepals and white corolla, 7-10mm broad, with erect oblong liner lobes. Characteristic pollen masses are cup-shaped, flattened and pendulous. Fruits are follicle, oblong to ovate, smooth and very hard.

The most prominent findings of the morphological studies of Solenostemma argel are corona and stylar heads with five gland-like pollen-carriers, alternating with adhering-to-another follicular fruits, flattened seed with silky coma and opposite extipulate leaves. The laticiferous tubes are intra-axillary phloem, with superficial development of the cork, a broad peri-cycle containing isolated-groups fibers, narrow medullary rays in the wood, anomocytic stomata, bilateral vascular bundles and non-glandular hairs are the most important anatomical characteristics of S. argel plant (Andrews, 1952; Mahran and Saber, 1964; Jatri and ElGadi, 1977; Tharib et al., 1986; ElKamali et al., 1996; ElGazali et al., 1998). Solenostemma argel has compound perennial leaves, with many sometimes broken, 1.5-5cm in length, 0.5-1.5cm in width, having five pubescence pressed hairs, more numerous on lower surface (1-7 mm).

4.1.8. Organoleptic Properties of Solenostemma argel Leaves

The color of the leaves is weak yellow to pale olive, 1-2cm; the odour is characteristic; and the taste is mucilage-like, and then slightly bitter.

4.1.9. Microscopic Characteristics of Solenostemma argel Leaves

Epidermis is with polygonal cells containing mucilage, unicellular, thick-walled trichomes, length up to 260mm, slightly curved at the base, warty, panacytic stomata on both surfaces; under the epidermal cells, a single row of palisade layer, cluster crystal of calcium oxalate distributed through the lacanose tissue on the edaxial surface, sclerenchymatous fibers and a gutter-shaped group of similar fibers an the abaxial side counting prismatic.

4.1.10. Determination of Microorganisms in Solenostemma argel Leaves

Medicinal plant materials normally carry a great number of bacteria and moulds, often originating in soil. While a large range of bacteria and fungi form the naturally occurring microflora of herbs, aerobic spore-forming bacteria frequently predominate.

**Determination of Escherichia coli in Solenostemma argel Leaves**

The examined plant material of Solenostemma argel leaves passed the test for the absence of Escherichia coli since no such colonies were detected, and the confirmatory biochemical reactions were negative (BP, 1988; WHO, 2000).

**Determination of Salmonella species in Solenostemma argel Leaves**

The examined plant material of Solenostemma argel leaves passed the test for the absence of Salmonella species as cultures of the type described (BP, 1988; WHO, 2000) did not appear in the primary test, and the confirmatory biochemical tests in the secondary test were negative.

4.1.11. Powdered Plant Material of Solenostemma argel Leaves

LXXVI
The examined powdered plant material of *Solenostemma argel* leaves was Light green to greenish yellow, with polygonal epidermal cells, showing panacytic stomata, unicellular trichomes conical shape, with warty walls, isolated or attached to fragments of epidermis, fragments of fibro-vesicular bundles with a crystal sheath containing calcium oxalate prisms, dusted installs isolated or in fragments of parenchyma.

4.2. Phytochemical Investigations of *Solenostemma argel* Leaves

4.2.1. The Used Plant Materials of *Solenostemma argel* Leaves

4.2.1.1. Powdered *Solenostemma argel* Leaves

The particle size and particle surface area play an important role in the physico-chemical and pharmaceutical properties of drug substances, and therefore particle size reduction and analysis of powdered *Solenostemma argel* leaves were carried out. Different fractions were obtained after milling of *S. argel* leaves using the hammer mill and sieve analysis of the powdered leaves. The amount of powder particles that passed through the 160-micron mesh was found to be 61.7%, which was in agreement with that reported by Mahmoud *et al.* (1996). This fraction of powdered *Solenostemma argel* leaves was used in the formulation of optimized *S. argel* tablets.

4.2.1.2. Water Extract of *Solenostemma argel* Leaves

The collected dried aqueous extract of *Solenostemma argel* leaves ranged between 20 and 30% of the total weight of the leaves. The dried aqueous extract of *Solenostemma argel* leaves was used in the preparation of optimized tablets of *S. argel* extract as one of the formulations produced in the present study.

4.2.1.3. Alkaloids Extract of *Solenostemma argel* Leaves

The collected dried alkaloids extract of *S. argel* leaves was brown in colour and more hygroscopic than the dried water extract of *S. argel*. The dried alkaloids content of *S. argel* leaves ranged from 2 to 5% of the total weight of *S. argel* leaves. The amount needed for formulation is lesser than that for the extract or the leaves powder. The two extracts of *S. argel* leaves were the dried chloroform-extracted fraction (brown in colour) and the alkaline aqueous layer evaporated to dryness and extracted with methanol. This dried methanol extract was yellowish-brown in colour. The presence of quaternary ammonium compounds and Nitrogen-containing compounds was indicated by Dragendorff's reagent, while the presence of alkaloids was confirmed by Mayer's reagent (Mandelic acid and iodine) (Figures 1-3).

4.2.1.4. Detection of Flavanoids in Extracts of *Solenostemma argel* Leaves

The presence of flavonoid compounds in dried aqueous and methanolic extracts of *Solenostemma argel* Leaves was detected by using different reagents including ninhydrin, antimony aluminum chloride, and natural substances reagent. They were examined under UV light on TLC columns and PC run in different systems, and compared with authentic samples of kaempferol, quercetin and rutin. Yellow spots were detected having similar Rf values to those of the authentic compounds and confirming the presence of such flavonoid compounds in the extracts of *S. argel* Leaves (Figures 4-6). Similar findings were reported by Khalid *et al.* (1979) and ElKamali (1996).

4.2.1.5. Separation of Flavanoids in Extracts of *Solenostemma argel* Leaves

The different types of flavonoid compounds in the dried aqueous and methanolic extracts of *Solenostemma argel* leaves were separated by using accelerated column of polyamide which was subjected to applied negative pressure (suction) from underneath, and a reservoir from upwards to enhance the continuity of the elution, keep the column intact, and not to get dry. The thick green extract material was placed at the top of the
polyamide column, 200ml of water was passed through the column, followed by increasing amounts of methanol (10%; 20%; 30%; 40%; 50%; 60%; 70%; 75%; 80%; 85%; 90%; 95%; and 100%), used as a solvent system with different polarity, which resulted in separation of different clotting material from each portion, and in fraction (40-50%), a persistent foaming, due to a saponin-like material, was observed (Figure 5).

Figure 1: Paper Chromatography of Solenostemma argel water extract
developed in B: A: W system, sprayed with Ninhydrin reagent and viewed under UV light, revealed seven areas.
Figure 2: (A) and (B) *Solenostemma argel* Ethyl acetate extraction with different separated fractions from Polyamide column, and eluted with different polar solvents system by increasing the methanol percentages, in (water: Methanol) system.
Figure 3: Solenostemma argel Alkaloids separation using Silica Gel TLC
Developed by different solvents systems:

a) Butanol: Buffered acetic acid (470:130)
b) Chloroform: Methanol: Ethyl Acetate (30 : 20 : 20)
c) Benzene: Methanol (2 : 1)
d) Chloroform: Ether: Methanol (30: 20: 20)
e) Benzene: Acetate Acid: Water (5.7: 2.8: 1.5)
f) Benzene: Ethyl Acetate: Methanol (60: 40: 30)
g) Methanol: Ammonia (99.5: 0.5)
and viewed under UV lamp.
Figure 4: *Solenostemma argel* Three Distinct Alkaloids Areas in Precoated Silica Gel TLC, Developed in (Methanol: Ammonia) (99.5: 0.5) System, Sprayed with Dragendorff’s Reagent; (A) Before, and (B) After color fixation with 5% 2M sulfuric acid alcoholic solution.
Figure 5: *Solenostemma argel* different fractions spots separated by elution with increased Polar systems on aluminum oxide column, started with 100% methanol and ended with 100% water systems, and sprayed with Dragendorff’s reagent.
4.2.1.6. Identification of Alkaloids in Extracts of Solenostemma argel Leaves

The alkaloids in extracts of Solenostemma argel leaves were applied to:

a) Silica gel TLC preparative and precoated, run in B: A: W system, with three yellow-orange colored spots detected after spraying with Dragendroff’s reagent. The colour was fixed by using 2M sulphuric acid ethanolic solution (Figure 4).

b) IGM alkaloids were eluted by different solvents with increasing polarity (ether, benzene, chloroform, ethyl acetate, methanol, and methanol-water). Their content was subjected to TLC on an aluminum oxide plate using methanol: ammonia, 0.5%, system. Different orange spots were detected by Dragendroff’s reagent (Figure 5).
4.3. Pharmacological Investigations of *Solenostemma argel* Leaves

4.3.1. The Small Intestine Models

The effects of the alkaloids of dried chloroform or aqueous extracts of *Solenostemma argel* were compared to those of freshly prepared solutions of tested reference drug substances (hyoscine, papaverine and authentic flavonoids) in different small intestine models.

Acetylcholine chloride, 1 µg/ml, or barium chloride, 0.002 mg/ml, were used as contractility inducers or enhancers in contractile rabbit small intestine model (CRSIM) (Figures 7, 8) or in guinea pig small intestine model (GPSIM) (Figure 9).

![Figure 7: Effect of *Solenostemma argel* Leaves Alkaloids on Spontaneous and Acetylcholine-Induced Contractility in Rabbit Small Intestine](image-url)
Figure 8: Effect of *Solenostemma argel* Alkaloids on Rabbit Small Intestine

Figure 9: Effect of *Solenostemma argel* Leaves Water Extract and Alkaloids on Barium Chloride-Induced Contractility in Guinea Pig Ileum.
Figure 10: Effects of Solenostemma argel Leaves Chloroform and Alkaloid Extracts on Acetylcholine Induced-Contractility in Rabbit Small Intestine.

The inhibitory effects of Solenostemma argel alkaloids of dried chloroform or aqueous extracts were compared with those of reference drug substances, including hyoscine, papaverine and authentic flavonoids, in different small intestine models. All these drug substances were used in freshly prepared solutions. Their inhibitory effects were tested either on the normal contractility of the small intestine or on contractility induced by either acetylcholine chloride, 1 µg/ml, or barium chloride, 0.002mg/ml, used as contractility inducers or enhancers in rabbit small intestine (CRSIM) (Figures 7, 8) or in guinea pig small intestine (GPSIM) (Figure 9).

The inhibitory effects of Solenostemma argel leaves alkaloids, 4mg/ml, on the contractility induced in rabbit small intestine model by acetylcholine at a sub-maximal dose of 1.0 µg/ml indicated an anti-cholinergic action of these S. argel alkaloidal extract (Figures 7, 8).

Furthermore, the inhibitory effects of Solenostemma argel leaves alkaloids, at a 4mg/ml concentration, on the normal contractility of the rabbit small intestine as well as on the contractility induced by acetylcholine, at a sub-maximal dose, further confirmed the anti-cholinergic action of S. argel alkaloids (Figure 8).

These inhibitory effects of Solenostemma argel leaves water extract, at 12mg/ml, and alkaloids, at 4mg/ml, were further reflected on barium chloride induced-contractility in guinea pig ileum model (Figure 9).

However, the inhibitory effects of Solenostemma argel leaves alkaloid extract, at 2- and 3-mg/ml concentrations, on acetylcholine induced-contractility in rabbit small intestine were in contrast to the stimulatory effect of chloroform extract, at 2 and 4mg/ml concentrations, and which were similar to those of acetylcholine itself (Figure 10). These findings reflected the contradicting effects of S. argel leaves ranging from inhibition to stimulation of rabbit small intestine.
4.3.2. Effects of *Solenostemma argel* Extracts on the Heart Models

4.3.2.1. Effects of *Solenostemma argel* Extracts on Rabbit Isolated Heart Model

Figure 11: Effect of *Solenostemma argel* Leaves Water Extract on Contractility and Heart Rate in Isolated Rabbit Heart Model.

*Solenostemma argel* leaves water extract, 12mg/ml, reduced both the contractility and the heart rate of rabbit isolated heart model (Figure 11). Likewise, *Solenostemma argel* leaves water extract (1), 12mg/ml, reduced both the contractility and the heart rate of intact African toad heart model for a longer period of time than the stem extract (2) or fruit extract (3) (Figure 12).

4.3.2.2. Effects of *Solenostemma argel* Extracts on African Toad Contact Heart

Figure 12: Effects of *Solenostemma argel* Extracts of Different Parts* on Contractility and Heart Rate in African Toad Heart Model

*Solenostemma argel* leaves water extract, 12mg/ml, reduced both the contractility and the heart rate of rabbit isolated heart model (Figure 11). Likewise, *Solenostemma argel* leaves water extract (1), 12mg/ml, reduced both the contractility and the heart rate of intact African toad heart model for a longer period of time than the stem extract (2) or fruit extract (3) (Figure 12).
4.3.2.3. Effect of Flavonoid Constituents of *Solenostemma argel* Leaves Extracts on Contracting Rabbit Small Intestine Model

The presence of flavonoid compounds was confirmed in the water or methanol extracts of *S. argel* by using paper chromatography (PC) or thin layer chromatography (TLC, silica gel) run in B:A:W system (40:10:50). The chromatograms were sprayed with natural substances reagent or ninhydrin (Figure 1).

Separation of the different flavonoid compounds was carried out by using an accelerated eluted solvent passage through a polyamide chromatographic column. A 20-gram sample of the powdered *S. argel* leaves was defatted with petroleum ether and dichloromethane several times, and extracted with 200ml of ethyl acetate for many times at room temperature (25°C), using a magnetic stirrer and a closed system to prevent evaporation.

The extract was concentrated with a Roto-vap to about 10ml which became as a thick green substance. After the application of the extract at the top of the column, 200ml of water were passed together with increasing amounts of methanol to change the polarity of the system. These systems with different polarities separated different materials in each eluted portion (Figures 2, 5).

The different pharmacological activities of these separated portions were checked on contracting rabbit’s small intestine (Figures 13-20).

Figure 13: Effect of Alcohol on the Contracting Rabbit’s Small Intestine Model
Figure 14: Effect of *Solenostemma argel* First Fraction, from the Base Band (1) in PC run in (B: A: W) System, on Contracting Rabbit's Small Intestine Model

Figure 15: Effect of *Solenostemma argel* Second Fraction, from the Base Band (2) in PC run in (B: A: W) System, on Contracting Rabbit's Small Intestine Model
Figure 16: Effect of *Solenostemma argel* Third Fraction, from the Base Band (3) in PC run in (B: A: W) System, on Contracting Rabbit's Small Intestine Model

Figure 17: Effect of *Solenostemma argel* Fourth Fraction, from the Base Band (4) in PC run in (B: A: W) System, on Contracting Rabbit's Small Intestine Model
Figure 18: Effect of *Solenostemma argel* Fifth Fraction, from the Base Band (5) in PC run in (B: A: W) System, on Contracting Rabbit’s Small Intestine Model

Figure 19: Effect of *Solenostemma argel* Sixth Fraction, from the Base Band (6) in PC run in (B: A: W) System, on Contracting Rabbit’s Small Intestine Model
4.3.3. Calculation of Median Effective Dose, ED<sub>50</sub>, of Hyoscine and Alkaloids of Solenostemma argel Leaves Using MedUSA System

The median effective doses (ED<sub>50</sub>) of the alkaloids of Solenostemma argel leaves and hyoscine were calculated using Medusa system, by recording their inhibitory effects on the contractility induced by a sub-maximal dose of acetylcholine (1µg/ml) in a guinea pig ileum model. A comparison was made of the reduction in contractility, due the actions of these two drug substances, and calculated from the means of seven readings of six preparations for each concentration (Table 1, and Figure 21).

MedUSA system was used to compare the therapeutic equivalence of Solenostemma argel alkaloids with authentic hyoscine. These two drug substances suppressed the sub-maximal dose response of barium chloride, 0.002mg/ml, on guinea pig ileum contractility, which was a concentration-dependent action. This sub-maximal stimulation due the enhancer's effect was found to be 35mm.

Table 1: Effects of S. argel Alkaloids and Hyoscine on Guinea Pig Ileum Contractility Induced by Submaximal Dose of Barium Chloride: Calculation of Median Doses, ED<sub>50</sub>, of S. argel Alkaloids and Hyoscine Using MedUSA System

<table>
<thead>
<tr>
<th>No.</th>
<th>S. argel Alkaloids Dose (mg)</th>
<th>Effect (%)</th>
<th>Hyoscine Dose (mg)</th>
<th>Effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.5000×10⁻³</td>
<td>5.400</td>
<td>2.500×10⁻⁴</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>5.0000×10⁻³</td>
<td>5.410</td>
<td>5.000×10⁻⁴</td>
<td>0.000</td>
</tr>
<tr>
<td>3</td>
<td>1.000×10⁻²</td>
<td>13.510</td>
<td>1.000×10⁻³</td>
<td>14.290</td>
</tr>
<tr>
<td>4</td>
<td>0.020</td>
<td>18.920</td>
<td>2.000×10⁻³</td>
<td>22.860</td>
</tr>
<tr>
<td>5</td>
<td>0.040</td>
<td>27.030</td>
<td>2.500×10⁻³</td>
<td>22.860</td>
</tr>
<tr>
<td>6</td>
<td>0.080</td>
<td>56.760</td>
<td>5.000×10⁻³</td>
<td>20.000</td>
</tr>
<tr>
<td>7</td>
<td>0.160</td>
<td>72.970</td>
<td>1.000×10⁻²</td>
<td>37.140</td>
</tr>
<tr>
<td>8</td>
<td>---</td>
<td>---</td>
<td>0.020</td>
<td>80.000</td>
</tr>
</tbody>
</table>
Clark’s classical model was used to draw the two graphs for hyoscine and *S. argel* alkaloids. For hyoscine, \( \text{PD}_2 \ (\text{-log ED}_{50}) = 2.240 \ (\text{sd} \pm 5.187 \ E^{-0.3}) \); and

For *Solenostemma argel* alkaloids = 1.297 (sd ± 0.020).

![Graph showing inhibition percentage vs. drug doses (mg)](image)

**Figure 21: Effects of *S. argel* Alkaloids and Hyoscine on Guinea Pig Ileum Contractility Induced by Submaximal Dose of Barium Chloride: Calculation of Median Doses, \( \text{ED}_{50} \), of *S. argel* Alkaloids and Hyoscine Using MedUSA System**

The concentration of *Solenostemma argel* alkaloids, which inhibited sub-maximal dose response to the enhancer, was found to be nearly eight times the concentration of hyoscine, which produced the same inhibitory effect on the contractility of the guinea pig ileum. Martin (1978) stated that the oral dose for hyoscine methyl nitrate was 2-4mg.

The maximal dose for *S. argel* alkaloids in MedUSA system = 0.275mg; and

The maximal dose for hyoscine in MedUSA system = 0.035mg.

**4.3.4. Bioequivalence of Papaverine and Alkaloids of *Solenostemma argel* Leaves**

Different preparations of *S. argel* leaves, such as ground leaves, water extract and alkaloids tablets, were compared with authentic papaverine sample, using rabbit small intestine model and acetylcholine (1.0µg/ml), at a sub-maximal dose, as a stimulant. Papaverine was used at a concentration of 2.5mg/ml, *S. argel* alkaloids at 4mg/ml, water extract, 8mg/ml, and powdered leaves, 16mg/ml in a 30ml-gut’s bath (Figure 22). In the previous pharmacological experiments, therapeutic comparisons were set between the anti-cholinergic and antispasmodic actions of reference drug substances and those of different *S. argel* preparations.

The used dose of papaverine that has caused complete inhibition in CRSIM experiment was 1.0mg, while that of alkaloids, extracts and powder of *S. argel* leaves were 4mg, 17.5mg and 40mg, respectively (Figures 21, 22). In the MedUSA system, the doses were 0.035 and 0.275mg for hyoscine and *S. argel* alkaloids, respectively, when using 0.002mg/ml barium chloride as sub-maximal dose.
4.3.5. Effects of Solenostemma argel Extracts on the Frog Foot Withdrawal Reflex Evaluation of Local Anaesthetic Activity Solenostemma argel Extracts

Another pharmacological action namely the local anesthetic effect was used to compare the activities of S. argel extracts, authentic flavanoids and lignocaine. Generally, there is a close relation between local anaesthetic action, anti-cholinergic activity and inhibition of peristalsis or spasm reduction, as well as anti-hypertensive activity (ElTahir et al., 1986; Khalaf Allah et al., 1996; EIAgib, 2001; Salah eldeen, 2003). These two pharmacological effects indicated the drug action and potency.

The local anaesthetic activities of the authentic samples, at 4mg/ml concentration, of kaempferol, quercetin, rutin, and the alkaloids of dried alcoholic extract of S. argel as well as rutin-like substances of S. argel were evaluated in comparison with lignocaine (4mg/ml) as standard. The effects of Solenostemma argel extracts and the authentic substances were evaluated using the frog foot withdrawal reflex. Each drug was tested in six frog preparations, and the local anesthetic onset mean time for each drug was determined. The local anesthetic onset time of lignocaine was found to be 3.33 minutes; this was the reference response, and the potencies of the other substances were compared to that of lignocaine as relative potencies of these drug substances (Table 2).

The median effective doses (ED50) of lignocaine and the other drug substances, based on local anaesthetic onset time as the response, were calculated and consequently the relative potencies were established (Table 2). The relative potency is the ratio of the potencies of the comparative drugs which will give the same response, i.e. local anaesthetic effect. The relative potency is the inverse ratio of experimental dose/standard dose, i.e. the ratio of doses that give equal responses, or the ratio of responses given by equal doses (Finnery, 1964).
There were similarities in local anaesthetic effects and relative potencies of the alkaloids of different extracts of *S. argel* leaves and the three authentic samples, especially kaempferol which had a 0.5 relative potency, compared to lignocaine as standard. This local anaesthetic activity might be used as a parameter to identify and evaluate different types of flavanoids, because the three authentic flavanoids and the three tested extracts showed variable local anaesthetic activities. The relative potencies of an authentic sample of kaempferol and *S. argel* alkaloids were found to be nearly the same and equal to half the potency of lignocaine (Table 2).

The first separated chloroform fraction might contain flavanoids with kaempferol being one of them (Khalid *et al.*, 1976). The dried aqueous extract of *S. argel* leaves had the same local anesthetic action and relative potency as procaine used as a reference drug (Khalaf Allah *et al.*, 1996).

### Table 2: Comparative Evaluation of Local Anaesthetic Activity of *Solenostemma argel* Extracts and Authentic Substances

<table>
<thead>
<tr>
<th>No.</th>
<th>Drug or Extract</th>
<th>Local Anesthesia Onset Time (minutes)</th>
<th>Relative Potency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lignocaine</td>
<td>3.33</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Kaempferol</td>
<td>6.71</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>9.28</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>Rutin</td>
<td>9.60</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>Alcoholic Extract of <em>S. argel</em></td>
<td>10.0</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td><em>S. argel</em> Alkaloids</td>
<td>6.65</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>Rutin-like Substances of <em>S. argel</em></td>
<td>12.6</td>
<td>26</td>
</tr>
</tbody>
</table>

4.3. 6. Diffusion of *Solenostemma argel* Alkaloids across Rabbit Small Intestine

The diffusion of *S. argel* alkaloids across the rabbit small intestine model was studied both in the absence and presence of sodium fluoride (0.01g). Concentrations of alkaloids diffusing at different time interval were determined; graphs were obtained by plotting concentration readings, at $\lambda$ 294.5nm, versus time (Table 3, Figure 23).

### Table 3: Diffusion of *Solenostemma argel* Alkaloids across Rabbit Small Intestine, and the Effects of Sodium Fluoride*

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Absence of Sodium Fluoride</th>
<th>Presence of Sodium Fluoride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean* ±SEM</td>
<td>Time (minutes)</td>
</tr>
<tr>
<td>5</td>
<td>0.58882 ±0.1555</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>0.9876 ±0.3149</td>
<td>10</td>
</tr>
<tr>
<td>15</td>
<td>1.7788 ±0.831</td>
<td>15</td>
</tr>
<tr>
<td>30</td>
<td>2.6526 ±1.3141</td>
<td>30</td>
</tr>
<tr>
<td>60</td>
<td>3.5220 ±2.61562</td>
<td>60</td>
</tr>
<tr>
<td>90</td>
<td>4.3066 ±0.4980</td>
<td>120</td>
</tr>
<tr>
<td>120</td>
<td>9.012 ±0.96280</td>
<td>180</td>
</tr>
<tr>
<td>150</td>
<td>11.964 ±2.192</td>
<td>240</td>
</tr>
<tr>
<td>180</td>
<td>10.592 ±1.9609</td>
<td></td>
</tr>
</tbody>
</table>

*For the mean, n=6  *Sodium Fluoride conc.  0.01g
These findings reflected the inhibitory effect of sodium fluoride on the S. argel alkaloids diffusion rates (Table 3, Figure 23). There was a significant retardation of diffusion of S. argel alkaloids across the rabbit small intestine model; results of alkaloids diffusion rates in the absence of sodium fluoride ranged between three-folds and ten-folds those recorded in the presence of sodium fluoride, at 0.01g/ml concentration.

![Figure 23: Effects of Sodium Fluoride on the Diffusion of Solenostemma argel Alkaloids across Rabbit Small Intestine,](image)

In order to determine whether active diffusion was involved beside passive diffusion in S. argel alkaloids diffusion in the rabbit small intestine model, 0.01g of sodium fluoride was added to the system. The decrease in the amounts of S. argel alkaloids diffusing, on the addition of sodium fluoride, indicated that the rate of diffusion was affected greatly due to the blocking of glycolysis enzymes. Sometimes the rate of diffusion would be retarded by 10-times that of the normal diffusion. This would indicate that beside the passive diffusion, there was an active diffusion taking place in S. argel alkaloids absorption. The in vitro measurement of diffusion rate of medicaments would resemble closely the in vivo situation (Rae, 1944).

The diffusion of S. argel alkaloids across the rabbit small intestine at different time intervals and the effects of sodium fluoride (0.01g) on this diffusion of S. argel alkaloids across the rabbit small intestine were also studied at different time intervals. This involved the evaluation of rates of diffusion, and absorption, of Solenostemma argel alkaloids before and after sodium fluoride addition. The means of six UV readings of samples of diffusion medium, before and after the addition of sodium fluoride, were determined at different time intervals (Table 3, Figure 23). The decrease in the rates of diffusion of S. argel alkaloids across the rabbit small intestine model that had been recorded due to the inhibitory effects of sodium fluoride may indicate an involvement of an active transport mechanism. From the curve, S. argel alkaloids had an active transport which was delayed greatly by using sodium fluoride. Therefore, there might be an intrinsic factor or factors which would affect greatly the absorption of S. argel. More investigations would be required to evaluate the possible role of these factors in formulations of S. argel preparations.
Diffusion is the migration of molecules from a region of high concentration, and is a result of Brownian movement of the solute molecules. This migration of solute molecules would take place in the absence of external forces. Such a movement of solution by concentration gradient is a measure of the escaping tendency of the solute to achieve an equilibrium state. A similar situation arises if a semi-permeable membrane separates two solutions of unequal concentrations, when the equilibrium is restored by the diffusion across the membrane, i.e. osmosis. Thus osmosis is concerned with escaping tendency of the solvent. The forces involved in the two processes are equal in magnitude but opposite in direction.

Fick’s first law of diffusion is analogous to processes involved in heat conduction. This law states that the amount of substance, $dm$, diffusing in $X$ direction in time, $dt$, across an area, $A$, is proportional to the concentration gradient $\frac{dc}{dx}$ in the plane of the area; $dm = -DA\frac{dc}{dx} dt$; The proportionality factor, $D$ is called the diffusion coefficient; the negative sign denotes that the diffusion takes place in the direction of decreasing concentration. Diffusion coefficient is not strictly a constant since it varies slightly with concentration, but it can be regarded as a mean value for the concentration range covered. The dimensions of $D$ are length/time.

Fick’s second law is derived from the first law by eliminating the dependant variable $m$, and expressing the rate of change of concentration at any point,

$$\frac{dc}{dt} = \frac{Ddc^2}{dx^2}$$

However, *Solenostemma argel* alkaloidal extracts are colloidal solutions, and the diffusion of colloidal particles is related to the frictional coefficient of the particles by Einstein’s law of diffusion: $Df = KT$, where $K$ is the Boltzmann constant, $T$ is absolute temperature, and for spherical particles, $f$, the frictional coefficient is given by Stokes law: $f = 4\pi\eta r$, $D = \frac{KT}{6\pi\eta r} = \frac{RT}{6\pi\eta rN}$, where $R$ is the gas constant, and $N$ is Avogadro’s number. The effect of the temperature and viscosity on diffusion is apparent from the equation, as $D\alpha(T)$ for a given solute. For non-spherical particles, the frictional coefficient increases with decreasing symmetry and with particle solution, e.g. hydration. The liquid in the beaker was kept constant, and freed from the influence of external disturbance, so that the transport of solute through the intestine was solely due to diffusion. Homogeneity of the separated phases was effected by slight convection currents by gentle stirring or rotation of the beaker for small concentration changes.

There are other factors, e.g. the pore size between the intestinal muscles, the network structure of the intestinal wall, the fatty constituents of this wall, and the amino acids content. In addition to these, there are the sieve-like actions of the gel, and other factors such as the viscosity of the mucous membrane, and the internal food and drug content. However, it is only the solubility of the material that will affect greatly the diffusion rate.

If the mesh of amino acids or fatty constituents possesses ionizable groups of opposite charge to that of the diffusing particle, adsorption or ion exchange reaction may occur; for instance anionic groups will interact with the cationic such as the basic dyes and quaternary ammonium compounds and retard their diffusion. So the sodium fluoride might act in this way on the *S. argel* compounds, or by inhibiting the enzymes and the active transport mechanism. Also the pH of the intestinal media, the mucus
content and the contact with walls cells would affect drug absorption. For the drug, the more sites and the more area contacted, the more absorption will happen. The gentle stirring was essential to confirm solution contact and homogenous constant concentration (Co-open et al., 1946).

**Passive drug transport may be characterized by the following:**

a) Drug molecules move from a region of relatively high concentration to one of lower concentration; b) The rate of transfer is proportional to the concentration gradient between the compartments involved in transfer; c) The transfer process achieves equilibrium when the concentration of the transferable species is equal on both sides of the membrane; and d) Drugs, capable of existing in both a charged and non-charged form, approach an equilibrium state primarily by transfer of non-charged species across the membrane. In contrast to this, an active process involves participation by a factor in the transfer of molecules between compartments. A carrier, which may be an enzyme or some other component of the membrane, is responsible for affecting the transfer by a process which may be represented as follows in the following sketch:

![Active transport sketch across a membrane](image)

When the drug in compartment A is picked up by the carrier in the moves across the membrane, the drug-carrier complex then moves across the membrane and the drug is changed to compartment B at the membrane surface open to B. The carrier then returns to the A compartment surface for another drug molecule. This transfer system has characteristics that are decidedly different from those listed for the passive system.

**These characteristics of active drug absorption include:**

a) This process consumes energy in the work done by the carrier; b) Since it involves consumption of energy, it may be subject to poisoning by metabolic poisons such as fluoride and dinitrophenol, lack of oxygen and so on; and c) Unlike the passive transfer process which depends on concentration gradient; the carrier may transport all of drug from one compartment to the other without regard to an equilibrium state, which was the end point in the case of a passive transport process. Indeed, the carrier transfer system will generally be a one-way transport process.

**Interactions of Drugs with Components of Gastrointestinal Mucosa**

Interactions of drugs with the substances present in, or secreted by, the gastrointestinal mucosa can be of considerable consequence in the absorption of drugs. This may be illustrated by a consideration of the gastrointestinal absorption of quaternary ammonium compounds, many of which are used as anti-cholinergic and hypotensive agents. It is known that many of these drugs are absorbed poorly and irregularly from the gastrointestinal tract (Levine, 1955). While this probably is related to some extent to their poor lipoid solubility, it has been shown that quaternary ammonium...
compounds form non-absorbable complexes with the polysaccharide acids of intestinal mucin (Levine, 1955). Once the mechanism for the poor absorption of quaternaries had been elucidated, formulation adjustments to circumvent this problem and to facilitate absorption could be made. A physiologically inert quaternary compound was co-administrated, which either interacted preferentially or competed with the pharmacologically active quaternary compound for interaction sites. This resulted in less binding and greater physiological availability of the quaternary compound drug, as shown in both animals and humans (Cavallito et al., 1958; 1959).

**Effect of Calcium on Diffusion of Solenostemma argel Alkaloids**

Gastrointestinal absorption of hypotensive quaternaries thus attained permitted a reduction of the administered dose, which is desirable not only for economic reasons but also because some of these drugs exhibit undesirable local actions on the gastrointestinal tract. The use of pharmacologically inert quaternaries to enhance drug absorption is not limited to hypotensive quaternaries, but is also applied to other quaternaries such as anti-cholinergics and antispasmodics. So this may be applicable for *Solenostemma argel* alkaloids.

Interest with respect to the regions of optimum drug absorption in the gastrointestinal tract is the observation that the drug-mucin interaction decreases at lower pH. It has been suggested that, for this reason, absorption of quaternary compounds may be most rapid from the upper part of the gastrointestinal tract. Delayed drug release from dosage forms could under such circumstances reduce drug absorption. One might also speculate that co-administration of antacids could interfere with the absorption of these quaternary drugs due to increased drug binding at higher pH. This is also of interest for studies if these *S. argel* alkaloids are designed for systemic sustained action. Along with the consideration of these types of interactions, which cause inhibition of drug absorption, it is appropriate to mention interactions leading to opposite effects. This encompasses all instances of active transport for which the interaction of drug with carrier is often postulated; of greater interest to the biopharmaceutics are interactions with sodium fluoride (Figure 23).

Diffusion and absorption may involve compounds or components which could be isolated and reasonably defined, such as the well-known interaction of vitamin B12 with the intrinsic quaternary ammonium compounds, or with phosphatido-peptide fractions of intestinal tissue. Since these physiologic materials can be isolated, they can be employed as dosage form additives to enhance the absorption of certain drugs. Little published work is as yet available in this area, but it would seem that it represents a unique and perhaps fruitful approach to improve drug formulations of *S. argel* which contains quaternary ammonium compound alkaloids.

Study of the type of diffusion of drug substances and factors that affect its rate would help in the proper choice of the substances which enhance the bioavailability and the therapeutic effectiveness. In the case of *S. argel* leaves tablets, this needed more investigations, such as concurrent administration of the drug with antacids, acidic food stuffs, or fruits which would affect the absorption of drugs and affect the binding site of the drugs; this is due to the quaternary ammonium compounds of *S. argel* alkaloidal constituents. The absorption of the active constituents of *S. argel*, which contain quaternary compounds, would be affected by concurrent administration with antacids, milk, and acidic beverages and foods. This might affect its therapeutic uses in different pharmacological applications as anti-cholinergic, local anesthetic, anti-rheumatic, anti-arrhythmic, and analgesic (AlAgib, 2001), and as anti-hypertensive from domestic use. Knowledge of the type of absorption of a drug and even its mechanism would help in predication of factors that would affect the bioavailability, absorption and possible drugs interactions, such as that of quaternary ammonium compounds and antacids, sodium fluoride-like substances and *S. argel* alkaloids (Figure 23).
4.4. Toxicological Studies of Solenostemma argel Leaves

4.4.1. Acute Peritoneal Toxicity of S. argel Leaves in Rabbits and Albino Rats: The Lethal Dose of Solenostemma argel Leaves in Rabbits and Albino Rats

This experiment was carried out on a group of four rats and another group of four rabbits. The mean lethal dose was calculated for each group, and was found to be 6.35g/kg for the rabbits and 5.49g/kg for the rats (n=4)).

4.4.2. Determination of Median Lethal Dose (LD_{50}) of Solenostemma argel Leaves

Sixty Albino rats were used and assigned to six groups, each group comprising ten animals. Each group was given a different dose. Starting from the recorded lethal dose of 5.49g/kg in the pervious experiment, each of the following doses was given to each animal in an assigned group: 25, 37.5, 50, 75, 87.5 and 100% of the lethal dose. The animals were observed for twenty-four hours. The number of deaths in each group was recorded as percentage of the whole number (sixty) (Table 4). The log dose was plotted versus the mean deaths; the log LD_{50} was obtained from Figure 24.

Table 4: Solenostemma argel Acute Peritoneal Toxicity in Albino Canadian Rats

<table>
<thead>
<tr>
<th>Group No</th>
<th>Number Of Rats</th>
<th>Dose (g/kg)</th>
<th>Log Dose</th>
<th>Number of Deaths</th>
<th>Death %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>6.350</td>
<td>0.8277</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>5.500</td>
<td>0.7403</td>
<td>8</td>
<td>80%</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4.730</td>
<td>0.6767</td>
<td>1</td>
<td>10%</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>3.937</td>
<td>0.5952</td>
<td>1</td>
<td>10%</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>3.123</td>
<td>0.4948</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>1.5625</td>
<td>0.0263</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

logdose
death

![Figure 24: Solenostemma argel alkaloid lethal dose in Canadian Albino Rats](image)

4.4.3. Toxic Effects of Solenostemma argel Oral Syrup in Young Nubian Goats: Acute Toxicity of S. argel Oral Syrup in Young Nubian Goats

A dose of 5g/kg of S. argel oral syrup was administered daily for 45 days to seven young Nubian goats; a control group of another seven animals was given water instead of the syrup. After this daily administration of S. argel oral syrup to the Nubian goats, one of them showed ruffled hair, but no deaths occurred. There was an increase in the weight of the treated group (mean weight 12kg) compared to the control group (mean
weight 10kg). At the end of the 45 days, the animals in the two groups were sacrificed and the following investigations were carried out:

### 4.4.3.1. Relative Weights of Some Vital Organs

The relative weights of the vital organs including brain, kidneys, heart, spleen, and liver were normal compared to those of the control group (Table 5). There were no significant (P<0.05) changes occurring in the investigated vital organs compared to those of the control group. This indicated that *S. argel* had a good margin of safety since no hepatomegaly, cardiomegaly or splenomegaly occurred as a result of a daily administration of a dose of 5g/kg of *S. argel* oral syrup for 45 days.

**Table 5: Daily Administration of A 5g/kg Dose of Solenostemma argel Oral Syrup in Young Nubian Goats: Effects on the Relative Weights of Some Vital Organs**

<table>
<thead>
<tr>
<th>Young Nubian Goats (Control Group)</th>
<th>Young Nubian Goats (Test Group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>------------</td>
<td>-------</td>
</tr>
<tr>
<td>1</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td>3</td>
<td>81</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
</tr>
<tr>
<td>7</td>
<td>83</td>
</tr>
<tr>
<td>Mean</td>
<td>82</td>
</tr>
</tbody>
</table>

### 4.4.3.2. Histopathological Investigations of Tissues Abnormalities

Histopathological examination of some vital organs of these Nubian goats, given an oral daily dose of 5g/kg of *S. argel* oral syrup for 45 days, revealed some abnormalities that had taken place in these organs.

Some of them had a congested heart; others had hyperemia of the intestinal tissues revealing catarrhal inflammation with lymphocyte infiltration, which might be due to irritation and lead to enteritis; the continuous irritation may lead to the intestinal epithelial cells damage and papillae damage (Figure 25). The small intestine tissues were reported to show damage of small intestine epithelial cells on administration of toxic doses of *S. argel* (Madani, 2000).

The liver tissues were checked for the histopathological changes which revealed necrosis of centrilobular hepatocytes, fatty cytoplasm vacuolation and
slight congestion of the sinusoids (Figure 26). The liver also showed
degeneration of hepatocytes, with the RBC among them indicating hemorrhages,
and there was a cytoplasm vacillation.

The kidneys tissues showed necrosis of renal tubules, pyknosis, karyolysis
of tubular epithelial cells, and interstitial mononuclear cells infiltration; also
necrosis of the glomeruli and degenerated renal tubules observed might suggest
renal dysfunction accompanied by increase in the concentration of urea and
inorganic phosphorus (at level P<0.01), although the creatinine level was not
affected; the control group showed no lesions (Tables and Figures). Similar
findings were reported by Murroy et al. (1988).

In order to avoid the toxic effects manifested in these experiments, it is
necessary to avoid the use of toxic doses of S. argel preparations, and to be very
careful with patients with renal or hepatic dysfunction, due to the possible
toxicity of high doses of Solenostemma argel, in particular to avoid taking it for
long periods, i.e. it has to be taken when it is necessary and for a short period of
time.
Figure 25: (A) Normal Nubian Goat's small intestine epithelial cells, (B) Damaged Nubian Goat's small intestine epithelial cells, after oral daily dose of 5g/kg of *Solenostemma argel* water extract for 45 days (stain H and Ex400)
Figure 26: Nubian Goat Liver Tissue showing:
(A) Degeneration of Hepatocytes;
(B) RBC between hepatocytes indicating hemorrhages;
(C) Cytoplasmic vacuolation; and
(D) Normal hepatocytes,
after oral daily dose of 5g/kg of
*Solenostemma argel* water extract for 45 days
Figure 27: (A) Nubian Goat Kidney Tissue showing: Necrosis of Renal Tubules, Pyknosis, Karyolysis of Tubular Epithelial Cells; Interstitial Mononuclear Cells Infiltration; and Necrosis of the Glomeruli, after oral daily dose of 5g/kg of *Solenostemma argel* Water Extract for 45 days; (B) Nubian Goat’s Normal Kidney Tissue showing: Normal Renal Tubules and Normal Glomeruli (Stain H and E x400)
Figure 28: (I) Nubian Goat's Kidney tissue section, after 45 daily use of toxic dose of 5g/kg S. argel, shows:
(A) Degeneration of the renal tubules;
(B) Necrosis of glomeruli;
(II) Normal Nubian Goat's Kidney tissues section shows:
(A) Normal renal tubules; and (B) Normal renal glomeruli,
(Stain H and Ex400)

4.4.3.3. Effects of Solenostemma argel Leaves Extract* on the Serum Constituents of Young Nubian Goats**

Effects of Solenostemma argel on the activities of alkaline phosphatase and aspartate aminotransferase, AST (SGPT), and on albumin, total protein, creatinine, calcium and phosphorus concentrations in the serum of the young Nubian goats were given in Table 6. In the case of the 5g/kg daily dose S. argel alkaloids, phosphorus, creatinine, calcium, urea concentrations, as well as alkaline phosphatase and aspartate aminotransferase, AST, had high levels in the treated animals than those of the normal animals.

The treated animals given a 5g/kg daily dose of S. argel had low levels of protein, and albumin compared to those of the control group (Table 6). These findings were similar, to some extent, to those reported by Madani (2001) in Nubian goats.

There was a significant increase in serum urea (P<0.05), AST enzyme and phosphate (P<0.01) when compared with those of the normal control group.

Table 6: Effects of Solenostemma argel Leaves Extract* on the Serum Constituents of Young Nubian Goats**
### Groups → Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I** Control</th>
<th>Group II** Test (5g/kg/day For 45 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>0.45±0.12</td>
<td>1.5±0.21a</td>
</tr>
<tr>
<td>Alkaline Phosphatase (IU)</td>
<td>220±11.55</td>
<td>336.4±12.1b</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.25±0.05</td>
<td>0.15±0.02c</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>22.0±2.21</td>
<td>32.0±0.081b</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>7.7±0.47</td>
<td>8.6±0.91c</td>
</tr>
<tr>
<td>AST (IU)</td>
<td>16.4±0.8</td>
<td>90±1.71a</td>
</tr>
<tr>
<td>GGT(IU)</td>
<td>1.6±0.29</td>
<td>2.1±0.12c</td>
</tr>
<tr>
<td>Total Protein (g/dl)</td>
<td>6.9±0.33</td>
<td>6.0±0.61c</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>5.5±0.65</td>
<td>3.61±0.085c</td>
</tr>
</tbody>
</table>

*: Daily administration of 5g/kg dose of *S. argel* extracts as a syrup for 45 days; **: No. of Young Nubian Goats in Each of the Control and Test Groups=7; a: P< 0.01; b: P< 0.05; c: N.S.

### 4.4.3.4. Effects of *Solenostemma argel* Leaves Extract* on the Haematological Parameters of Young Nubian Goats**

There were significant changes in the hematological parameters, except with the MCHC, there was no significant change (Table 7). The haemoglobin (P<0.01), packed cell volume, and red blood cells count (P<0.05) as haematological parameters were significantly decreased; mean corpuscular volume was significantly increased in the treated animal compared to the control group (Table 7)

### Table 7: Effects of *Solenostemma argel* Leaves Extract* on the Haematological Parameters of Young Nubian Goats**

<table>
<thead>
<tr>
<th>Parameter→Group</th>
<th>Hb (g/dl)</th>
<th>MCV (m³)</th>
<th>PCV (%)</th>
<th>RBCs (X10⁶ mm³)</th>
<th>MCHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I** Control</td>
<td>8.61±0.3</td>
<td>34.6±0.61</td>
<td>30.4±0.66</td>
<td>8.71±0.10</td>
<td>30.1±1.2</td>
</tr>
<tr>
<td>Group II** Test (5g/kg/day For 45 days)</td>
<td>7.5±0.03a</td>
<td>38.5±0.80b</td>
<td>25.8±0.61b</td>
<td>8.2±0.70b</td>
<td>29.01±0.77c</td>
</tr>
</tbody>
</table>

*: Daily administration of 5g/kg dose of *S. argel* extracts as a syrup for 45 days; **: No. of Young Nubian Goats in Each of the Control and Test Groups=7;
4.4.4. Effects of *Solenostemma argel* Alkaloids on White Albino Canadian Rats

Three *S. argel* alkaloids were administered to white Albino Canadian rats at doses of 640, 320, and 160mg/kg for 7, 15, and 35 days, and examined for tissues abnormalities in the following tissues:

4.4.4.1. Effects of *Solenostemma argel* Alkaloids on the Relatives Weights of Some Vital Organs of White Albino Canadian Rats

There were no significant changes in the relative weights of the vital organs of the animals treated with *S. argel* alkaloids, including the brain, kidneys, heart, spleen, and liver when compared to those of the control group. This further confirmed that *S. argel* had a good margin of safety since no significant alterations occurred in these vital organs as a result of a daily administration of a dose of 640mg/kg of *S. argel* alkaloids for 35 days.

4.4.4.2. Effects of *Solenostemma argel* Alkaloids on Serum Constituents of White Albino Canadian Rats

The blood serum biochemistry of the three treated rats was compared with that of the control group, and the following changes were observed: there were significant increases in urea (at level P<0.05), and in creatinine, calcium, phosphorous, alkaline phosphatase, and aspartate aminotransferase (at level P < 0.01). There were no significant changes recorded in both total protein and albumin concentrations (Table 8).

The effects of *S. argel* on the activities of alkaline phosphatase, aspartate aminotransferase, AST, and SGOT, and on albumin, total protein, creatinine, calcium, and phosphorus concentrations in the serum of the treated animals were determined and compared with those of the control group, as given in Table. Phosphorus, creatinine, calcium, total protein and albumin concentrations, and alkaline phosphatase and aspartate aminotransferase, AST, activities were at high levels in animals given the high dose of 0.63gm/kg of *S. argel* alkaloids, when compared to those of the normal rats. The median effective dose had low protein and albumin alkaline phosphatase.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Urea mg/dl</th>
<th>Creatinine mg/dl</th>
<th>Calcium mg/dl</th>
<th>Phosphorus mg/dl</th>
<th>Total protein g/dl</th>
<th>Albumin g/dl</th>
<th>Alkaline Phosphatase, iu/L</th>
<th>Aspartate Transf erase, iu/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. argel Dose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum Dose</td>
<td>32.57 ± 10.66</td>
<td>0.53 ± 0.8a</td>
<td>7.4 ± 41a</td>
<td>1.32 ± 0.27a</td>
<td>6.44 ± 0.43</td>
<td>2.59 ± 1.27</td>
<td>134.00 ± 49.11a</td>
<td>11.00a ± 1.22</td>
</tr>
<tr>
<td>Median Dose</td>
<td>34.86 ± 7.56</td>
<td>0.53 ± 0.0a</td>
<td>0.00 ± 0.0b</td>
<td>1.00 ± 0.19</td>
<td>5.87 ± 0.63</td>
<td>2.92 ± 0.46</td>
<td>65.60 ± 23.77b</td>
<td>7.00 ± 4.00</td>
</tr>
<tr>
<td>Effective Dose</td>
<td>30.43 ± 6.35</td>
<td>0.34 ± 13.0b</td>
<td>7.3 ± 10.0a</td>
<td>0.90 ± 0.07b</td>
<td>6.14 ± 0.42</td>
<td>2.99 ± 0.19</td>
<td>93.00 ± 9.29b</td>
<td>7.50 ± 2.12</td>
</tr>
<tr>
<td>Control</td>
<td>22.00 ± 2.58</td>
<td>0.27 ± 1.7b</td>
<td>7.16 ± 1.3</td>
<td>0.89 ± 0.12b</td>
<td>6.07 ± 0.21</td>
<td>2.97 ± 0.24</td>
<td>72.86b ± 13.75</td>
<td>5.17 ± 1.60</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>29.96 ± 8.54</td>
<td>0.41 ± 3.39</td>
<td>5.16 ± 2.3</td>
<td>1.01 ± 0.47</td>
<td>6.13 ± 0.68</td>
<td>2.86 ± 0.68</td>
<td>91.72b ± 36.86</td>
<td>7.63 ± 3.16</td>
</tr>
<tr>
<td>Signi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.4.4.3. Changes in Haematological Components

These experiments were carried out to study the possible haematological changes that may occur when doses four, eight, or even sixteen times the stated effective dose of *S. argel* alkaloids were administered daily for a period of 35 days.

In these Albino rats, the blood parameters of the treated rats were compared with those of the control group, and the following changes were observed: for the haemoglobin and packed cell volume, PCV, there were significant decreases with the maximum dose of 640mg/kg and effective dose of 160 mg/kg, while there was an increase with the median effective dose of 320 mg/kg for seven days.

At 35 days, there were significant increases in the haemoglobin value and PCV with all three doses, at the level of p<0.05, in comparison with those of the control group. At 15 days, there were significant differences, at the level of p<0.01, with an increase with the 640mg/kg dose, and a decrease with the other two doses.

For the maximum dose of 640mg/kg, there was an increase in both hemoglobin value and PCV at 7, 15 and, 35 days; i.e. during the entire period of the drug administration. There were also increases in hemoglobin and PCV values at seven days and at 35 days periods of drug treatment.

### Table 9: Effects of Different Doses of *Solenostemma argel* Alkaloids on Haemoglobin Concentration in Albino Rats

<table>
<thead>
<tr>
<th>Time</th>
<th>Doses</th>
<th>7 days (g/dL)</th>
<th>15 days (g/dL)</th>
<th>35 days (g/dL)</th>
<th>Dose (overall mean g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum Dose 640mg</td>
<td>9.07 ±1.44</td>
<td>11.88 ±0.66</td>
<td>12.80 ±1.33</td>
<td>11.58 ±1.82</td>
</tr>
<tr>
<td></td>
<td>Median Effective Dose 320mg</td>
<td>12.23 ±1.16</td>
<td>10.24 ±1.92</td>
<td>13.12 ±1.12</td>
<td>11.90 ±1.90</td>
</tr>
<tr>
<td></td>
<td>Effective dose 160mg</td>
<td>10.65 ±1.85</td>
<td>10.35 ±1.56</td>
<td>12.36 ±1.51</td>
<td>11.22 ±1.77</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>11.53 ±0.50</td>
<td>11.53 ±0.50</td>
<td>11.53 ±0.50</td>
<td>11.53 ±0.44</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>10.83 ±0.50</td>
<td>10.97 ±0.50</td>
<td>12.58 ±0.50</td>
<td>11.57 ±0.50</td>
</tr>
<tr>
<td>Dose</td>
<td>7 Days (%)</td>
<td>15 Days (%)</td>
<td>35 Days (%)</td>
<td>Dose (Overall Mean)</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>------------</td>
<td>-------------</td>
<td>-------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>Maximum Dose 640mg</td>
<td>26.33 ±2.08</td>
<td>31.60 ±2.88</td>
<td>40.60 ±3.05</td>
<td>33.85 ±6.47</td>
<td></td>
</tr>
<tr>
<td>Median Effective Dose 320mg</td>
<td>35.00 ±5.29</td>
<td>26.80 ±4.76</td>
<td>39.17 ±5.42</td>
<td>33.86 ±1.90</td>
<td></td>
</tr>
<tr>
<td>Effective dose 160mg</td>
<td>30.50 ±2.89</td>
<td>30.00 ±5.42</td>
<td>34.80 ±3.96</td>
<td>32.00 ±1.77</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>32.00 ±1.00</td>
<td>31.33 ±0.58</td>
<td>32.00 ±1.00</td>
<td>31.78 ±0.83</td>
<td></td>
</tr>
<tr>
<td>Time (Overall Mean)</td>
<td>30.92 ±4.17a</td>
<td>29.76 ±4.19b</td>
<td>37.26 ±4.94b</td>
<td>32.98 ±5.61</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of 7 rats for each treatment
Means in column with the same superscribe are not significantly different
*: Significant at level P< 0.05; **: Significant at level P< 0.01; N.S.: Not significant
4.5. Pharmaceutical Aspects

*Solenostemma argel* leaves are used in folklore medicine in four forms: decoctions; Leaves powder swallowed using water, or macerated in a drinkable liquid; externally as a local poultice; and inhaling its smoke, after ignition, as in measles treatment. In order to simulate the most common folkloric uses of *Solenostemma argel*, i.e. orally as solid (powder) or liquid (extract), the powdered leaves, powdered dried extracts, and dried alkaloids extracts were used after being incorporated into tablet dosage forms. Tablets were selected as a dosage form since they represent the preferred drug delivery system because of their several advantages including:

a) They are unit dosage forms, and therefore, they deliver the dose accurately;

b) The ease of their preparation, evaluation, control, storage, and dispensing;

c) Their improved dosage accuracy and precision, a more reliable therapy, providing convenience of use and compliance of patients, and ease of product identification;

d) Their inherently greater chemical stability, compared to other dosage forms, and have the longest shelf life;

e) The prevention or reduction of incompatibilities between constituents is almost

the best in tablets compared to other dosage forms;

f) The cost of fabricating the entire compressed tablet, particularly if a simple tablet granulation process is employed, is lesser than that of filling capsules and other operations; in addition to that modern high-speed tablet compression machines are much faster than the full-automatic capsule filling machines;

g) They are easier to carry, compact and easy to store, and lightest in weight per dosage unit, and hence the cheapest to package and strip;

h) They are the preferred dosage form by pharmaceutical manufacturers, because they best lend themselves to rapid mass production and are the least expensive of the solid dosage forms; and

i) They can readily be designed to provide controlled drug dissolution so that the rate of drug release, and not the inherent absorbability of the drug, determines the rate of absorption.

As expected, the bioavailability of the drug substances would affect greatly their bioequivalence. The rate and extent to which a drug in a dosage form becomes available for biological absorption or utilization may depend to a great extent upon the materials utilized in the formulation, the method of manufacture, or the order of addition of excipients (e.g. before or after granulation). Thus the same drug when formulated in different dosage forms may be found to possess different physicochemical properties and variable bioavailability characteristics, and thus exhibits different levels of clinical effectiveness. For example, two seemingly identical or equivalent products, of the same drug in the same dosage strength and in the same dosage form type, but differing in particle size of the drug or excipients, and formulation materials or method of manufacture, may vary widely in bioavailability and thus in their clinical effectiveness.

The following terms were used to define the type or level of equivalency between drug products (Food and Drug Administration, 1979):

a) Pharmaceutical equivalency is used for those products that contain the same amount of the same therapeutically active ingredient(s) in the same dosage form;
b) Biological equivalency is used for those pharmaceutical equivalents which when administered to the same individuals in the same dosage regimen, will result in comparable bioavailabilities; and

c) Therapeutic equivalency is used for those pharmaceutical equivalents which when administered to the same individuals in the same dosage regimen, will provide essentially the same therapeutic effect.

Therefore, the effects of additives (including diluents, binders, disintegrants, lubricants or glidants) on the physicochemical properties must be carefully studied, in addition to the method of preparation, the order of excipients addition, and the particle size of drug substances and additives. These variables in tablet processing would affect greatly the disintegration, dissolution, and consequently affect the diffusion, bioavailability, absorption and therapeutic effectiveness.

Although pharmaceutically equivalent products contain the same amount of the same therapeutically active ingredients in the same dosage form, they may have a different biological equivalence and would not give the same therapeutic equivalence.

4.5.1. Microbial Contamination of *Solenostemma argel* Leaves Preparations:

Determination of *Escherichia coli* and *Salmonella* species

Examined *Solenostemma argel* leaves powders, extracts and alkaloids passed the tests for the absence of *Escherichia coli* and *Salmonella* species since no such colonies were detected, and the confirmatory biochemical reactions were negative (BP, 1980; WHO, 2000). Total bacterial count did not exceed 200cfu/g in the different tablets prepared and stored (Tables)

4.5.2. Alkaloids Content of *Solenostemma argel* Leaves Preparations

*Solenostemma argel* leaves preparations, including powders, extracts and alkaloids were assessed for alkaloidal contents. The alkaloids contents of *S. argel* leaves preparations ranged from 2 to 5% of the total weight of *S. argel* leaves. The amounts of the alkaloids needed for formulation were lesser than those for the extracts or the leaves powders. The two extracts of *S. argel* leaves used were the dried chloroform-extracted fraction (2-5%, brown in colour) and the alkaline aqueous layer evaporated to dryness (20-30%), and extracted with methanol. This dried methanol extract was yellowish-brown in colour.

The alkaloids contents of *S. argel* leaves powders and extracts as well as all types of *S. argel* leaves preparations and tablets of the different formulae were determined by using the standard calibration curve of *Solenostemma argel* alkaloids (Table 11, Figure 29).

4.5.2.1. The Calibration Curve of *Solenostemma argel* Alkaloids

According to Beer, Bouguer and Lambert law, concerned with the light absorbance and solution concentrations, the fraction of radiation absorbed by a solution of an absorbing analytical agent can be quantitatively related to its concentration; i.e. absorbance is directly proportional to the concentration.

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Absorbance Readings, at UV 294.5 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>0.343 0.417 0.615 0.545 0.550 0.531</td>
</tr>
<tr>
<td>500</td>
<td>0.175 0.172 0.312 0.343 0.370 0.330</td>
</tr>
</tbody>
</table>

Table 11: Absorbance Readings of Different Concentrations of *Solenostemma argel* Alkaloids at 294.5nm
The calibration curve of a reference standard was generated (Figure 29; Table 11); the value of the determination coefficient ($R^2$) between different concentrations, and the equation of the best fit line of high value ($R^2=0.9978$) were as shown in Figure 29.

Accordingly, in the range of 10 µg/ml to 1000 µg/ml, concentrations vary linearly with the absorbance, with a high correlation coefficient of ($R^2 = 0.9978$); with an intercept near to zero equation of a straight line as follows:

$$y = ax + b$$

Where ($y$) stands for absorbance and ($x$) for *S. argel* alkaloids concentration in the solution (Figure 29). Value of $R^2$, determination coefficient, indicates the precision of the analytical method. The calibration curve of the reference standard *S. argel* constituents was diluted to one liter of water and stirred for 45 minutes. Each data point is the average of six determinations. Error bars are not shown. This analytical method is considered reproducible when dealing with analysis of different formulae. They gave the same results when comparison was held between them.

The absorbance of each diluted solution was measured at UV $\lambda$ 294.5nm. Accordingly, the other different concentrations were calculated from this standard calibration curve. Compared with the absorbance of a pure material, without additives, having known concentrations and the percentage of dissolution calculated at different times to know the rate of dissolution (at 15, 30, and 45 minutes) as follow:

**Percentage of the dissolved constituents =**

$$\text{Absorbance of sample/Absorbance of standard}\times100;$$

Not less than 75% of the labeled amount of *S. argel* alkaloids was dissolved in one liter stirred for 45 minutes. The same procedure was done with the extract, but there was no reflux, and only dissolving the powder in sufficient methanol and water (4:1) mixture. To know the mean alkaloids contents of the *S. argel* tablets of the different formulae, the same procedure was followed either for the total alkaloids contents or alkaloids released at the specified time intervals in the dissolution tests.

<table>
<thead>
<tr>
<th></th>
<th>0.047</th>
<th>0.0777</th>
<th>0.083</th>
<th>0.095</th>
<th>0.111</th>
<th>0.058</th>
<th>0.0786</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.027</td>
<td>0.0316</td>
<td>0.057</td>
<td>0.05</td>
<td>0.034</td>
<td>0.027</td>
<td>0.0378</td>
</tr>
<tr>
<td>25</td>
<td>0.012</td>
<td>0.0167</td>
<td>0.027</td>
<td>0.03</td>
<td>0.028</td>
<td>0.017</td>
<td>0.0218</td>
</tr>
<tr>
<td>10</td>
<td>0.0078</td>
<td>0.012</td>
<td>0.013</td>
<td>0.013</td>
<td>0.012</td>
<td>0.008</td>
<td>0.0103</td>
</tr>
</tbody>
</table>

*: Mean of six determinations.
Figure 29: Standard Calibration Curve* of Solenostemma argel Alkaloids

*: The (x) axis for concentrations of S. argel Alkaloids, in micrograms
The y-axis for Readings under UV at λ 294.5nm

4.5.3. Solenostemma argel Leaves Pharmaceutical Preparations

4.5.3.1. Role of Particle Size

Although the use of powders as a dosage form has declined recently, they are the starting point of the manufacture of other dosage forms, particularly tablets. Powders have become increasingly important, and along with this has come the recognition of the importance of particle size (Felmeister, 1970). Particle size can have a significant effect on the physical and therapeutic properties of drug (Lees 1963). As an aspect of material properties which is common to most compacted products is the effect of particle size on tablet properties. Research on sieved fractions has been done by many workers, and it is generally accepted that the strength of granular materials increases with decreasing particle size or to an effect of particle size during the strength measurement (Benbon, 1979).

Particle size effects may account for differing mechanical properties of tablets prepared from various sized materials above 60 mesh (Bassam et al., 1988). The formulation of a disintegrating tablet is a complex process depending on many interacting variables affecting the in vitro drug dissolution, and include the characteristics of the drug itself particularly its particle size (Tuladhar–Ularly et al., 1983). Particle size was found to have influence on wetting of the powder mixture in the first stage of the granulation process (Jaiyeoba and Spring, 1980). The amount of liquid required to form granules from different granulates of lactose was inversely related to the mean particle size. Fine particles of...
hydrophiles result in stronger larger granules being formed, and coarser hydrophobics result in the production of weaker granules (Jaiyeaba and Spring, 1980).

Particle size also has an important influence on the flow properties of powders. The frictional and cohesion resistance to the flow of powders become more significant as the particle size is reduced, because they are too cohesive to flow through orifices; such behavior is a result of the van der Walls forces that often predominate over gravitational forces at such size of particles (Augsberger, 1974).

4.5.3.2. Preparation of Solenostemma argel Leaves Powder

The particle size and particle surface area of powdered substances play an important role in the physico-chemical and pharmaceutical properties of drug substances, and therefore particle size reduction and analysis of powdered Solenostemma argel leaves were carried out to obtain powders of specific size and surface area.

4.5.3.2.1. Size Reduction of Solenostemma argel Leaves

Solenostemma argel leaves were powdered using a hammer mill which is quite popular in pharmaceutical industry for particle size reduction. It was used to mill the dried S. argel leaves to a particle size and distribution that would flow freely. It is compact with high capacity and size reduction of (60, 80, and less than 80 mesh screens) (Table 12).

In addition, small particles would pass through the screen almost as fast as they were formed, and thus a hammer mill tends to yield a relatively narrow size distribution. It is also simple to operate, easy to clean and may be operated as a closed system to reduce dust and explosion hazards.
4.5.3.2.2. Sieve Analysis and Separation of *Solenostemma argel* Leaves Powder

This method is applicable to particles in the size range of 5 to 2000 microns. It could be used both as a method of particle size analysis and for separating a sample of powder into various sizes ranges (Allen, 1975; Parrot, 1986).

Many workers have carried out research on sieved fraction. Two size fractions of paracetamol were separated and the drug particle size had significant effects on the tensile strength of the paracetamol tablets produced (Sanderson *et al*., 1984).

The process of size reduction was followed by size classification of the ground *S. argel* material. Although the comminuting machinery may be adjusted to produce materials within a specified range, it was often necessary to subject the ground material to some procedure that would screen out very fine particles or over-size materials.

For determination of particle size separation and distribution of the ground *S. argel* leaves, 2.6kg of the powdered leaves were placed on the upper sieve of a series of successively smaller sieves, and the sieve set was then placed on the mechanical sieve shaker which was operated for 15 minutes. The portion of the powders retained on each sieve was weighed and the results were shown in Table 12. From the data obtained, the particle size analysis and distribution were determined (Lloyd, 1979; Bassam *et al*., 1988) (Table 12).

For separation of the powders into different sieve fractions, the above mentioned process was carried out several times until sufficient amount of each fraction was obtained and stored in well-closed plastic containers for further utilization during the course of this work. The amount of powder that passed through the 160-micron mesh was found to be 63% (Table 12), and this is in agreement with the findings on senna leaves reported by Mahmoud *et al*. (1996). This fraction of powdered *Solenostemma argel* leaves was mainly used in the formulation of optimized *S. argel* tablets.

**Table 12: Sieve Analysis and Separation of *S. argel* Leaves Powder**

<table>
<thead>
<tr>
<th>Mesh</th>
<th>Particle Size (microns)</th>
<th>Powder Weight (g) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 80</td>
<td>≤ 160</td>
<td>1640, (63%)</td>
</tr>
<tr>
<td>80</td>
<td>250-160</td>
<td>420, (16%)</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>&gt; 250</td>
<td>540, (21%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2600, (100%)</td>
</tr>
</tbody>
</table>
4.5.4. Preparation of *Solenostemma argel* Leaves by Wet Granulation Method

Granulation is the generic name used for particle size enlargement and is seen as an answer to different flow problems to ensure better results when mixing different powders to reduce dust hazard problems, to insure uniform fill in a tablet machine, and to produce tablets conforming to the requirements and monograph specifications.

The principal granulation methods available for production of tablets are:

a) Direct compression method that involves the direct compaction of the powdered material into tablets, without major modification of the physical characteristics of the drug substance or excipients; it is suitable for thermolabile or moisture-sensitive drug substances, but depends on the use of rather expensive additives.

b) Double compression method, as a dry granulation process that consists of two compression cycles, the first is intended to prepare slugs, which are crushed, and the resultant particles are further re-sized, lubricated, and prepared for the second compression process into the required tablets. It is suitable for moisture-sensitive or thermolabile drugs since it involves neither moisture nor heat in the preparation of the granules or the final tablets.

c) Wet granulation method as the most widely used method of tablet preparation or production, involves wetting of the mixed powders with a suitable binding agent and screening the moist mass, drying, and sizing of the dried granules. It has the advantages of improving the physical properties of the drug substances, such as flowability, compressibility, wettability, and solubility, but it is not suitable for thermolabile or moisture-sensitive drug substances. Its chief disadvantages are the number of separate steps involved as well as the time and labour necessary to carry out the procedure especially on large scale production.

d) Moist granulation method is a newly-developed method which closely resembles the wet granulation method in that the powdered material is moistened, but using only an aqueous solution. Drying of the granules is carried out by using sodium carboxymethylcellulose as a water-withdrawing agent, which makes this granulation method suitable for thermolabile drugs, but inappropriate for moisture-sensitive drugs.

In the present work aimed at production of granules of *Solenostemma argel* leaves preparations, the wet granulation method was used because of its popularity in local pharmaceutical factories, and the several reported advantages, in addition to the increased probability that the granulation will meet all the physico-chemical requirements for the compression of good tablets.

4.5.4.1. Preparation of Granules for *Solenostemma argel* Leaves Tablets

Different granule batches of 250g of mixtures of *S. argel* leaves powders, of different sieve fractions, or powdered dried aqueous extracts, or dried alkaloids extracts, were prepared, evaluated and used in the preparation of different batches of *Solenostemma argel* tablets.

A very important step in the preparation of good granules and tablets compliant to the quality requirements and specifications is the successful mixing, which is defined as a process that tends to result in randomization of dissimilar particles. This is acknowledged to be one of the more difficult unit operations, because unlike situations with liquids, perfect homogeneity is practically unattainable. All that is possible is to
achieve a maximum degree of randomness in the arrangement of the individual components of the mix (Marsh, 1979).

The effects of particle size and shape on the mixing of powder layers flowing down a chute were assessed, and it was found that at constant particle size of the upper layer the mixing rate was increased (Ridgeway and Ruff, 1971). It is generally accepted that intense shear mixing is needed in addition to mass movement of the bulk mixing, and recently there have been significant advances in equipment. However, with all mixers, it is necessary that acceptable degree of homogeneity must be reached (Heisery and Cook, 1974).

Size classification may be achieved by centrifugal type of motion. Sifting, sieving or screening are terms used interchangeably to describe a process of passing powdered material through openings in a sieve or screen to separate coarser from fine particles (Kaning, 1965; Allen, 1975).

Maize starch and lactose, in different ratios, and with or without microcrystalline cellulose, were used to prepare different granules, and in addition to the other excipients, they were compressed into *S. argel* tablets of different formulae. The preparation of the granules of the different formulae involved the preparation and use of various binders including different types of starches.

The wet masses of the different formulations were forced through a No. 12-mesh screen manually in the case of small batches, or by using Erweka granulating machine with large batches (Cherhan, 1988). The prepared granules were dried in a hot air oven at 50°C overnight, and the dried granules were sized by passing through a No. 14-mesh screen, to facilitate the granules flow and compressibility (Parrot and Saski, 1971).

For wet granulation with the moist binder, the method of preparation was to add the known required amount of binder as an aqueous solution near to the beginning of the granulation process; after this pure water was sprayed in monitoring the torque till the required torque level was reached.

When granulation was complete, mixer granulator with a sensor was developed to determine when the end point was reached. The best measure of the end point was the torque needed to turn the mixer shaft. Experiments had shown that at the same torque, not only did the granules had the same size distribution, but also the same flow properties, the same behavior on compaction and the same disintegration time of the resultant tablet (Cliff, 1988).

In their study of the pressure-dependence of several physical properties of the tablets prepared with similar composition using different granulation techniques, Cooper and Rees (1972) concluded that wet granulated tablets were
most resistant to fracture and attrition and possessed longer disintegration times than direct compression or dry granulation formulations.

4.5.5. Preparation of Solenostemma argel Tablets of the Different Formulae

4.5.5.1. The Codes of Solenostemma argel Tablets of the Different Formulae

The Formulae Codes of Solenostemma argel leaves tablets were as follows:

**Formulas (A)** included the use of diluent (70% lactose and 30% maize starch) together with 5% MCC before and after granulation, in addition to using different types of binding agents. 0.5% Magnesium stearate and 1.5% talc powder were included as lubricant and glidant agents. The *S. argel* leaves tablets prepared were either 200mg, using a 9mm-diameter punches, or 500mg using a 12-mm diameter dies.

**B formulae (B)** were the same as A formulae in all aspects, except the composition of the diluent used, which included 50% lactose and 50% maize starch.

**C formulae (C)** were prepared without a diluent.

For Extracts (A) and (B) were the same, but (C) was without a binding agent. The concentration of the *S. argel* water extract in each tablet was 175mg, and using 9-mm-diameter dies. For Alkaloid (A) the same, without 5% MCC, and alkaloid in each tablet was 80mg, using 9mm diameter dies. Because the physicochemical properties of the prepared *S. argel* tablets were affected by the different types and concentrations of the additives, their initial letters were included in the codes of the different formulae used.

The codes for the chosen bases were as follows:

1. SPC, for 4% PVP, Formula C;
2. SSC, for 8% Sorghum, 2%PVP, Formula C;
3. SMC, for 8% Maize Starch, 2% PVP, Formula C;
4. SZC, for 8% Zora Starch, 2% PVP, Formula C;
5. SGC, for 6% Guar Starch, 2% PVP, Formula C;
6. SEB, for *S. argel* Water Extract, with 2%PVP, Formula B;
7. SAA, for *S. argel* Alkaloids, with 2%PVP, Formula A, Without MCC.

Following granules sizing, the granules were mixed with the other formulation ingredients, such disintegrants, lubricants and glidants, and compressed into tablets. Using a single-punch tableting machine (Korch, Erweka), tablets were made by compressing unit volumes of particles (BP, 1993); plain flat-face or convex punches were used. The tablets produced by the convex punches were more friable and produced more powder; most formulae without diluents did not pass the friability test on stability testing (Tables 33-45). This might be due to the uneven distribution of the applied compression forces on an uneven surface.

Twenty five formulations were first prepared for optimization of the physicochemical properties of Solenostemma argel dried extracts tablets by adjusting the types and quantities of the different additives. The dried aqueous extract by itself, without a binding agent, had resulted in tablets with a very long disintegration time (51 minutes) and a very low dissolution rate.

For example, the addition of external 2% guar, i.e. post-granulation, reduced the disintegration time from 51 minutes to 17 minutes only, while internal 2% guar, i.e. before granulation, increased the disintegration time to 62 minutes. The addition of 2% guar solution before granulation reduced the disintegration time to 48 minutes. Therefore, the state of guar, in solid or solution, and the time of its addition, before or post granulation, had affected the disintegration time of *S. argel* extract tablets (Table 14).
The addition of a binding agent, e.g. polyvinylpyrrolidone, PVP, starches, and guar and the percentage of the binding agent added had an influence on the physicochemical properties of the tablets (Table 14).

Likewise, the addition of a disintegrating agent, e.g. microcrystalline cellulose, MCC (external and internal), 2% sugar or 2% starches as external agents, were studied to optimize the physicochemical properties of the S. argel extracts tablets (Table 14).

### 4.5.5.2. *Solenostemma argel* Leaves Tablets of the Different Formulae

Different formulae of *Solenostemma argel* tablets were attempted by compressing unit volumes equivalent to 500mg of the granulation or leaves powders in direct compression method, and 250mg of the granulation in the case of both the dried extracts or alkaloids of *S. argel*. The compression was achieved by 12mm or 9mm diameter, stainless steel dies fitted with normal flat-face punches in a single-punch tableting machine (Korch, Erweka) (Armstrong, 1988).

When granulation was first prepared through mesh-10, large granules were produced which were firstly reduced to small granules by passing through mesh-14 (≤1400 micron) to facilitate the continuous movement of the powder, and an adequate filling of the die, to give an even and accurate weight for each tablet, minimizing the weight variations in the batch.

### 4.5.5.3. *Solenostemma argel* Leaves Extracts Tablets of the Different Formulae

The formula B was used with equal amounts of lactose and maize starch as diluents. The tablet weight was 250mg; the dried water extract of *S. argel* leaves used was equal to 175mg; the dried water extract of the different decoctions was found to be 20 to 30% of the whole leaves weight. Polyvinylpyrrolidone, PVP (2-4%), was used as a binding agent in *S. argel* tablets. The standard physiochemical properties were as shown in the following Table 13.

#### Table 13: Physicochemical Properties of *Solenostemma argel* Leaves Tablets

<table>
<thead>
<tr>
<th>Character</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tablet Diameter</td>
<td>9.0 ± 0.5mm</td>
</tr>
<tr>
<td>Tablet Thickness</td>
<td>3.0 ± 0.5mm</td>
</tr>
<tr>
<td>Tablet Hardness</td>
<td>6.3kg/inch²</td>
</tr>
<tr>
<td>Mean Tablet Weight</td>
<td>250 ± 50mg</td>
</tr>
<tr>
<td>Disintegration Time</td>
<td>12 minutes</td>
</tr>
<tr>
<td>Friability</td>
<td>0.019%</td>
</tr>
</tbody>
</table>

Dissolution rate at 37°C ± 0.5 for 45 minutes readings under UV light, at λ 294.5 nm = 250 ± 50mg, using codes for the different tablets formulae, according to the different additives; code SEA. *Solenostemma argel* leaves extract tablets were prepared according to formulae with diluents 70% lactose and 30% starch, 5% microcrystalline cellulose, MCC, added as internal or external disintegrant; and 1.5% talc powder and 0.5% magnesium stearate as lubricant and glidant system (Table 14).

SEA is the formula (A) with 70% lactose and 30% starch without binding agent. SEB is this formula (B) with 50% of each of the diluents. In the formula B, microcrystalline cellulose, MCC, was used internally and externally as a disintegrating agent, with 1.5% talc powder as well as 0.5% magnesium stearate as glidant and lubricating agents.

### 4.5.6. Optimization of the Prepared *Solenostemma argel* Tablets

Optimization Techniques in Pharmaceutical Formulation and Processing
The word “optimize” is defined as follows: To make a product as perfect, effective or functional as possible. The last phrase (as possible) leads are immediately into the area of decisions making since in Optimization one might ask: ‘perfect’ by whose definition with respect to what characteristics and under what conditions. The term “Optimization” is used often in pharmacy with respect to formulation and processing.

4.5.6.1. Types of Optimization Problems: the Constrained and Unconstrained.

Constrained Optimization are those restrictions placed upon the system due to physical limitation or perhaps due to simple practicality, or economic considerations, whereas unconstrained optimization is almost non-existent in pharmaceutical systems. There are always restrictions which the formulator wishes to place or must place on his system and in pharmaceuticals. Many of these restrictions are competing such as hardness and disintegration of tablets.

It is sometimes necessary to sacrifice one characteristic for another. Thus the primary objective may not be optimizing absolutely, but to compromise effectively, and therefore produce the best formulation under a given set of restrictions (Schwartz, 1979).

A lot of trials were required for the complete experimental design of work for optimization based on: a) The particle size of *S. argel* leaves powders; b) Type and quantity of binder, (Doelker et al., 1977); c) Type and quantity of diluents, (Stanelywood, et al., 1979); d) Disintegrating agent type and time of its addition, before or after granulation, (Esezobo et al., (1976); and e) Lubricant and glidant agents (MCC, Talc, and magnesium stearate). These included: i) the effects of particle size of *S. argel* leaves powders on tablet hardness, disintegration time, and dissolution rate; ii) the effects of the type and quantity of the binding agents on these physico-chemical properties (Abdelaziem et al., 1987); iii) the effects of MCC and its addition before and after granulation on these physico-chemical properties; in addition to iv) the effects of different types of starch on the powders passing mesh 80, \( \leq 160 \mu \); and v) the effects of aging on some of these physicochemical properties of *Solenostemma argel* tablets.

4.5.6.2. Role of Processing Conditions

The processes used in tablet making are of equal importance as the materials involved.

4.5.6.2.1. Effect of Mixing

Successful mixing, defined as a process that tends to result in randomization of dissimilar particle, involves randomization of dissimilar particles as a criterion of successful mixing. It is acknowledged to be one of the more difficult unit operations, because unlike situation with liquids, perfect homogeneity is practically difficult to attain. All that is possible is to achieve a maximum degree of randomness in the arrangement of the individual components of the mix (Marsh, 1979).
The effects of particle size and shape on the mixing of powder layers flowing down a chute were assessed, and it was found that at constant particle size of the upper layer increased the mixing rate (Ridgway and Ruff, 1971). It is generally accepted that intense shear mixing is needed in addition to mass movement of the bulk mixing.

Recently, there have been significant advances in equipment; however with all mixers, it is necessary that acceptable degree of homogeneity must be reached (Heisery and Cook, 1974). Size classification may be of greatly centrifugal type. Sifting, sieving and screening are terms used interchangeably to describe a process of passing powered material through openings in a sieve or screen to separate coarser from fine particles (Kaning 1965; Allen, 1975).

4.5.6.2.2. Effect of Size Reduction and Sieve Analysis

This method is applicable for particles in the size range of 5 to 2000 microns. It could be used both as a method of particle size analysis and for separating a sample of powder into various sizes ranges (Allen, 1975; Parrot, 1986). Many workers have done research on sieved fractions. Two-size fractions of paracetamol were separated, and the effects of drug particle size were studied. It was found that the drug particle size had significant effect on the tensile strength of the paracetamol tablets produced (Sanderson et al., 1984). Sieving is widely used in sizing granules, a step involved in tablet manufacture, and for measuring particle size distribution, because it is simple, inexpensive, rapid, and reproducible with little variation between operators.

The procedure involves the mechanical shaking of a sample through a series of successively smaller sieves, and the weighing of these portions of powder samples retained on each sieve. The type of motion influences sieving; vibratory motion is the most efficient. Time is also an important factor in sieving.
which in turn is influenced by load of powder or thickness. The time required to sieve a material is roughly proportional to the load placed on the sieve (Parrot, 1986).

The effect of the powder particle size, without making analysis or sorting of the particle size, and using different sizes powder mixtures to produce tablets showed mottling, non-homogenous colour, uneven distribution, harsh, tough, and rough surface to touch (Table 14).

After analysis of particle size, the use of 160 micron particle size, passing mesh number 80, to produce tablets having a very long disintegration time, which might be attributed to cohesive forces, mainly due to its van der Waals. The fine powder prevented the penetration of the solvent to the tablet content. It forms a clump moving together, without passing the mesh of the disintegration apparatus. This was shown with different used binding agents (Table 14).

As well, the fine powders would affect greatly the mixing with the binder itself, so there is a need of adding internal MCC before granulation to enhance the contact of granulating agents’ solutions, and its even distribution through the powder before granulation. Also the dissolution rate would be enhanced due to this contact with particle size, and the particle shape would affect greatly the mixing of the leaves powders and additives properties like binding solution.

To have a smooth tablet surface, with even colour distribution, without mottling, particle size analysis and separation must be carried out for selection of the suitable size as an important step in the production of S. argel leaves tablets.

Size reduction is an important preliminary stage in the preparation of compressed tablets, and the particle sizes of different excipients have a major role in the properties of the finished tablets. Control of particle size is an important aspect of obtaining proper powder flow properties. The main object in most size reduction operations is to obtain a product smaller than a certain specified size. Size reduction increases the surface area per unit weight, which is known as a specific surface, and the therapeutic efficiency of the drug is affected on mixing and blending if the ingredients are approximately of the same size (Procedfest, 1988). Lubricants used in compressed tablets function by virtue of their ability to coat the surface of granulation or powder, and a fine particle size is essential for lubricants to function properly (Danish and Parrott, 1971).

**Table 14: Effects of Different Particle Sizes and Different Additives on the Physicochemical Properties of Solenostemma argel Leaves Tablets, Formula B**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th>Disinteg</th>
<th>Dissol</th>
</tr>
</thead>
</table>

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4.5.6.2.3. Granulation

Solid, spherical, free-flowing granules, with low friability, can be prepared by spherization of extruded pellets of wet granulate using suitable process or equipment with careful controlling of degree of wetting and solvent content (Reynolds, 1970). The smaller the tablet, the finer the granulation to enable more uniform filling of the die cavity, while large granules give an irregular fill to a comparatively small die cavity. The size of the granulated material and the disintegration time in the case of compressed tablets of sodium bicarbonate, lactose and magnesium trisilicate, whereas for sulphasiazole, the particle size distribution did not appear to influence hardness or disintegration (Levine and Schwartz, 1985). For Solenostemma argel leaves tablets, the particle size played a great role in the magnitudes of the physicochemical properties. The slight swelling that occurred was due to the rapid hydration step and the slower sorption of additional water step (Lowenthal and Wood, 1973).

Formulae also showed the effects of increasing the binder concentration to 20%, with or without 2% starch as disintegration agent, and after the addition of 2% PVP. The effects of different concentrations of PVP as the binder on the A formulae, and the reduction of PVP concentration would lead to decrease in the tablet hardness or resistance to break and inherent disintegration time property. In A formulae, 10% concentration of each binder, except 1% guar, with the addition of 2% starch would optimize the physicochemical properties of S. argel leaves tablets to a great extent, especially the disintegration time, although the majority of them had an increase in the tablet hardness, but 0.1% guar as a binder resulted in more fragile and friable tablets. Furthermore, there was more chance for microbial growth, or instability might be unpredictable when the product was subjected to moisture. This might be also applicable to gum as a binder which had been restricted by BPC (1973).

4.5.6.2.4. Powder Flow Properties

Pharmaceutical powders may be broadly classified as free-flowing or cohesive, i.e. non-free-flowing. Most flow properties are significantly affected by charges on particles, particle size, density, shape, electrostatic charge, and adsorbed moisture which may be left behind from processing or formulation. As a result, a free-flowing drug candidate may become cohesive during development, thus necessitating an entirely new formulation strategy. Reformulation powder flow investigations should quantitatively assess the pharmaceutical consequences of each process improvement and provide
direction for the formulation development project team. This direction may consist of a formulation recommendation such as granulation or densification via slugging, the need for special hanger feed equipment in flow brought about by formulation. This subject becomes paramount when attempting to develop a commercial solid dosage form containing a large percentage of cohesive materials.

Free-flowing powders may be recognized by a simple flow rate apparatus consisting of a grounded metal tube from which the drug flows throughout onto an electronic balance, which is connected to a strip chart recorder. Several flow rate (g/sec) determinations of each variety of orifice size should be made. The greater the standard deviation between multiple flow rate measurements, the greater is the weight variation in products produced from these powders.

The die-fill process is based on continuous and uniform flow of granulation from the hopper though the feed frame. When the granulation does not flow readily, it tends to move sporadically through the feed frame and that some dies are incompletely filled properly when the machine speed is in excess of the granulations flow capabilities. With poor flow, the addition of the glidant such as talcum or colloidal silica, or increase in the amount already present, may be helpful. Also available are induced die feeds, which mechanically force the granulation down into the die cavities as they pass beneath the feed frame.

Poor flow through the feed frame is usually a sign that the granulation is not flowing properly out of the hopper. As particulate solids move under the force of gravity through progressively smaller openings, they are subjected to uneven pressures from the mass above and along-side. Depending on the geometry of the hopper, the situation may give rise to one or another of two causes for poor flow: arching or bridging, and rate-holding. When poor hopper flow occurs, it may be controllable with vibrators attached to the hopper sides to enhance the granulation flow.

Devices designed to improve poor flow characters of materials often introduce another problem, however. Since most tablet granulations consist of materials with a range of particles sizes, the vibration or mixing action of the flow-promoting devices may induce segregation and stratification of the particles, with the larger particles accumulating downward. Not only can the resulting classification of particle sizes cause appreciable changes in tablet weight but also weight variation as distributed between the larger and smaller particles.

Poor particulate flow may be caused not only by granulation formulation, but by poor design of granulation hopper, which can be exaggerated by dents that effectively cut off the flow. Poor weight variation can also be caused by surges of excessive flow. Direct compression granulations fed through typical wet granulation hoppers and feed frames are prone to this type of flow. Often restricting the flow out of the hopper connects the problem.

Recently, a patent was issued for a new feed frame design that accommodated excessive flow from the hopper without compromising uniform weight variation. Poor mixing is another cause of this poor powder flow; sometimes, the lubricants and glidants are not thoroughly distributed, the flow of the particles is then impaired, and the granules do not move efficiently into the dies. There is a tendency to minimize the mixing time during lubricant addition to prevent or reduce granule friability; however, inadequate mixing at this stage can result in unsatisfactory granulation flow.

4.5.6.3. Formulation Ingredients of the Prepared *Solenostemma argel* Tablets

Formulation factors affecting the physicochemical properties of *Solenostemma argel* leaves or extract tablets were studied, particularly the effects of different types and
concentrations of binders, as well as the different types of disintegrants and the time of their addition (pre- or post-granulation).

4.5.6.3.1. Effects of Binding Agents on the Physicochemical Properties of *Solenostemma argel* Leaves Tablets, of the Different Formulae

The effects of binding agent, type and concentration, was investigated. Both type and concentration of binders would affect greatly the physicochemical properties of *S. argel* extract tablets formula B, SED (Tables 15-18).

Investigations were carried out to determine the effects of the addition of starch and polyvinylpyrrolidone, PVP, on the dissolution rate of *S. argel* tablets, formula C, with 5% MCC added before and after granulation to improve the physicochemical properties of the drug particle size > 250micron, 160-250 micron and ≤ 160micron (Tables 14-18).

**Table 15: Effects of Different Additives on the Physicochemical Properties of *Solenostemma argel* Leaves Tablets, Formula A, with a Diluent**

<table>
<thead>
<tr>
<th>Binder</th>
<th>Hardness (kg/inch²)</th>
<th>Hardness (kg/inch²) (+2%Starch)</th>
<th>Friability (%)</th>
<th>Disintegration Time (minuets)</th>
<th>Dissolution Rate (UV Readings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%Gum</td>
<td>8.0</td>
<td>9.12</td>
<td>0.0191%</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>10%Starch</td>
<td>6.1</td>
<td>8.2</td>
<td>0.4%</td>
<td>29</td>
<td>26</td>
</tr>
<tr>
<td>5%PVP</td>
<td>6.8</td>
<td>7.5</td>
<td>0.001%</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>0.1%Guar</td>
<td>3.45</td>
<td>3.3</td>
<td>6.9%</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>5%PVP</td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>29</td>
<td>-</td>
</tr>
<tr>
<td>4%PVP</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>18</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 16: Effects of Different Binders on the Physicochemical Properties of *Solenostemma argel* Leaves Tablets, 12 mm-diameter, Formula (B)*

<table>
<thead>
<tr>
<th>Parameter Binder</th>
<th>Average Tablet Weight (mg)</th>
<th>Hardness (kg/inch²)</th>
<th>Diameter (mm)</th>
<th>Thickness (mm)</th>
<th>Disintegration Time (min)</th>
<th>Dissolution Rate (UV Reading)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPB 4%PVP</td>
<td>530 ±17E-02</td>
<td>4.59 ±0.98</td>
<td>12.1 ±0.63E02</td>
<td>4.1 ±0.05</td>
<td>27</td>
<td>0.465 ±1.94E-02</td>
<td>0.04</td>
</tr>
<tr>
<td>SMB 8%Starch + 2%PVP</td>
<td>513 ±16.8E-02</td>
<td>3.06 ±0.829</td>
<td>12.20 ±0.05</td>
<td>4.1 ±0.05</td>
<td>9</td>
<td>0.47 ±1.473E-02</td>
<td>23.0</td>
</tr>
<tr>
<td>SGB 6%Guar + 2%PVP</td>
<td>513 ±16.8E-02</td>
<td>2.30 ±0.954</td>
<td>12.15 ±0.05</td>
<td>4.05 ±0.05</td>
<td>12</td>
<td>0.468 ±1.23E-02</td>
<td>29.4</td>
</tr>
<tr>
<td>SSB 8%Sorghum + 2%PVP</td>
<td>510 ±16.7E-02</td>
<td>4.5 ±0.664</td>
<td>12.05 ±0.05</td>
<td>4.1 ±0.05</td>
<td>18</td>
<td>0.454 ±1.115E-02</td>
<td>19.43</td>
</tr>
<tr>
<td>SZB 8%Zora + 2% PVP</td>
<td>507 ±20.2E-02</td>
<td>3.95 ±0.17</td>
<td>12.05 ±0.05</td>
<td>4.1 ±0.05</td>
<td>20</td>
<td>0.543 ±2.38E-02</td>
<td>18.0</td>
</tr>
</tbody>
</table>

*: Particle size ≤ 160microns

**Table 17: Effects of Different Binders on the Physicochemical Properties of *Solenostemma argel* Extract Tablets, 9mm-Diameter, Formula (B)*
Table 18: Effects of Different Types of Starch on the Physico-chemical Properties of *Solenostemma argel* Leaves Tablets, Formula B

<table>
<thead>
<tr>
<th>Parameter Binder</th>
<th>Average Tablet Weight (mg)</th>
<th>Hardness (kg/inch²)</th>
<th>Diameter (mm)</th>
<th>Thickness (mm)</th>
<th>Disintegration Time (min)</th>
<th>Dissolution Rate (UV Reading)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPC 4%PVP</td>
<td>232</td>
<td>4.9</td>
<td>9.1</td>
<td>3.1</td>
<td>9</td>
<td>0.275 ±0.058</td>
<td>0.19</td>
</tr>
<tr>
<td>SMC 8%Starch 2%PVP</td>
<td>235</td>
<td>3.1</td>
<td>9.0</td>
<td>3.0</td>
<td>11</td>
<td>0.223 ±0.075</td>
<td>0.35</td>
</tr>
<tr>
<td>SGC 6%Guar 2%PVP</td>
<td>235</td>
<td>2.83</td>
<td>9.2</td>
<td>3.0</td>
<td>28</td>
<td>0.293 ±0.082</td>
<td>0.44</td>
</tr>
<tr>
<td>SSC 8%Sorghum PVP</td>
<td>240</td>
<td>4.26</td>
<td>9.1</td>
<td>3.0</td>
<td>24</td>
<td>0.263 ±0.033</td>
<td>0.23</td>
</tr>
<tr>
<td>SZC 8%Zora + 2%PVP</td>
<td>235</td>
<td>4.26</td>
<td>9.1</td>
<td>3.2</td>
<td>21</td>
<td>0.256 ±0.103</td>
<td>1%</td>
</tr>
</tbody>
</table>

*: Particle size < 160 microns

Different formulations were first prepared for optimization of the physicochemical properties of *Solenostemma argel* dried extracts tablets by adjusting the types and quantities of the different additives. The dried aqueous extract by itself, without a binding agent, had resulted in tablets with a very long disintegration time and a very low

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dissolution rate (Tables 14-18). For example, the addition of external 2% guar, i.e. post-granulation, reduced the disintegration time from 51 minutes to 17 minutes only, while internal 2% guar, i.e. before granulation, increased the disintegration time to 62 minutes. The addition of 2% guar solution before granulation reduced the disintegration time to 48 minutes. Therefore, the state of guar, in solid or solution, and the time of its addition, before or post granulation, had affected the disintegration time of S. argel extract tablets (Table 18).

The addition of a binding agent, e.g. polyvinylpyrrolidone, PVP, starches, and guar and the percentage of the binding agent added had an influence on the physicochemical properties of the tablets (Tables 15-18). Likewise, the addition of a disintegrating agent, e.g. microcrystalline cellulose, MCC (external and internal), 2% sugar or 2% starches as external agents, were studied to optimize the physicochemical properties of the S. argel extracts tablets (Table 19).

The Formulations, based on the powdered leaves of Solenostemma argel, included as diluent 70% lactose and 30% starch; 2% PVP as a binder in both the leaves and extract tablets. Furthermore, 1.5% talc and 0.5% magnesium stearate and 10% MCC, 5% external and 5% internal, were added to the powdered leaves tablets (Tables 19-23). These formulae were used to achieve optimization of the physicochemical properties of S. argel tablets, with the addition of different diluents and additives to comply with the monographs of the compressed tablets requirements (USP, 1990; BP, 1990).

So for different formulae, using different binders and without external addition of disintegrant after granulation, ten formulas were studied to determine the effects of different types of binders and their different concentrations on the physicochemical properties of S. argel tablets (Tables 15-23).

The effects of 2% starch as a disintegrating agent, added after the granulation process, on these physicochemical properties; addition of starch resulted in tablets with long disintegration time and low dissolution rate, while guar also had resulted in a low dissolution rate (Tables 24, 25).

Addition of 2% starch affected the tablet hardness and the disintegration time and dissolution rate. Reduction of PVP percentage as a binding agent reduced both the disintegration time and tablet hardness for further optimization of the physicochemical properties of the S. argel leaves tablets. For 4% PVP, tablet hardness was 6.8—4 kg/inch², and the disintegration time was 35—18 minutes.

**From these data, it could be concluded that:**

a) A combination of binding agents was needed and a reduction of binder concentration was a must to optimize A formulas (Table);

b) The use of a disintegrating agent, before and after granulation, in the S. argel leaves tablets, e.g. 2% starch, also optimized A formulae;

c) PVP preparations showed lower friability, followed by gum, starch and guar;

d) PVP preparations had the best dissolution rate properties of S. argel leaves tablets; PVP was followed by gum, guar, and starch had the least rate;

e) A formulae that included S. argel leaves powders without particle size analysis or separation resulted in tablets with mottling of the color, and had coarse surfaces which were tough to touch because their particles were not equal.

So C formulae were adopted as a base to other formulas to investigate the effects of different particle sizes powders and additives on the physicochemical properties of Solenostemma argel leaves tablets.

Table 19: Effects of Starch as a Binding Agent on the Different Physicochemical Properties of S. argel Leaves Tablets, Formula C, Different Particle Sizes

<table>
<thead>
<tr>
<th>Particles</th>
<th>Starch</th>
<th>MCC</th>
<th>MCC</th>
<th>PVP</th>
<th>Hard-</th>
<th>Disinteg</th>
<th>Dissol</th>
</tr>
</thead>
</table>

CXXIX
<table>
<thead>
<tr>
<th>Particles size (microns)</th>
<th>Zora (Binding Agent) (%)</th>
<th>MCC (Internal) (%)</th>
<th>MCC (External) (%)</th>
<th>PVP (%)</th>
<th>Hardness (kg/inch²)</th>
<th>Disintegration Time (min)</th>
<th>Dissolution Rate (UV Reading)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 250</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>10</td>
<td>2</td>
<td>-</td>
<td>16</td>
<td>0.103</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>250-160</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>10</td>
<td>2</td>
<td>-</td>
<td>23</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>≤ 160</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>23</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>10</td>
<td>2</td>
<td>-</td>
<td>11</td>
<td>0.209</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>3.2</td>
<td>12</td>
<td>0.268</td>
</tr>
</tbody>
</table>

Table 20: Effects of Zora as a Binding Agent on the Different Physicochemical Properties of S. argel Leaves Tablets, Formula C, Different Particle Sizes

<table>
<thead>
<tr>
<th>Particles size (microns)</th>
<th>Sorghum (Binding Agent) (%)</th>
<th>MCC (Internal) (%)</th>
<th>MCC (External) (%)</th>
<th>PVP (%)</th>
<th>Hardness (kg/inch²)</th>
<th>Disintegration Time (min)</th>
<th>Dissolution Rate (UV Reading)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 160</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>4</td>
<td>15</td>
<td>0.161</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>12</td>
<td>0.230</td>
</tr>
</tbody>
</table>

Table 21: Effects of Sorghum as a Binding Agent on the Different Physicochemical Properties of S. argel Leaves Tablets, Formula C, Different Particle Sizes

Table 22: Effects of Different Binding Agents on the Physico-chemical Properties of Solenostemma argel Leaves Tablets*, 12mm-Diameter, Formula (B)
<table>
<thead>
<tr>
<th>No</th>
<th>Parameter Additives</th>
<th>Average Tablet Weight (mg)</th>
<th>Average of 20 Tablets (Mean±SEMmg)</th>
<th>Hard ness (kg/inch²)</th>
<th>Dia meter (mm)</th>
<th>Thickness (mm)</th>
<th>Disintegration Time (minutes)</th>
<th>Dissolution Rate (UV Readings)</th>
<th>Fri ability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SPB 4% PVP</td>
<td>526</td>
<td>520 ± 17</td>
<td>4.59 ±0.977</td>
<td>12.16 ±0.63 E-02</td>
<td>4.1 ±0.05</td>
<td>27</td>
<td>0.477</td>
<td>0.04</td>
</tr>
<tr>
<td>2</td>
<td>SMB 8% Malze Starch + %2PVP</td>
<td>538</td>
<td>537 ±1.2 E-02</td>
<td>3.06 ±0.829</td>
<td>12.2 ±0.05</td>
<td>4.1 ±0.05</td>
<td>9</td>
<td>0.473</td>
<td>23</td>
</tr>
<tr>
<td>3</td>
<td>SGB 6%Guar +2%PVP</td>
<td>517</td>
<td>510 ±.68 E-20</td>
<td>3.2 ±0.954</td>
<td>12.15 ±0.05</td>
<td>4.05 ±0.05</td>
<td>12</td>
<td>0.466 ±1.23E-20</td>
<td>29.5</td>
</tr>
<tr>
<td>4</td>
<td>SSB Sorghum +2%PVP</td>
<td>505</td>
<td>507 ±1.67E-20</td>
<td>3.26 ±0.664</td>
<td>12.05 ±0.05</td>
<td>4.1 ±0.05</td>
<td>18</td>
<td>0.454 ±1.115E-20</td>
<td>19.4</td>
</tr>
<tr>
<td>5</td>
<td>SS(B)</td>
<td>539</td>
<td>507 ±2.02E-20</td>
<td>4.26 ±1.7</td>
<td>12.05 ±0.05</td>
<td>4.1 ±0.05</td>
<td>20</td>
<td>0.534 ±2.38E-20</td>
<td>18</td>
</tr>
</tbody>
</table>

*: Particle size < 160microns

Table 23: Effects of Different Binding Agent on the Physico-chemical Properties of Solenostemma argel Leaves Tablets*, 9 mm-diameter, Formula (B)

<table>
<thead>
<tr>
<th>No</th>
<th>Parameter Additives</th>
<th>Average Tablet Weight (mg)</th>
<th>Average of 20 Tablets (Mean±SEMmg)</th>
<th>Hard ness (kg/inch²)</th>
<th>Dia meter (mm)</th>
<th>Thickness (mm)</th>
<th>Disintegration Time (minutes)</th>
<th>Dissolution Rate (UV Readings)</th>
<th>Fri ability %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SPB 4% PVP Formula (B)</td>
<td>232</td>
<td>225 ±9</td>
<td>9.24 ±0.08 E-20</td>
<td>4.1 ±0.145</td>
<td>9.0 ±0.05</td>
<td>3.45 ±0.28</td>
<td>0.275 ±1.445E-20</td>
<td>23.0</td>
</tr>
<tr>
<td>2</td>
<td>SMB Starch +%2PVP</td>
<td>235</td>
<td>231 ±10E-02</td>
<td>9.13 ±0.7E-20</td>
<td>6.71 ±0.45</td>
<td>7.0 ±0.05</td>
<td>2.65 ±0.62</td>
<td>0.259 ±2.28E-30</td>
<td>29.5</td>
</tr>
<tr>
<td>3</td>
<td>Guar +2%PVP</td>
<td>235</td>
<td>210 ±7E-20</td>
<td>9.2 ±0.95E-20</td>
<td>12.15 ±0.05</td>
<td>4.05 ±0.05</td>
<td>12</td>
<td>0.466 ±1.23E-020</td>
<td>39.5</td>
</tr>
<tr>
<td>4</td>
<td>Guar +2%PVP</td>
<td>235</td>
<td>229 ±7E-20</td>
<td>9.06 ±0.62E-20</td>
<td>4.33 ±0.10</td>
<td>9.1 ±0.05</td>
<td>2.87 ±0.205</td>
<td>0.271 ±4.5E-30</td>
<td>19.4</td>
</tr>
<tr>
<td>5</td>
<td>SS(B)</td>
<td>236</td>
<td>231 ±22E-20</td>
<td>9.12 ±0.27E-20</td>
<td>4.2 ±0.01</td>
<td>4.1 ±0.05</td>
<td>1.5</td>
<td>0.285 ±2.4E-02</td>
<td>18</td>
</tr>
</tbody>
</table>

*: Particle size < 160microns

After different trials for the physicochemical properties and tablets characters optimization, different tests were conducted to confirm the resistance to crushing of Solenostemma argel tablets. Tablet characters optimization included, in addition to
improved tablet hardness, other characters required by the official monographs (friability, uniformity of diameter, thickness, weight, and content, and disintegration and dissolution specifications).

Formulations, based on the powdered leaves of *Solenostemma argel*, included as diluent 70% lactose and 30% starch; 2% PVP as a binder in both the leaves and extract tablets. Furthermore, 1.5% talc and 0.5% magnesium stearate and 10% MCC, 5% external and 5% internal, were added to the powdered leaves tablets. These formulae were used to achieve optimization of the physicochemical properties of *S. argel* tablets, with the addition of different diluents and additives to comply with the monographs of the compressed tablets requirements (Tables 15-25) (USP, 1990; BP, 1990).

Table 24: Effects of Different Binders, in Different Concentrations, and Starch* on Physicochemical Properties of *Solenostemma argel* Tablets, Formula A

<table>
<thead>
<tr>
<th>Binder Parameter</th>
<th>0.1% Guar</th>
<th>10% Gum</th>
<th>10% Starch</th>
<th>4% PVP</th>
<th>5% PVP</th>
<th>10% PVP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hardness (kg/in²)</td>
<td>3.45</td>
<td>8.2</td>
<td>6.1</td>
<td>4.0</td>
<td>6.0</td>
<td>6.8</td>
</tr>
<tr>
<td>Hardness (kg/in²) (±2%Starch)</td>
<td>3.30</td>
<td>9.12</td>
<td>8.20</td>
<td>-</td>
<td>-</td>
<td>7.2</td>
</tr>
<tr>
<td>Disintegration Time(min)</td>
<td>11</td>
<td>18</td>
<td>29</td>
<td>18</td>
<td>29</td>
<td>35</td>
</tr>
<tr>
<td>Disintegration Time(min) (±2%Starch)</td>
<td>7</td>
<td>20</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>31</td>
</tr>
<tr>
<td>Dissolution Rate, UV Reading (λ 294.5)</td>
<td>0.124</td>
<td>0.205</td>
<td>0.189</td>
<td>-</td>
<td>-</td>
<td>0.269</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>6.90</td>
<td>0.019</td>
<td>0.40</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
</tr>
</tbody>
</table>

So for formulae A, using different binders and without external addition of disintegrant after granulation, ten formulas were studied to determine the effects of different types of binders and their different concentrations on the physicochemical properties of *S. argel* tablets (Table 24). The effects of 2% starch as a disintegrating agent, added after the granulation process, on these physicochemical properties; addition of starch resulted in tablets with long disintegration time and low dissolution rate, while guar also had resulted in a low dissolution rate (Table 24).

Addition of 2% starch affected the tablet hardness and the disintegration time and dissolution rate. Reduction of PVP percentage as a binding agent reduced both the disintegration time and tablet hardness for further optimization of the physicochemical properties of the *S. argel* leaves tablets. For 4% PVP, tablet hardness was 6.8—4 kg/inch², and the disintegration time was 35 — 18 minutes (Tables 24, 25).

Table 25: Effects of Different Binders, in High Concentrations, and Starch on Physicochemical Properties of *Solenostemma argel* Tablets, Formula A
From these data, it could be concluded that:

a) A combination of binding agents was needed and a reduction of binder concentration was a must to optimize A formulas (Tables 15-25); b) The use of a disintegrating agent, before and after granulation, in the S. argel leaves tablets, e.g. 2% starch, also optimized A formulae; c) PVP preparations showed lower friability, followed by gum, starch and guar (Tables 19-24); d) PVP preparations had the best dissolution rate properties of S. argel leaves tablets; PVP was followed by gum, guar, and starch had the least rate; and e) A formulae that included S. argel leaves powders without particle size analysis or separation resulted in tablets with mottling of the color, and had coarse surfaces which were tough to touch because their particles were not equal.

Comparison of the Effects of Three Different Starches as Binding Agents on the Dissolution Rates of S. argel Tablets was conducted, Formula C. The comparisons were between three binding agents, 10% of the Different Starches, on the dissolution rate of S. argel Tablets, formula C, when 5% MCC was added before and after granulation (Tables 26, 27).

Optimization of physicochemical properties of S. argel tablets, formula C, without diluent and using maize starch as the binding agent, and other additives, was investigated in a series of trials involving S. argel tablets of formula C. The effects of MCC addition on the formulas using maize starch as a binding agent in mesh 60 particles had a good dissolution rate which would be reduced or retarded to some extend by the addition of 2% PVP, and extended after granulation addition of 10% MCC, but 5% MCC would keep the physicochemical property, i.e. dissolution rate; when using >160micron particles, 5% MCC before and after granulation, with 2% PVP, would enhance the dissolution rate (Table 28).

10% MCC external and 2% PVP enhanced the dissolution rate of particles (<160); improved all the physicochemical properties (dissolution rates and disintegration time; Tables 28-30).

4.5.6.3.2. Effects of Disintegrating Agents on the Physicochemical Properties of
**Solenostemma argel** Leaves Tablets, of the Different Formulae

Disintegrants are materials which are added to tablets to cause them to breakup and fragment after ingestion usually in the stomach. They are added even for tablets which are to disperse in water before administration (Banker 1974). Disintegrants are incorporated to overcome the cohesive strength that is introduced into the tablet by compression or by any binder present. Examples of disintegrants include external MCC, sugar, starch, and guar. There are several types of disintegrants acting by different mechanisms. They include: those which enhance the action of capillary forces in producing a rapid uptake of aqueous liquids; those which release gases to disrupt the tablet structure; those which melt at body temperature; and those which destroy the binder by enzymatic action.

The addition of 5% microcrystalline cellulose, MCC, or 2% starch, sugar or guar after granulation would optimize the disintegration time (Table). Polyvinylpyrrolidone, PVP 2%, with MCC had a high dissolution rate.

The physicochemical properties of **S. argel** tablets were optimized using different particle sizes of the drug substance, and employing 5% microcrystalline cellulose, MCC, before and after granulation. Microcrystalline cellulose was acting as a disintegrating agent and glidant, as well in the formula C, without a diluent. The addition of 5% MCC pre- and post-granulation enhanced all the physicochemical properties of **S. argel** tablets prepared by using the fine powder of **S. argel**. In all C formulas, using fine powders (≤160micron), resulted in tablets with even surfaces, good texture, homogenous green color and no mottling were observed. For C formulae, every particle must pass mesh 80, and the binding agents used were 4% PVP, 2% PVP, with 8% other binding agents, such as the starches, or 0.6% guar), together with 5% MCC, before and after granulation, to produce tablets with the desired characters.

Investigations were carried out to study the effects of the addition of 5% MCC, before and after granulation, or 10% MCC after granulation, and 2% PVP on the tablet hardness and disintegration time of Solenostemma argel tablets, formula C.

Microcrystalline cellulose, MCC, changed greatly the disintegration time (without a binding agent) in formula C. Using 2% PVP as a binding agent in formula C improved tablet hardness for all three mesh sizes used.

| Table 26: Effects of Three Different Starches as Binding Agents on the Dissolution Rates of **Solenostemma argel** Tablets, Formula C |
|-----------------|-----------------|-----------------|-----------------|
| **Starch Type** | **Sorghum**     | **Maize**       | **Zora**        |
| Dissolution Time (15min) | 0.108 ±6.093E-02 | 0.0983 ±6.103E-02 | 0.103 ±6.54E-02 |
| (5% MCC before and 5% after granulation) | | | |
The disintegration time and tablet hardness were decreased with decreasing the concentration of the binding agents, e.g. PVP (10%, 5%, 4%); the addition of 2% starch external after granulation increased the tablet hardness (Table).

Microcrystalline cellulose, MCC, often refereed to by trade name ‘Avicel’, is one of the most commonly used binders. It is a directly compressible material, but in the present work it has been used as a disintegrant, and a physicochemical MCC exists in two tablet grades: pH 10.1 (powder) and pH 10.2 (granules). MCC is somewhat a unique excipient in that while producing cohesive compacts, it is also acts as a disintegrating agent. It is, however, a relatively expensive material when used as diluent in high concentration. So it is added internally and externally to improve disintegration and cohesive compact.

Addition 10% MCC after granulation optimized both disintegration time for formula C, when different sizes of S. argel powders were used with 10% Zora as binding agent. Addition of 2% PVP optimized the dissolution rate at 45 minutes.

Addition of Avicel to the other tableting excipients has been shown to modify the effect of the granulating agent (PVP 4%, starch, or guar 10% mucilage) in wet granulation and on the stability of S. argel tablets at different storage conditions. The
Tablets were compressed on a single-punch press without pressure control. The time and method of addition of MCC as a disintegrant has also received attention, particularly the possibility of adding the disintegrant before or after the granulation process (Shotton and Leonard, 1972).

There are advantages to be gained in dividing the disintegrant into an extra-granular and intra-granular portion. The extra-granular portion ensures rapid disintegration. Intra-granular fraction leads to harder tablets and finer size distribution on dispersion. This technique gave the best overall performance in tablets of *Solenostemma argel* leaves powders since it shortened the time of disintegration after the addition of MCC, a finding similar to that reported by Rubinstein and Bodey (1974).

**Table 28: Effects of 5% or 10% MCC, before and/or after Granulation, and 2% PVP on the Tablet Hardness and Disintegration Time of *S. argel* Tablets, Formula C**

<table>
<thead>
<tr>
<th>Particle size (microns)</th>
<th>Disintegration Time (min) (without 10% MCC)</th>
<th>Disintegration Time (min) (10% MCC after granulation)</th>
<th>Hardness (kg/inch²) (10% MCC after granulation)</th>
<th>Disintegration Time (min) (5% MCC before and 5% after granulation)</th>
<th>Hardness (kg/inch²) (5% MCC before and 5% after granulation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 250</td>
<td>5</td>
<td>12</td>
<td>1.25±0.33</td>
<td>16</td>
<td>2.72±0.72</td>
</tr>
<tr>
<td>≥ 160</td>
<td>75</td>
<td>28</td>
<td>2.15±0.59</td>
<td>15</td>
<td>4.0±0.3</td>
</tr>
<tr>
<td>≤ 160</td>
<td>240</td>
<td>28</td>
<td>3.5±0.93</td>
<td>11</td>
<td>4.5±0.45</td>
</tr>
</tbody>
</table>

Microcrystalline cellulose, some form of MCC, has been shown to be highly porous with strong wicking tendencies, and therefore makes a good disintegrant. In addition to this property, it is an excellent binder used to improve significantly the mechanical strength of some weak formulations. One disadvantage with MCC is that the dissolution performance may be adversely affected at higher compressional forces (Sixmith, 1977).

Starch 2% remains the most popular disintegrant of all and it is now widely accepted that its mode of action is probably more by inducing water uptake in the tablet than by swelling. It was observed that rupture of the tablet surface was postulated as the mode of action, and that water hydrates the hydroxyl groups of the starch molecule and causing them to move apart. There is some evidence to suggest that the fat content of a particular starch can also influence its performance as disintegrant, or its other physicochemical properties as a binding agent or diluent. Recently, expltab, a commercial disintegrant based on starch was introduced in the market.

Polyvinylpyrrolidone, PVP, is characterized as a white-colored, odourless or almost odourless, hygroscopic powder. It is soluble in water, alcohol and chloroform. It can be used in alcoholic solutions to granulate water-sensitive substances (BP, 1988). It has been reported that PVP complexes and binds drugs, although this polymer is not reducing drug availability or disintegration time or solubility (Higuchi *et al.*, 1954).

Another group of tablet additives include lubricants, glidants and anti-adherents. These three classes of material are frequently described together because of their overlapping functions. Materials that are primarily described as antiadherent are typically as a lubricant and with some glidant properties as well.

Lubricants like talc and magnesium stearate were incorporated in these formulae to reduce the friction between the tablet surface and the walls of the die cavity in which the tablets were formed. Other agents used include hydrogenated vegetable oils and polyethylene glycols, PEG, (Rudnic and Schwartz, 1990). Lubricants are required to act at the tooling/material interface, and therefore they should be incorporated at the end of
any recompression stage, and over-mixing should be avoided so that the maximum amount is retained on the surface of the particles. However this surface coating by the lubricant may have an adverse effect on the properties of other excipients in the formula. The compatibility of the microcrystalline cellulose, MCC, was found to be adversely affected by the formation of surface coating of magnesium stearate (Ahmed and Staniforth, 1988).

Many of the commonest lubricants are hydrophobic, and consequently they might affect the release of the medicaments. It was found that hydrophobic tablet lubricant magnesium stearate retarded dissolution rate of salicylic acid tablets, while a water-soluble, surface active lubricant, such as sodium lauryl sulfate, enhanced the dissolution rate (Levy and Gumtow, 1963). Also, MCC improved the dissolution rate of S. argel leaves tablets. Lubricants may also significantly reduce the mechanical strength of the tablets. It was found that the crushing strength of paracetamol tablets, formed from egg albumin-coated particles, was decreased by the use of magnesium stearate as a lubricant (Torrado-Duram et al., 1995).

Published formulae showed that the level of these lubricants is 1-4%, but there is evidence to show that they could be reduced to as little as 0.5% and 1.5%. Therefore 0.5% magnesium stearate, a widely used lubricant, was used in the formulae of the present work. Its use is often problematic in that the compound exhibits significant batch-to-batch variation in its lubricant properties.

Effects of chemical composition and moisture content on the lubricant properties of magnesium stearate were examined. It was concluded that none of these variables appeared to have any influence on lubrication, but when the effect of moisture content was further examined using three hydrates of pure magnesium stearate; it appeared that the lubricant properties were influenced by crystal structure of the compound (Ertel and Carstensen, 1988).

Sodium sulphate has been shown to have a very significant opposite effect to that of magnesium stearate on the dissolution rate of drugs from tablets. A physical mixture of these two lubricants, sodium sulphate and magnesium stearate, can lead to the best compromise in terms of lubricity, tablet strength and disintegration. A further investigation has shown that magnesium stearate-sodium sulphate mixture had improved powder flow as indicated in terms of weight uniformity in capsules and tablets (Augesberger and Shangraw 1966).

Stearic acid is also widely used as a lubricant but it doesn’t generally provide adequate lubrication for high speed tableting machines, and therefore, it is important to investigate the effect of the combination of the stearic acid and magnesium stearate (Chowhan et al., 1992).

Talc powder, 1-1.5%, is added as a glidant, which is added to improve the flow characteristics of a powder mixture to be fed to the die, and sometimes to aid particles rearrangement within the die during the early stages of compression.

Glidants may act by interposing their particles between the particles of other components, and also by lowering the overall inter-particulate friction of the system. In addition, they may be of some effect due to rounding off the surfaces of irregular particles by adsorption of the glidant into irregularities, and this role also might be done by adding MCC after granulation.

Talc is widely used and has advantage in minimizing any tendency for the material to stick to the punch surface, and this property is sometimes classified as anti-adherent. Other popular glidants used include potato and corn starches as well as colloidal silicone dioxide.

4.5.6.3.3. Effects of Different Additives on the Dissolution Rate of Solenostemma argel Leaves Tablets, of the Different Formulae
Effects of different additives on dissolution rate were investigated due to the particular importance of dissolution rate parameter in the quality of tablets and its effects on bioavailability of drug substances and their therapeutic response.

For example, effects of PVP, starch and guar on the dissolution rate of *Solenostemma argel* leaves extract tablets, formula (B), without MCC was studied (Tables 18-25).

Guar had a least dissolution rate; in both formulae A and B, at 10% and 4%, it had high dissolution rates at 45 minutes (Tables 26-32).

The effects of different additives on the physicochemical properties of *S. argel* extracts tablets are shown in these Tables 29-32.

### Table 29: Effects of Starch and Polyvinylpyrrolidone, PVP, on the Dissolution Rates of *Solenostemma argel* Tablets, Formula C*, Drug Particle Size $\geq 250$micron

<table>
<thead>
<tr>
<th></th>
<th>Dissolution Rate (15min)</th>
<th>Dissolution Rate (30min)</th>
<th>Dissolution Rate (45min)</th>
<th>Dissolution Rate (60min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% Starch + 2% PVP 10% MCC after</td>
<td>0.0867</td>
<td>+0.0035</td>
<td>+0.0025</td>
<td>+0.0025</td>
</tr>
<tr>
<td>8% Starch + 2% PVP 5% MCC before and 5% after granulation</td>
<td>0.1865</td>
<td>+0.0075</td>
<td>+0.0235</td>
<td>+0.0005</td>
</tr>
<tr>
<td>10% Starch Only</td>
<td>0.195</td>
<td>+5.87E-02</td>
<td>+4.9E-02</td>
<td>+5.2E-02</td>
</tr>
</tbody>
</table>

5% MCC added before and after granulation to improve the physicochemical properties of powder particles sizes $\geq 250$micron.

### Table 30: Effects of Starch and Polyvinylpyrrolidone, PVP, on the Dissolution Rates of *Solenostemma argel* Tablets, Formula C*, Drug Particle Size 160-250micron

<table>
<thead>
<tr>
<th></th>
<th>Dissolution Rate (15min)</th>
<th>Dissolution Rate (30min)</th>
<th>Dissolution Rate (45min)</th>
<th>Dissolution Rate (60min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% Starch</td>
<td>0.165</td>
<td>+2.09E-02</td>
<td>+1.36E-02</td>
<td>+1.501E-02</td>
</tr>
<tr>
<td>8% Starch + 2% PVP 5% MCC before and 5% after granulation</td>
<td>0.1865</td>
<td>+0.0075</td>
<td>+0.0235</td>
<td>+0.0005</td>
</tr>
<tr>
<td>8% Starch + 2% PVP 10% MCC</td>
<td>0.1715</td>
<td>+0.0155</td>
<td>+0.0095</td>
<td>+12</td>
</tr>
</tbody>
</table>

5% MCC added before and 5% MCC after granulation to improve the physicochemical properties of powder particles sizes 160-250micron.

### Table 31: Effects of Starch and Polyvinylpyrrolidone, PVP, on the Dissolution Rates of *Solenostemma argel* Tablets, Formula C*, Drug Particle Size $\leq 160$micron

<table>
<thead>
<tr>
<th></th>
<th>Dissolution Rate (15min)</th>
<th>Dissolution Rate (30min)</th>
<th>Dissolution Rate (45min)</th>
<th>Dissolution Rate (60min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\leq 160$ Micron</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10% Starch & +4.39E-03 & 0.112 & +3.02E-02 & 0.148 & +1.79E-02 & 0.156 & +0.0065

| 8% Starch + 2% PVP & 5%MCC before and 5% MCC after Granulation |
|---------------------|-----------------------------------|
| 0.1575 & +0.1575 & 0.165 & +0.011 & 0.223 & +0.020 & - & - |

| 8% Starch + 2% PVP & 10% MCC |
|---------------------|-----------------|
| 9.155 & 0.1915 & +0.0095 & 0.203 & +0.020 & - & - |

5% MCC added before and 5%MCC after granulation to improve the physicochemical properties of powder particles sizes ≤ 160micron.

Investigations were carried out to study the effects of guar as a binding agent on the dissolution rates of Solenostemma argel tablets, formula C. Therefore, the effects of guar as a binder on the Solenostemma argel leaves formulations were studied.

Guar had a critical concentration for its effects on the inherent dissolution rates and the release of S. argel leaves alkaloids (Table 32). It seemed from these experiments that 6% guar was the best for dissolution rate at the different time intervals, 15, 30, and 45 minutes. For 6% guar, for a longer period of time as 60 minutes, equilibrium would be established, while for 8% guar, after 90 minutes, there was a great increase in drug dissolution rate. For 5% guar, tablet hardness was 3.1 kg/inch²; disintegration time was 14 minutes; 5% MCC before and 5% MCC after granulation; dissolution rate = 0.223+2.7E02 (Table 32)

<table>
<thead>
<tr>
<th>(IV)</th>
<th>Dissolution Rate(15 minutes)</th>
<th>Dissolution Rate(30 minutes)</th>
<th>Dissolution Rate(45 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% Guar</td>
<td>0.0741+0.010</td>
<td>0.094+0.01</td>
<td>0.121+0.005</td>
</tr>
<tr>
<td>2% Guar</td>
<td>0.055+0.008</td>
<td>0.078+0.005</td>
<td>0.1121+0.001</td>
</tr>
<tr>
<td>4% Guar</td>
<td>0.072+0.01</td>
<td>0.077+0.01</td>
<td>0.138+0.005</td>
</tr>
<tr>
<td>6% Guar</td>
<td>0.162+0.003</td>
<td>0.225+0.005</td>
<td>0.269+0.01</td>
</tr>
<tr>
<td>8% Guar</td>
<td>0.087+0.002</td>
<td>0.116+0.005</td>
<td>0.202+0.01</td>
</tr>
<tr>
<td>10% Guar</td>
<td>0.064+0.01</td>
<td>0.087+0.005</td>
<td>0.1661+0.001</td>
</tr>
</tbody>
</table>

4.5.7. Evaluation the Physicochemical Properties of Solenostemma argel Leaves Tablets

The compressed tablet specifications evaluated included general appearance, diameter size, shape thickness, weight, hardness, friability, disintegration and dissolution (Tables 18-32). The diameter and shape depended on the die and punches selected for the compression of the tablets. Generally the tablets are discoid in shape although they may be oval, oblong, round, cylindrical
or triangular. The tablets may be in halves or quadrants to facilitate breakings scored if smaller dose is desired.

Tablets, which are subjected to individual monographs in the BP, are circular in shape and do not contain flavoring agent, unless otherwise indicated in the individual monograph. The surface of the tablets is flat or convex and may have line or break marks, a symbol or other markings (BP, 1980).

4.5.7.1. Uniformity of Tablet Weight

The volumetric fill of the die cavity, in the tableting machine, determines the weight of the compressed tablets. The weight of the tablet is the quantity of the granules, which contains the labeled amount of the active ingredients. The USP (1980) weight variation is carried out by weighing twenty tablets individually, calculating the average weight and comparing the individual tablet weight to the average.

The USP has provided tolerance for the average weight variation of uncoated tablets, e.g. for tablets with average weight less than 130mg, the maximum percentage difference allowed is 10%, and 75% for those with average weight more than 324mg.

The tablets meet the USP test if no more than two tablets are outside the percentage limit, and if no tablet differs from the average by more than two times the percentage limit (USP, 1980).

Furthermore, the weight variation test would be a satisfactory method for determining the drug content of tablets if the tablets were all or essentially all, 90-95%, active ingredients (Airth et al., 1967), or if the uniformity of the drug distribution in the granulation from which the tablets were made was perfect.

4.5.7.2. Uniformity of Tablet Diameter
For tablets which are not sugar-coated or enteric-coated, a deviation of ± 5% from the standard stated is allowed, except for diameters exceeding 12.5mm, the deviation allowed is ± 3% (BP, 1980). Average tablet weight, weight variation test, thickness and diameter, and other physicochemical properties of the different tablets were evaluated with reference to the BP (1980) or USP (1980) monographs requirements (Tables 16, 17, 22, 23).

4.5.7.3. Uniformity of Tablet Thickness

The tablet thickness is carefully controlled during production and from batch to batch (Tables 16, 17, 22, 23). Tablet thickness can vary with no change in weight due to differences in density of the granulation and the applied pressure as well as the speed of tabulating machine. Tablet thickness uniformity is important in production batches which will be usable with selected packaging components. Tablet thickness is determined with caliper or micrometers, and the thickness should be controlled within ± 5% variation of the standard value (Banker and Anderson, 1986).

4.5.7.4. Tablet Hardness

To avoid confusion of the term ‘strength’ with ‘potency’, the mechanical strength of pharmaceutical tablets is commonly referred to as hardness, although this term is by correct definition is a surface property (Cooper and Rec, 1972). Tablets require a certain degree of hardness to withstand mechanical shocks of handling in manufacture, packaging and shipping. In addition, tablets should be able to withstand reasonable abuse when in hands of the consumer.

Historically, the strength of a tablet was determined by breaking it between the second and the third fingers with the thumb acting as fulcrum. Tablet hardness has been defined as the force required for breaking a tablet in diametric
compression test, and when it stands falling or throwing from 2 meters height on the floor. In the tablet hardness test, the tablet is placed between two anvils and force is applied; the crushing strength that just causes the tablet break is recorded (Tables19-23).

There are several devices that operate in this manner, and have been and continue to be used to test tablet hardness; these include: the Monsanto tester, Strong-Cob tester, Pfizer tester, Erweka tester, Scheuniger tester, and other new electrically-operated testers (Goodhart et al., 1973). Unfortunately these testers do not produce the same result for the same tablet. Studies have shown that operator variation, lack of calibration, spring fatigue and manufacture variation could contribute greatly to the lack of uniformity (Newton and Stanley, 1977).

4.5.7.5. Tablet Friability

Tablet hardness is not an absolute indicator of strength, since some formulations when compressed into very hard tablets tend to chop or attrition loosing their crown portions. Another measure of tablet strength is the term ‘friability’ which is often measured particularly for tablets that tend to powder, clip and fragment when handled. This would result in inaccurate dose, lack of elegance and consumer acceptance, and can create excessively dirty processing areas of manufacture such as coating and packaging, and they can add to tablet’s weight variation and lack of content uniformity.

The friability tester, commonly known as Roche friabilator, is a device which subjects a number of tablets to the combined effect of abrasion and shock by utilizing a plastic chamber that revolves at 25rpm, dropping the tablets a distance of six inches with each revolution. The test is performed by placing pre-weighed tablets in the friabulator which is then operated for 100 revolutions, the tablets are then placed on No.10 sieve and removing any loose dust with the aid of pressure
or soft brush, and then the tablets are weighed (Tables 15-17, 22, 23). For tablets weighing up to 650 mg each, or a 6.0 to 6.5g sample, a minimum number of 20 tablets should be used in any test. However, for tablets weighing over 650 mg each, 10 tablet sample is sufficient. Generally, the test is run once, but if results are doubtful or if weight loss is greater than 1%, the test should be repeated twice and mean of the three tests is determined. A maximum weight loss of not more than 1% is considered acceptable for most products (BP, 1993).

4.5.7.6. Tablet Disintegration Time

The average of the disintegration time of six tablets, rather than upper time stated in companion monographs, was recommended (Allen and Parrot, 1971). Tablets have been classified, according to their disintegration by Lowenthal (1972), as sublingual tablets which dissolve or disintegrate in three minutes; uncoated tablets intended to disintegrate in the stomach in 10 to 120 minutes maximum; uncoated tablets intended to disintegrate in water at room temperature within five minutes; and hypodermic tablets within one minute limit. The Indian Pharmacopoeia (1996) stated that for herbal tablets, the disintegration time is one hour. Solenostemma argel tablets, of the different formulae, had disintegration time 5 to 20 minutes (Tables 14-18, 19-23).

4.5.7.7. Tablet Dissolution Test

Dissolution is the process by which a solid of only fair solubility characteristics enters into solution (Abdou, 1989). Dissolution testing is used in the quality control of manufactured dosage forms, in comparative studies of different forms and in studies of new forms (Esbelin, et al., 1991). So, it was applied to confirm the quality of Solenostemma argel tablets and their content uniformity (Tables 14-18, 29-32).
Noyes and Whitney's (1897) developed an equation, based on Fick's second law, to describe the dissolution phenomena.

\[
\frac{dc}{dt} = k(c_s - c) \quad (1)
\]

Where: \(\frac{dc}{dt}\) is dissolution rate, \(k\) = proportionality constant, \(ct\) = the concentration at time \((t)\); \(c_s\) = the saturation concentration (maximum solubility) and \(c_s - c\) = concentration gradient. The above equation was modified to incorporate the surface area.

\[
\frac{dc}{dt} = k_s(c_s - c_i) \quad (2)
\]

Equation (2) was expanded to include the diffusion coefficient \(D\), the thickness of the stagnant diffusion layer \(h\), and the volume \(V\), of the dissolution medium producing:

\[
\frac{dc}{dt} = k_s \frac{os}{vh} c_s - c_i \quad (3)
\]

\(k_2\) = is called the intrinsic dissolution constant (Abdou 1989). So there is variation in different size formulations, \(\geq 250, \alpha \leq 80\alpha \leq 80\) of \textit{S. argel} tablets.

Determination of the percentage dissolved of \textit{S. argel} constituents of the different tablets, under UV spectra \(\lambda 294\) nm, was carried out (Table 29-32).

The dissolution test was carried out following the procedure mentioned before in the experimental chapter. No correlation was detected between disintegration time and dissolution rate (Table 15-19). Similar findings were reported by Jacob and Plein (1968) and Rubinstein and Wells (1977).
However, the formulae containing 8% starch, without 2% PVP, and with 10% MCC added after granulation, with relatively smaller drug particle size ≤ 180micron, were found to release a comparatively low percentage of the drug in the dissolution medium than did the formulae with larger particle size ≥ 250micron (Tables 14, 19-21).

This behavior contradicted with Noyes-Whitney equation (2) (1897), since Solenostemma argel smaller particles floated on the surface of the dissolution medium, and disintegration as well as the effective surface area may subsequently be decreased.

As a result of this decreased effective surface area and reduced rate of wetting, the disintegration time increased to 240 minute and the dissolution rate decreased (Table). Such a behavior was encountered with the hydrophobic drug phenacetin (Finholt, 1974; Martin et al., 1983; Sanderson et al., 1984). These defects in these formulae could be amended by the addition of PVP to the binder, and inclusion of 5% MCC before and after granulation.

The formulae containing PVP, starch, and guar, when compared on the basis of the dissolution rate after 45 minutes, seemed to comply with the drug concentration or content, but those of Sorghum did not comply with the standard qualities and specifications.

A possible explanation of these effects of guar, maize starch, sugar, and Zora in improving the S. argel tablets disintegration and dissolution rate is that they are containing many large granules (13microns) that allow a more porous and loose mound net-work to form. This would enable the dissolution medium to circulate easily through the mound (layer around the particle).
The comparatively slower dissolution rate of maize starch formulae could be due to a more compact mound as a result of much smaller particle size (9microns) of sorghum starch. The compact mound would make it more difficult for the dissolution medium to transverse and circulate. Similar to these findings, different types of starch were found to have variable effects on the dissolution of salicylic acid from tablets (Underwood and Cadwallader, 1972).

In the case of the different types of starch, on internal and external addition of 5% MCC, sorghum had a higher hardness and lower dissolution rate, followed by starch (4 and 3.45 kg/inch² for hardness; and for dissolution, 0.161 and 0.186 UV readings, respectively; Tables 26-32). It is worth-mentioning that with the different starches, the use of 2% PVP as a binder, or 4% PVP, with 5% MCC before granulation and 5% MCC after granulation, would optimize greatly the physicochemical properties of the formulae.

The defects due to unsuitable particle size would be corrected to produce improved tablets with relatively higher resistance to crushing, lower percentage of friability, reasonable disintegration time and dissolution rate as well. Therefore, the appropriate particle size as a pharmaceutical property of drug substances should be checked and corrected during the manufacturing processes.

The method of amending this defect of inappropriate particle size involved the use of additives that can be adjusted. These included using different types of binder combination, and addition of a disintegrant before and after granulation. The validation of particle size has been recently considered as one of the important factors in the quality of the pharmaceutical preparations, and applied advanced techniques to separate particles of different materials, such as infra-red spectra method (Otsuka and Matsuda, 1996.) or sieving for particle separation and analysis.
All the physicochemical properties deviations due to particles size or binder inherent characters has been adjusted by using 2% PVP with binder and adding 5% microcrystalline cellulose before and after granulation of the formula.

The addition of 2% PVP, besides its optimization of the physicochemical properties of the *S. argel* leaves tablets, improved and quickened the solubility of the starches used as binding agents, especially guar starch in hot water. The use of different particle sizes (≥ 250, ≥ 160, and ≤ 160 microns) of drug substances affected the physicochemical properties of *S. argel* tablets (Tables 19-21, 27-32).

Also the dissolution rate can be affected by popular hydrophobic lubricants, e.g. 0.5% magnesium stearate incorporated in these formulae may impart hydrophobicity to the formulations at different levels, depending upon the extent of mixing. The randomness of the mixture affinity and fineness was reported to affect the distribution of magnesium stearate or talc powder between the granules (Sanderson *et al.*, 1984).

The variations in dissolution rates of the above formulae were explained, and the optimization with MCC or other disintegrants taking into consideration that incorporation of magnesium stearate was found to produce a decrease in dissolution rate (Levy and Gutmow, 1963; Rubinstein, 1988).

In the case of the binding agents in formula C, guar had resulted in high friability, whereas PVP and gum Arabic the least friability, followed by sorghum, maize and Zora (Tables 26-32).

When comparing the different types of starch, following addition of 5% MCC before and after granulation, guar had a long disintegration time and also maize starch. However, guar and PVP had resulted in a high dissolution rate (Tables 26-
This was adjusted by addition of a disintegrating agent, e.g. 5% MCC or 2% starch or sugar.

Sometimes, there is an increase the disintegration time when using starch as a binding agent; this has been adjusted or optimized by using 8% starch and 2% PVP as a binder instead of 10% starch alone (Tables 24-26).

It is generally accepted that the strength of the granular materials increases with decreasing particle size; such an effect has not been fully explained, whether it is due to an increase in the total inter-particle surface area with decreasing particle size or to an effect of particle size during the strength measurement (Benahow, 1979). Fine particles of hydrophiles result in stronger, larger granules being formed, and coarser hydrophobics result in the production of weaker granules (Jaiyeoba and Spring, 1980).

The formulation of a disintegrating tablet is a complex process depending on many interacting variables. These include the characteristics of the drug itself particularly its particle size, and would affect the in vitro drug dissolution (Tuladhar et al., 1983).

Particle size was found to have an influence on wetting of the powder mixture in the first stage of the granulation process (Jacyeoba and Springs, 1980), and would affect greatly the granules hardness as well as disintegration. This would increase the area of the interface between the S. argel leaves granules and the solvent, allowing better solvent penetration for the fine powder (after 80 mesh ≤ 180 micron).

It was found that addition of different starches, sugar or MCC had great effects on optimization of the physicochemical properties of S. argel tablets.
(leaves or extracts), by diminishing the effects of size variation or size reduction (250 ≤ 180 micron) (Tables 14, 19-21).

These additives might act as hydrophilic lubricants as well as disintegrating agents by enhancing water absorption rate and quick swelling, thus enhancing its break into fragments and dissolution in the medium.

However, there were a lot of discrepancies that were attributed to drugs particle sizes variable in the formulation, and which could be optimized by sugar or any disintegrating agent and glidant using MCC or starch before and after granulation. But this is different with the different types of starch which might give different rates of dissolution according to the nature and the physico-chemical properties of these starches (Tables 19-21, 26). Starches are usually more cheap and available than lactose, and so the effects of different diluents were studied (formula B, Tables 19-26).

The addition of microcrystalline cellulose affected the in vitro dissolution and other physico-chemical properties because:

a) it might change the characteristics of the powder itself being cohesive, or the hydrophobic nature of the fine powder itself, by changing its shape and size (Clark et al., 1977; Tutadhar et al., 1983);

b) inclusion of MCC before and after granulation might affect greatly the physico-chemical properties, porosity, density and viscosity of S. argel tablets when mixed with water, and poor wettability contributed to the general dissolution problems of flocculation and agglomeration (Wagner, 1961); and

c) It resulted in enhancement of dissolution and narrowing of the venation between particle size fractions and shapes (retarded above 60, 80 and passing 80
mesh as fine powder) to an extent where the variations due to particle size may be marked (Badwan et al., 1995) (Tablets 14, 19-21).

The reduction of particles to a size that passed mesh 80 retarded the disintegration rate (Tables 14, 19-21); this might be due to the reduction or prevention of the drug-solvent contact, because the fine particles of this size agglomerate together, and thus reducing the contact surface. Microcrystalline cellulose might increase the effective drug solvent interfaced area by changing the surface characteristic of the powders and granules, which would result in increasing wettability and decreasing the disintegration time (Tables 19-21).

The tablets content uniformity of the different formulae was confirmed, and they had the same content of active constitutions of S. argel when evaluated at $\lambda$ 294 nm. So there were no variations in the tablet content uniformity (Tables 17, 22, 23) (Rudnic and Schwartz, 1990), taking into account the processing difficulties, variations in granulation particle sizes and amounts of additives for powdered leaves, extracts and alkaloids reflecting the whole active ingredients in any of these S. argel tablets.

The fractional binder solution, made from natural products such as acacia, tragacanth, guar, starch or gelatin, should be freshly prepared and even sometimes in need of using homogenizer mill, especially in the case of guar. Also it requires centrifuging to remove debris, or addition of PVP as has been done in combination of 2% PVP with other binding natural products. Acacia has been largely superseded for tablet manufacture because of the possibility of contamination and its tendency to prolong the disintegration time of the product.

However, when used, visual examination of the powder should be carried out, and samples showing dark particles should be rejected (BPC, 1973). In
addition, acacia was found to be less used than maize and sorghum starch as a binding agent (Deshpaude and Panya, 1987).

Starch paste has historically been one of the most common granulating agents. It is prepared by dispersing starch into water which is heated on a water-bath for some specified time. During heating, the starch undergoes hydrolysis to dextrins and glucose. A properly made paste is translucent rather than clear, and produces cohesive tablets when properly formulated (Parrott and Saski, 1970).

While the starch from different plants is used as an adjuvant in the formulation of solid dosage forms, starch from sorghum appears not to have been used because it has a characteristic offensive odour which is increased by time due to the deterioration of the starch.

However, Deshpaude and Panya (1987) examined the usefulness of sorghum starch as a binder and disintegrant for the formulation of tablets containing organic and inorganic substances. The comparison of the physical characteristics of these tablets with those prepared using acacia and maize starch as binders showed that it is equally as good as maize starch in its binding and it is better than acacia for binding.

Povidone, polyvinylpyrrolidone or PVP, is frequently used as a binder. It is a mixture of essentially linear synthetic polymers of 1-vinylpyrrolid-2-one, of different chain lengths and molecular weights. It resulted in tablets with good physico-chemical characters, without deterioration with time and under drastic condition at high temperature and humidity (Tables 14, 15, 29-32). There were no smell or colour changes observed during the test period on tablets behavior. One effect appears to be an increase in the stability of certain drugs in tablet form.
A device has been shown to increase the hardness of tablets and lower the ejection forces (Sixmith, 1977). These results were explained in terms of the non-adherent properties of the celluloses. This explained the magic rule in adjusting the particle size variations in the physicochemical properties of tablets. One generalization that is sometimes ignored is that the required tablet hardness is often most readily achieved by judicious choice and optimum concentration of the granulating agent or adding a combined binding agent as used in these formulae (Tables 15-17, 29-32).

Increased binder concentration of 2% guar, acacia, starch, or PVP resulted in increased tablet hardness and decreased dissolution rate of S. argel tablets. Therefore, selection of the binder type and concentration should be properly controlled so that the medicament will be completely released for quick physiological availability (Jacob and Plein, 1968).

Most studies involving binders are concerned with the properties of the finished tablet. Comparison of the mechanical properties of granules did not drive a definite conclusion because of the large scattered results attached to symmetry of granules prepared in a conventional way. Later, to obviate this difficulty, a template method was used to obtain cylindrical granules and the effects of the binders, namely PVP and maize starch, were investigated (Doelker and shottan 1977).

The effects of volume, type and concentration of the different types of binders, namely water, alcohol, PVP, guar, gum, and starch on the granules were studied. It was found that the mean diameter of the granules prepared with alcohol was 50% that prepared with water and povidone exhibited the same particle diameter as alcohol but friability decreased (Abd-alim et al., 1987). In this study, disintegration tests were done because they would reflect the easiness of
dispersion of these formulae ready for dissolution, and disintegrants used were MCC, starch, sugar or guar.

4.6. Quality Assurance Studies

4.6.1. Studies of Stability in Formulation and Manufacturing

The scope and design of a stability study vary according to the product and the manufacturer concerned. Ordinarily the formulator of a product first determines the effects of temperature, light, air, pH, moisture, and traces metals, and commonly used excipients or solvents on the active ingredients. From this information, one or more formulations of each dosage from were prepared in suitable containers, and stored under a variety of environmental conditions, both exaggerated and normal.

Therefore, stability studies are based on the use of varying degrees of temperature, time, humidity, light intensity, and particle vapour pressure, and their application to the product in question. Types of stability tests are:

4.6.2. Stability Consideration in Formulation Practice

Stability is defined as the extent to which a product retains, within specified limits, and through its period of storage and use, i.e. its shelf-life, the same properties and characteristics that it possessed at the time of it manufacture.

Five type of stability are generally recognized as criteria for acceptable levels of stability:

a) Chemical Stability: a condition maintained throughout the shelf life of the drug product. Each active ingredient retains its chemical integrity and labeled potency, within the specified limits;

b) Physical Stability: with the original physical properties including appearance, palatability, uniformity, dissolution and suspendability being retained;

c) Microbiological Stability: as sterility or resistance to microbial growth being retained according to the specified requirements. Antimicrobial agents present retain the effectiveness within the specified limits;

d) Therapeutic Stability: with the therapeutic effects remaining unchanged; and e) Toxicological Stability: with no significant increase in the toxicity occurring.

4.6.3. Stability Testing

These are a series of tests designed to obtain information on the stability of pharmaceutical products for definition of shelf life and utilization period under specific packaging and storage conditions. At appropriate time intervals, samples of the products were assayed for potency by use of a stability indicated methods, observed for physical changes. Such a study in combination with clinical and toxicological results enables the manufacturer to select the optimum formulation and container and to assign recommended storage conditions and an expiration date for each dosage form on its package.

4.6.4. Accelerated Stability Testing

These are studies designed to increase the rate of chemical degradation and physical change of drug by using exaggerated storage conditions, and container/closure systems. They are applied in:

a) development of the product to select an adequate formulation and container/closure system, and in

b) development and registration dossier to determine shelf-life and storage conditions optimum for product stability.
They should be used consistently throughout a particular stability study because the results would vary with specific mode of the tests apparatus. Stability of tablets can be followed by appropriate colorimeter or reflectometer with heat, sun light and intense artificial light employed to accelerate the colour determination. These experiments involved the use of normal room light, with two electrical lamps being added.

Disintegration test was used in stability studies to detect periodic gross changes in the physical characteristics of tablets, but these tests were followed by dissolution rate study of tablet product content, to see whether it is affected or not (Tables 33-45).

Also the uniformity of weight, diameter, odour, texture, mottling, cracks, or friability, and powder contents in the containers were observed during the stability studies. Paper chromatography, PC, and thin layer chromatography, TLC, in BAW were used to look for the similar spots when sprayed with Dragendorff’s reagent.

The different S. argel tablets were found always stable, where no changes occurred in these S. argel tablets contents at different temperatures and relative humidities (Tables 33-45).

Disintegration test may be used to detect periodic gross changes in physical characteristics of tablet, but these tests must be correlated with dissolution rate study of a particular tablet product. Uniformity of weight, odour and texture, drug content and moisture are also investigated during a stability study of tablets (Linter 1982).

Colour stability of tablets can be followed by appropriate colorimeter or reflectometer with heat, sunlight and intense artificial light employed to accelerate the colour determination in this experiment and just using ordinary room light.

### 4.6.5. Stability of Solenostemma argel Tablets of the Different Formulae

The stability of a pharmaceutical product may be defined as the capability of a particular formulation, in a specific container/closure system, to remain within its physical, chemical, microbiological, therapeutic and toxicological specifications.

Stability of a drug can also be defined as the time from the date of manufacture and packaging of the formulation until its chemical or biological activity is not less than predetermined level of labeled potency, and its physical characteristics have not changed appreciably or deleteriously (Linder, 1985).

Eschkovski (1994) defined stability as the ability of a pharmaceutical product to retain its properties within specified limits throughout its shelf life, and the aspects of stability to be considered are chemical, physical, microbiological, and biopharmaceutical.

Long-term stability is defined as evaluation of experiments for physical, chemical, biological, and microbiological characteristics of a drug during and beyond the expected time of shelf life and storage of samples at the expected condition in the intended market (Echkovski 1994).

This may be studied by storage of the products at 45°C and at room temperature for two years. Starch and PVP are reported to have stable physicochemical properties.

In general, the quality control tests, together with the real-time study for stability, and in the development phase, accelerated stability tests, were carried out to compare in short-term experiments alternative formulations, packaging and manufacturing processes.

As soon as the final formulation and manufacturing process have been established, the manufacturer would carry out a series of accelerated stability tests, which will permit prediction of the stability.

Tests conditions are determined by the intended climatic zones in which the drug product would be used, as well as by the type of dosage forms. As a rule, accelerated studies are less suitable for semi-solids and emulsions.
In hot climatic zones, the product can be stored at 40°C and 75% RH for 6 months. Storage at higher temperatures for shorter time periods may be applied, e.g. three months at 45-50°C and 75% RH, especially in hot climatic zones.

In the stability studies of different S. argel tablets, the effects of different storage conditions, e.g. room temperature, 30±15°C and RH 60±15%, or 37±2°C and RH 70±5% or 50±5°C and RH 70±5%, were followed for 6, 12, 24, 36 and 48 months to determine the effects of these conditions on the physicochemical properties of these tablets.

In the stability studies of tablets, stable tablets would retain their original physical properties, such as size, shape, weight, and color, under normal handling and storage conditions throughout their shelf-life; in addition the in vitro availability of the active ingredient should not change appreciably with time.

Excessive powder or solid at the bottom of the container, cracks or chips on the surface of the tablets, or appearance of crystals on the surface of the tablets or container walls are indication of instability of uncoated tablets.

All these instability features were not detected in all S. argel tablets of the different formulae during different storage conditions, except for zone in some formulae and the powders in others.

The scope and design of stability study vary according to the product and the manufacturer concerned. Ordinarily, the formulator of a product first determines the effects of temperature, light, air, pH, moisture, and trace metals, in addition to the commonly used excipients or solvents on the active ingredients.

Based on this information, one or more formulations of tablets of different formulae were prepared in suitable containers and stored under a variety of environmental condition, both exaggerated and normal.

These stability tests were carried out by placing 100 tablets of each formula at room temperature at 30°C±15°C and RH 60%±15%; in an incubator at 37°C±2°C and RH 70%±5%; and in another incubator at 50°C±5°C and RH 70%±5%, for intervals extending from 6 months to 48 months, 50°C±2°C within a full dish of water to keep humidity at (70%±5%) for 6 month.

The evaluation of the physicochemical properties was done at zero time, and at the specified time intervals. The results were tabulated for each formula as in Tables 33-45.

Evaluation of the relative importance of the formulation and process variables was followed. At appropriate time intervals, samples of the products were assayed for potency by using stability-indicated methods, and observed for physicochemical characters and properties changes including: uniformity of tablet weight, diameters, thickness, microbial growth, and other organoleptic observations, e.g. odor, color, texture and hygroscopicity of tablets, tablet hardness, friability, disintegration time, dissolution rate of tablet constituents carried out by a UV spectrophotometer, powder contents estimation with time, and mottling, capping, and lamination, if any, were recorded.

In the tested tablets, only in certain formulae, there were changes in the hardness, odor, friability and powder contents. This might be due to the amount and type of the constituents (plant material whether leaves, extracts or alkaloids), diluents, additives, or even the punches diameters (Tables 33-45).

Such stability studies, in combination with clinical and toxicological investigations, enable the workers to select the optimum formulations and to assign recommended storage conditions and expiration date for each dosage form in its package. The paper packaging and plastic containers used for the packaging of different formulae were found to be affected by time at the normal room temperature, while it was to some extend more stable in glass-type containers.
There was no observed changes in the constituents for forty eight months, and this was deduced from the dissolution readings recorded (Tables 33-45).

Loss of potency usually results from chemical changes, the most common chemical reactions being hydrolysis, oxidation, reduction, and photolysis. Chemical changes may occur also through interactions of the formulation ingredients within the product, and rarely between the products and containers.

A parented loss of potency in the active ingredients may result from diffusion of the drug substances into or its combination with the surface of the container-closure system. The chemical potency of the active ingredients was required to remain within the limits specified in the monograph definition; potency was determined by means of an assay procedure (USP, 2000).

The different drug products have to conform to and meet the prevailing standards of Federal registration requirements (1973). With respect to a drug product effectiveness, the bioavailability has been defined as the extent and rate of absorption from a dosage form as reflected by a time-concentration curve of the administered drug product in the systemic circulation (Panel, 1974).

Table 33: Stability Studies of *Solenostemma argel* Leaves Tablets*
At Room Temperature (30°C), Formula C,
Using PVP as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Zero Time</th>
<th>Six months</th>
<th>Twelve months</th>
<th>Twenty four Months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>300±40</td>
<td>310±50</td>
<td>305±24</td>
<td>305±50</td>
<td>300±50</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>11.0±0.4</td>
<td>11.0±0.5</td>
<td>11.0±0.4</td>
<td>11.0±0.5</td>
<td>30.0±0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for <em>Escherichia coli</em> Salmonella spp.</td>
<td>Negative Negative</td>
<td>Negative Negative</td>
<td>Negative Negative</td>
<td>Negative Negative</td>
<td>Negative Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography In B:A:W**</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
</tr>
<tr>
<td>6</td>
<td>Total Plate Count (CFU)</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
</tr>
<tr>
<td>7</td>
<td>Thickness</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>No.</td>
<td>Parameter</td>
<td>Zero time</td>
<td>Six months</td>
<td>Twelve months</td>
<td>Twenty four Months</td>
<td>Specifications</td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------------------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------</td>
<td>-------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>1</td>
<td>Average Weight</td>
<td>300 ±40</td>
<td>300 ±50</td>
<td>304 ±20</td>
<td>310 ±50</td>
<td>300 ±50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time</td>
<td>11.0 ±0.4</td>
<td>11.0 ±0.5</td>
<td>11.0 ±0.4</td>
<td>11.0 ±0.4</td>
<td>30.0 ±0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for <em>Escherichia coli</em> and <em>Salmonella spp.</em></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography In B:A:W**</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
</tr>
<tr>
<td>6</td>
<td>Total Plate Count (CFU)</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>No.</td>
<td>Thickness (±SEMmm)</td>
<td>3.0 ±0.5</td>
<td>3.0 ±0.5</td>
<td>3.0 ±0.5</td>
<td>3.0 ±0.5</td>
<td>3.0 ±0.5</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>8</td>
<td>Diameter (±SEMmm)</td>
<td>9.0 ±0.5</td>
<td>9.0 ±0.5</td>
<td>9.1 ±0.5</td>
<td>9.1 ±0.5</td>
<td>9.0 ±0.5</td>
</tr>
<tr>
<td>9</td>
<td>Friability (%)</td>
<td>0.035</td>
<td>0.035</td>
<td>0.038</td>
<td>0.040</td>
<td>0.038</td>
</tr>
<tr>
<td>10</td>
<td>Hardness (±SEMkg/inch)</td>
<td>4.5 ±0.5</td>
<td>4.5 ±0.5</td>
<td>4.2 ±0.5</td>
<td>4.0 ±0.5</td>
<td>4.5 ±0.5</td>
</tr>
<tr>
<td>11</td>
<td>Mottling</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Picking Sticking</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Lamination and Capping</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Batch Number: PO100, Code: SPC; Manufacturing Date: Jan, 2000; Formula: C, with PVP, 4%; Packaging: Plastic Containers; Storage Conditions: At Temperature, 37°C ± 2°C; Relative Humidity, 70% ± 5%; Storage Period: 24 Months; **: 7 standard areas detected under UV

Table 35: Accelerated Stability Studies of Solenostemma argel Leaves Tablets*
At Temperature 50°C, Formula C, Using PVP as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Time Parameter</th>
<th>Zero Time</th>
<th>One Month</th>
<th>Two months</th>
<th>Three months</th>
<th>Six months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>290 ±20</td>
<td>300 ±30</td>
<td>305 ±50</td>
<td>300 ±40</td>
<td>305 ±50</td>
<td>300 ±50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>11.0 ±0.5</td>
<td>11.0 ±0.5</td>
<td>11.0 ±0.5</td>
<td>11.0 ±0.5</td>
<td>11.0 ±0.5</td>
<td>11.0 ±0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for Escherichia coli Salmonella spp.</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography In B:A:W**</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
</tr>
<tr>
<td>6</td>
<td>Total Plate Count (CFU)</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
<td>&lt;200</td>
</tr>
<tr>
<td>7</td>
<td>Thickness</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>2.9</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>No.</td>
<td>Parameter</td>
<td>Zero Time</td>
<td>Six Months</td>
<td>Twelve months</td>
<td>Twenty four Months</td>
<td>Specifications</td>
<td></td>
</tr>
<tr>
<td>-----</td>
<td>-----------------------------------</td>
<td>-----------</td>
<td>------------</td>
<td>---------------</td>
<td>--------------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>251 ±50</td>
<td>250 ±50</td>
<td>252 ±50</td>
<td>251 ±50</td>
<td>250 ±50</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>12.0 ±0.5</td>
<td>12.0 ±0.5</td>
<td>12.0 ±0.5</td>
<td>11.0 ±0.5</td>
<td>12.0 ±0.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Escherichia coli Salmonella spp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography In B:A:W**</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Total Plate Count (CFU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Batch Number: PO100, Code: SPC; Manufacturing Date: Jan, 2000; Formula: B, with PVP, 4%; Packaging: Plastic Containers; Storage Conditions: At Temperature, 50°C±2°C; Relative Humidity, 70%±5%; Storage Period: Six Months.

**: 7 standard areas detected under UV

Table 36: Stability Studies of Solenostemma argel Extract Tablets*
At Room Temperature (30°C), Formula B, Using PVP as a Binding Agent
<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Thickness (±SEMmm)</th>
<th>Diameter (±SEMmm)</th>
<th>Friability (%)</th>
<th>Hardness (±SEMkg/inch)</th>
<th>Mottling</th>
<th>Picking Sticking</th>
<th>Lamination and Capping</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td></td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.019</td>
<td>6.3 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.019</td>
<td>6.5 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.019</td>
<td>6.5 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.019</td>
<td>6.5 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>Mottling</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Picking Sticking</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Lamination and Capping</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Batch Number: EO100, Code: SEB; Manufacturing Date: Jan, 2000; Formula: B, with PVP, 4%; Packaging: Plastic Containers; Storage Conditions: At Room Temperature, 30°C±15°C; And Relative Humidity, 60%±15%; Storage Period: 24 Months; **: 7 standard areas detected under UV

Table 37: Stability Studies of *Solenostemma argel* Extract Tablets*
At Temperature 37°C, Formula B, Using PVP as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Zero Time</th>
<th>Six Months</th>
<th>Twelve months</th>
<th>Twenty four Months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>251 ± 50</td>
<td>251 ± 50</td>
<td>252 ± 50</td>
<td>252 ± 50</td>
<td>250 ± 50</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>12.0 ± 0.5</td>
<td>12.0 ± 0.5</td>
<td>12.0 ± 0.5</td>
<td>11.0 ± 0.5</td>
<td>12.0 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for <em>Escherichia coli</em> &amp; <em>Salmonella spp.</em></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography In B:A:W**</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
</tr>
<tr>
<td>No.</td>
<td>Parameter</td>
<td>Zero Time</td>
<td>One Month</td>
<td>Two Months</td>
<td>Three Months</td>
<td>Six Months</td>
</tr>
<tr>
<td>-----</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>------------</td>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>251 ±20</td>
<td>250 ±30</td>
<td>252 ±50</td>
<td>252 ±44</td>
<td>252 ±50</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>12.0 ±0.5</td>
<td>12.0 ±0.5</td>
<td>12.0 ±0.5</td>
<td>12.0 ±0.5</td>
<td>11.5 ±0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for <em>Escherichia coli</em> <em>Salmonella spp.</em></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography In B:A:W**</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
<td>7 standard areas</td>
</tr>
</tbody>
</table>

*:Batch Number: EO100, Code: SEB; Manufacturing Date: Jan, 2000; Formula: B, with PVP, 4%; Packaging: Plastic Containers; Storage Conditions: At Temperature, 37°C±2°C; Relative Humidity, 70%±5%; Storage Period: 24 Months; **: 7 standard areas detected under UV

Table 38: Accelerated Stability Studies of *Solenostemma argel* Extract Tablets*
At Temperature 50°C, Formula B,
Using PVP as a Binding Agent
<table>
<thead>
<tr>
<th>No.</th>
<th>Total Plate Count (CFU)</th>
<th>Thickness (±SEMmm)</th>
<th>Diameter (±SEMmm)</th>
<th>Friability (%)</th>
<th>Hardness (±SEMkg/inch)</th>
<th>Mottling</th>
<th>Picking Sticking</th>
<th>Lamination And capping</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.019 ± 0.019</td>
<td>6.5 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.019 ± 0.019</td>
<td>6.5 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.020 ± 0.020</td>
<td>6.3 ± 0.3</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.020 ± 0.020</td>
<td>6.3 ± 0.3</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.021 ± 0.019</td>
<td>6.2 ± 0.1</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.019 ± 0.019</td>
<td>6.3 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.020 ± 0.020</td>
<td>6.2 ± 0.1</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>&lt; 200</td>
<td>3.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>0.021 ± 0.019</td>
<td>6.3 ± 0.5</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Batch Number: EO100, Code: SEB; Manufacturing Date: Jan, 2000; Formula: B, with PVP, 4%; Packaging: Plastic Containers; Storage Conditions: At Temperature, 50°C ± 2°C; Relative Humidity, 70% ± 5%; Storage Period: Six Months.

**: 7 standard areas detected under UV

---

Table 39: Shelf Life Stability of *Solenostemma argel* Leaves Tablets*
At Room Temperature (30°C), Formula C, Using Guar as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Time Parameter</th>
<th>Zero Time</th>
<th>Six months</th>
<th>Twelve months</th>
<th>Twenty four Months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>310 ± 50</td>
<td>305 ± 50</td>
<td>300 ± 50</td>
<td>300 ± 50</td>
<td>300 ± 50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>15.0 ± 0.5</td>
<td>15.0 ± 0.5</td>
<td>14.0 ± 0.4</td>
<td>14.0 ± 0.4</td>
<td>30.0 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for <em>Escherichia coli</em> Salmonella spp.</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography</td>
<td>7 standard</td>
<td>7 standard</td>
<td>7 standard</td>
<td>7 standard</td>
<td>7 standard</td>
</tr>
</tbody>
</table>

---
In B:A:W**

<table>
<thead>
<tr>
<th></th>
<th>Total Plate Count (CFU)</th>
<th>areas</th>
<th>areas</th>
<th>areas</th>
<th>areas</th>
<th>areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td></td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>7</td>
<td>Thickness (±SEMmm)</td>
<td>3.0</td>
<td>±0.5</td>
<td>3.0</td>
<td>±0.5</td>
<td>3.1</td>
</tr>
<tr>
<td>8</td>
<td>Diameter (±SEMmm)</td>
<td>9.2</td>
<td>±0.5</td>
<td>9.1</td>
<td>±0.5</td>
<td>9.2</td>
</tr>
<tr>
<td>9</td>
<td>Friability (%)</td>
<td>2.9</td>
<td>±0.5</td>
<td>2.6</td>
<td>±0.5</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>Hardness (±SEMkg/inch)</td>
<td>4.75</td>
<td>±0.5</td>
<td>4.5</td>
<td>±0.5</td>
<td>5.0</td>
</tr>
<tr>
<td>11</td>
<td>Mottling</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Picking Sticking</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Lamination and Capping</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Batch Number: GO100, Code: SG; Manufacturing Date: Jan, 2000; Formula: C, with Guar, Packaging: Plastic Containers; Storage Conditions: At Room Temperature, 30°C±15°C; And Relative Humidity, 60%±15%; Storage Period: 24 Months; **: 7 standard areas detected under UV.

Table 40: Stability Studies of Solenostemma argel Leaves Tablets*
At Temperature 37°C, Formula C, Using Guar as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Time Parameter</th>
<th>Zero Time</th>
<th>Six months</th>
<th>Twelve months</th>
<th>Twenty four Months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>300 ±50</td>
<td>300 ±50</td>
<td>291 ±40</td>
<td>290 ±20</td>
<td>300 ±50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>15.0 ±0.5</td>
<td>15.0 ±0.5</td>
<td>15.0 ±0.4</td>
<td>14.0 ±0.4</td>
<td>30.0 ±0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for Escherichia coli Salmonella spp.</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper Chromatography</td>
<td>7 standard</td>
<td>7 standard</td>
<td>7 standard</td>
<td>7 standard</td>
<td>7 standard</td>
</tr>
</tbody>
</table>

CLXIII
<table>
<thead>
<tr>
<th>No.</th>
<th>In B:A:W**</th>
<th>areas</th>
<th>areas</th>
<th>areas</th>
<th>areas</th>
<th>areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Total Plate Count (CFU)</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>7</td>
<td>Thickness (±SEMmm)</td>
<td>3.1  ±0.5</td>
<td>3.0  ±0.5</td>
<td>3.0  ±0.5</td>
<td>3.0  ±0.4</td>
<td>3.0  ±0.5</td>
</tr>
<tr>
<td>8</td>
<td>Diameter (±SEMmm)</td>
<td>9.01 ±0.5</td>
<td>9.02 ±0.5</td>
<td>9.01 ±0.5</td>
<td>9.01 ±0.5</td>
<td>9.0  ±0.5</td>
</tr>
<tr>
<td>9</td>
<td>Friability (%)</td>
<td>2.5</td>
<td>2.5</td>
<td>2.8</td>
<td>2.8</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>10</td>
<td>Hardness (±SEMkg/inch)</td>
<td>4.5  ±0.5</td>
<td>4.5  ±0.5</td>
<td>4.7  ±0.5</td>
<td>4.3  ±0.5</td>
<td>4.5  ±0.5</td>
</tr>
<tr>
<td>11</td>
<td>Mottling</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Picking Sticking</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Lamination and Capping</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Batch Number: GN100, Code: SGC; Manufacturing Date: Jan, 2000; Formula: C, with Guar, Packaging: Plastic Containers; Storage Conditions: Temperature, 37°C ± 2°C; Relative Humidity, 70% ± 5%; Storage Period: 24 Months;
**:7 standard areas detected under UV

Table 41: Accelerated Stability Studies of Solenostemma argel Leaves Tablets*
At Temperature 50°C, Formula C,
Using Guar as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Time Parameter</th>
<th>Zero Time</th>
<th>One Month</th>
<th>Two months</th>
<th>Three months</th>
<th>Six months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>300 ±50</td>
<td>300 ±30</td>
<td>300 ±50</td>
<td>295 ±40</td>
<td>290 ±50</td>
<td>330 ±50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
<td>Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>15.0 ±0.5</td>
<td>15.0 ±0.5</td>
<td>15.0 ±0.5</td>
<td>15.0 ±0.5</td>
<td>14.0 ±0.5</td>
<td>15.0 ±0.5</td>
</tr>
<tr>
<td>4</td>
<td>Microbiological Tests for Escherichia coli, Salmonella spp.</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Paper</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

CLXIV
<table>
<thead>
<tr>
<th>No.</th>
<th>Chromatography In B:A:W**</th>
<th>standard areas</th>
<th>standard areas</th>
<th>standard areas</th>
<th>standard areas</th>
<th>standard areas</th>
<th>standard areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Total Plate Count (CFU)</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>7</td>
<td>Thickness (±SEMmm)</td>
<td>2.9 ± 0.5</td>
<td>2.95 ± 0.5</td>
<td>3.0 ± 0.5</td>
<td>3.0 ± 0.5</td>
<td>3.0 ± 0.5</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>Diameter (±SEMmm)</td>
<td>9.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
<td>9.0 ± 0.5</td>
</tr>
<tr>
<td>9</td>
<td>Friability (%)</td>
<td>2.5</td>
<td>2.4</td>
<td>3.9</td>
<td>2.6</td>
<td>2.7</td>
<td>≤ 1.0</td>
</tr>
<tr>
<td>10</td>
<td>Hardness (±SEMkg/inch)</td>
<td>4.5 ± 0.5</td>
<td>4.5 ± 0.4</td>
<td>4.2 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>4.0 ± 0.35</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>11</td>
<td>Mottling</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Picking Sticking</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Lamination And capping</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Batch Number: GO100, Code: SGC; Manufacturing Date: Jan, 2000; Formula: C, with Guar; Packaging: Plastic Containers; Storage Conditions: Temperature, 50°C ± 2°C; Relative Humidity, 70% ± 5%; Storage Period: Six Months.

**: 7 standard areas detected under UV.

Table 42: Stability Studies of *Solenostemma argel* Leaves Tablets*
At Room Temperature (30°C), Formula C, Using Maize Starch as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Time Parameter</th>
<th>Zero Time</th>
<th>Six Months</th>
<th>Twelve Months</th>
<th>Twenty Four Months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>306 ± 50</td>
<td>310 ± 50</td>
<td>298 ± 40</td>
<td>290 ± 20</td>
<td>360 ± 50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time (SEM±min)</td>
<td>12.0 ± 0.5</td>
<td>12.0 ± 0.5</td>
<td>12.0 ± 0.4</td>
<td>13.0 ± 0.4</td>
<td>30.0 ± 0.5</td>
</tr>
</tbody>
</table>

CLXV
Table 43: Stability Studies of Solenostemma argel Leaves Tablets*
At Temperature 37°C, Formula C,
Using Maize Starch as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Zero Time</th>
<th>Six months</th>
<th>Twelve months</th>
<th>Twenty four Months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>300 ±50</td>
<td>310 ±50</td>
<td>310 ±40</td>
<td>320 ±50</td>
<td>300 ±50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration Time</td>
<td>12.0</td>
<td>12.0</td>
<td>12.0</td>
<td>13.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

*: Batch Number: MO100, Code: SMC; Manufacturing Date: Jan, 2000; Formula: C, with Methylcellulose**, Packaging: Plastic Containers; Storage Conditions: Room Temperature, 30°C±15°C; And Relative Humidity, 60%±15%; Storage Period: 24 Months; **: 7 standard areas detected under UV.
### Table 44: Accelerated Stability Studies of *Solenostemma argel* Leaves Tablets*

At Temperature 50°C, Formula C, Using Maize Starch as a Binding Agent

<table>
<thead>
<tr>
<th>No.</th>
<th>Time Parameter</th>
<th>Zero time</th>
<th>One month</th>
<th>Two months</th>
<th>Three months</th>
<th>Six months</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Average Weight (±SEMmg)</td>
<td>290 ±50</td>
<td>290 ±30</td>
<td>295 ±50</td>
<td>295 ±40</td>
<td>295 ±50</td>
<td>300 ±50</td>
</tr>
<tr>
<td>2</td>
<td>Colour</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
<td>Grayish Green</td>
</tr>
<tr>
<td>3</td>
<td>Disintegration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Batch Number: MO100, Code: SMC; Manufacturing Date: Jan, 2000; Formula: C, with Methylcellulose, Packaging: Plastic Containers; Storage Conditions: Temperature, 37°C±2°C; Relative Humidity, 70%±5%; Storage Period: 24 Months; **: 7 standard areas detected under UV.
<table>
<thead>
<tr>
<th></th>
<th>Time (SEM±min)</th>
<th>12.0 ±0.5</th>
<th>12.0 ±0.5</th>
<th>12.0 ±0.5</th>
<th>13.0 ±0.5</th>
<th>12.0 ±0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Microbiological Tests for <em>Escherichia coli</em> and <em>Salmonella spp.</em></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Paper Chromatography In B:A:W**</th>
<th>7 standard areas</th>
<th>7 standard areas</th>
<th>7 standard areas</th>
<th>7 standard areas</th>
<th>7 standard areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Total Plate Count (CFU)</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
<td>&lt; 200</td>
</tr>
<tr>
<td>6</td>
<td>Thickness (±SEMmm)</td>
<td>3.1 ±0.5</td>
<td>3.1 ±0.5</td>
<td>3.1 ±0.5</td>
<td>3.1 ±0.5</td>
<td>3.0 ±0.5</td>
</tr>
<tr>
<td>7</td>
<td>Diameter (±SEMmm)</td>
<td>9.1 ±0.5</td>
<td>9.1 ±0.5</td>
<td>9.01 ±0.5</td>
<td>9.02 ±0.5</td>
<td>9.01 ±0.5</td>
</tr>
<tr>
<td>8</td>
<td>Friability (%)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>9</td>
<td>Hardness (±SEMkg/inch)</td>
<td>4.75 ±0.5</td>
<td>4.75 ±0.4</td>
<td>4.75 ±0.5</td>
<td>4.5 ±0.5</td>
<td>4.5 ±0.5</td>
</tr>
<tr>
<td>10</td>
<td>Mottling</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>11</td>
<td>Picking</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>12</td>
<td>Sticking</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>13</td>
<td>Lamination And capping</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Batch Number: MO100, Code: SMC; Manufacturing Date: Jan, 2000; Formula: C, with Methylcellulose; Packaging: Plastic Containers; Storage Conditions: Temperature, 50°C±2°C; Relative Humidity, 70%±5%; Storage Period: Six Months.

**: 7 standard areas detected under UV.

### 4.6.6. On-Going Stability Studies (Four Years) Results

Table 45: On-going Stability Studies, for Four-years, of *Solenostemma argel* Tablets, of the different Formulae, stored at Room Conditions, (Temperature 30°C±15°C and RH 60%±15%)

<table>
<thead>
<tr>
<th>Formula</th>
<th>Binder (%)</th>
<th>Diameter (mm)</th>
<th>Hardness kg/in²</th>
<th>Disintegration Time (min)</th>
<th>Dissolution Rate (UV Reading)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Aqueous Alkaloids Extract</td>
<td>-</td>
<td>4</td>
<td>14</td>
<td>0.2530</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-------</td>
<td>-----</td>
<td>----</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>4% PVP</td>
<td>9</td>
<td>4.5</td>
<td>19</td>
<td>0.2475</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4%PVP</td>
<td>12</td>
<td>2.6</td>
<td>15</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>8% Starch +4%PVP</td>
<td>9</td>
<td>4.5</td>
<td>27.5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4% PVP</td>
<td>9</td>
<td>8.7</td>
<td>9</td>
<td>0.2475</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>8% Starch +2%PVP</td>
<td>12</td>
<td>2.108</td>
<td>9</td>
<td>0.445</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2% Guar</td>
<td>12</td>
<td>1.75</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>6% Guar +2%PVP +10%DiSol +5%MCC</td>
<td>-</td>
<td>2.0</td>
<td>38</td>
<td>0.314</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>10%PVP +5%MCC +10%DiSol</td>
<td>-</td>
<td>3.1</td>
<td>31</td>
<td>0.312</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2% Guar +2%PVP</td>
<td>9</td>
<td>3.0</td>
<td>23</td>
<td>&lt; 0.5</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2% Guar +2%PVP</td>
<td>12</td>
<td>1.9</td>
<td>3.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4%PVP</td>
<td>12</td>
<td>2.5</td>
<td>14</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>8%Starch +2%PVP</td>
<td>12</td>
<td>2.0</td>
<td>12</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4%PVP</td>
<td>9</td>
<td>3.0</td>
<td>12</td>
<td>0.223</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>8%Starch +4%PVP</td>
<td>9</td>
<td>3.2</td>
<td>10</td>
<td>0.267</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>4%PVP</td>
<td>12</td>
<td>2.18</td>
<td>10</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Alkaloids Extract</td>
<td>-</td>
<td>15.0</td>
<td>7</td>
<td>0.268</td>
<td></td>
</tr>
</tbody>
</table>
4.7. Clinical Studies

Before starting these clinical trials, both on healthy volunteers and on patients, a notice of claimed investigational exemption for new drug (IND) was claimed before the drug can be clinically tested on human beings. The clinical trials were conducted in two phases, the first one including ten health male volunteers, and the second one involving hundred patients of both genders.

These trials were performed for the evaluation of the safety and effectiveness of Solenostemma argel tablets and the assurance of safety and protection of the human subjects participating in these investigations, and if possible the determination of TD50/ED50, i.e. the therapeutic window or therapeutic ratio of Solenostemma argel tablets. This was done because this drug, if proven safe and effective, should be adopted for clinical use and would be a fairly straightforward task. The trial would be started with a low dosage, and gradually increasing the dose cautiously, and watching carefully for toxic effects. If the deduced therapeutic effect could be obtained with little toxicity in a large number of patients, it would be acceptable to increase the dosage well beyond the therapeutically effective range, with the aim of assessing by how much the therapeutic dose could be exceeded before toxicity ensued.

Broadly speaking any adverse drug effect may be thought of as a manifestation of drug toxicity. It will be useful, however to consider separately and in considerable detail, the following special categories of adverse drug effects: idiosyncrasy, drug allergy, tolerance and physical dependence, mutagenesis, teratogenesis and carcinogenesis. It is important to consider the adverse effects that are dose related, and experienced by most or all of the exposed population, usually at drug levels (or doses) in excess of those associated with a therapeutic action. Sometimes a toxic effect is simply an extension of the therapeutic effect at a higher dose level; often on the other hand, toxicity takes the form of a side effect more or less unrelated to the primary drug action. Examples are dryness of mouth seen frequently with atropine or hyoscine, and tachycardia and breathlessness with S. argel toxic doses.

If quantitative estimates of efficacy and toxicity could be carried out in humans, the decision whether a new drug should be adopted for clinical use would be a fairly straightforward decision.

4.7.1. A Questionnaire (II) Investigating the Practice of Herbal Medicines Use for Spasms and Colics

This questionnaire (II) was distributed among individuals who had the practice of using herbal medicines. The following findings were recorded:

Concerning the effectiveness of the medicinal herb used, sixty percent of the participants felt completely relieved of pain after a single administration of the herbal medicine; twenty six percent used the drug several times due to repeated attacks of pain; four percent had mild pain relief; and fifteen percent did not feel relieved of their colics and pains.

With regard to the origin of instructions to use a herbal medicine, seventy percent were advised by their parents and relatives, twenty-one percents by a pharmacists, and only four percent by doctors.

With respect to the herbal medicine source or supply, sixty two percent obtained it from the Sudanese herbal medicines local market and thirty eight from abroad.

When considering the part of the medicinal plant used, thirty percent of used the whole plant, twenty two percent the roots, fifteen percent the leaves, ten percent the stems, nine percent the seeds, six percent the fruits, two percent the flowers, and six percent used other parts of the plants or plant products.
With regard to the quantity or dose of herbal medicine to be used, eighty five percent used the preparation just by guessing or used an arbitrary quantity, while fifteen percent either measured or weighed specified amount of the herbal medicine.

Concerning the medicinal herb preparation, seventy five percent prepared the preparation by themselves or by their relatives at home, while twenty five percent of these preparations were dispensed by an herbal doctor.

When considering the types of herbal preparations used, forty five percent were decoctions, thirty eight percent were powder type, seventeen percent were macerated plants preparations.

4.7.2. Phase I

4.7.2.1. Monitoring Possible Adverse Reactions in Healthy Volunteers Receiving *Solenostemma argel* Leaves, Extracts and Alkaloids Tablets

Ten healthy male subjects, from the staff and students of the Faculty of Pharmacy, University of Khartoum, participated in these clinical trials at their own choice and will. They either use *Solenostemma argel* leaves in their ordinary life as a decoction or with milk, as a beverage and flavour, or as a medicine for treatment of various gastro-intestinal disturbances or minor ailments.

These subjects were well informed about the purpose and nature of the investigation, the preparations they were going to administer, the potential risk involved, and the possible side effects or adverse reactions which were likely to occur.

Following this information delivered to them, each of these volunteers signed a written consent indicating his participation in this study at his own will and choice. Each of these healthy subjects was subjected to a medical examination by a clinician; blood pressure and heart beats were measured before and after treatment, and all of them proved to be in very good health. Then, to monitor any possible side effects or adverse reactions to *S. argel* preparations, these trials were conducted under the supervision of a clinician, and started with low doses, e.g. one tablet of one of *S. argel* products, three times daily, and gradually increased till the highest dose used was attained, which was four tablets three times a day, for each of the leaves, extracts or alkaloids tablets.

<table>
<thead>
<tr>
<th>Participant No.</th>
<th>Age (year)</th>
<th>Heart Beats</th>
<th>Blurred Vision</th>
<th>Nausea and/or Vomiting</th>
<th>Mouth Dryness</th>
<th>Stomach Upset</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>28</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>45</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>45</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

*: Two Tablets of Each Formula Three Times for One Day
These volunteers were monitored or followed up for the occurrence of any side effects or adverse reactions following the administration of the specified doses of S. argel tablets. None of the volunteers complained of blurred vision, dryness of the mouth, change in heart beats (tachycardia or bradycardia), palpitation, headache or vomiting during the period of the trial. Only one of these patients complained of dryness of the mouth and another patient complained of stomach upset felt at the time of administration of the leaves tablets. None of the participants complained of any other side effects. Furthermore, no side effects were recorded in these subjects on using Solenostemma argel herb as a medicine for a week.

These observations and findings indicated the very high tolerability of Solenostemma argel tablets, of the different formulae, as no signs of adverse reactions or side effects were recorded when these tablets were administered in the doses stated.

4.7.3. Phase II

4.7.3.1. Therapeutic Effectiveness and Adverse Reactions of Solenostemma argel Leaves, Extracts and Alkaloids Tablets Administered to Patients

These clinical trials were organized in three teaching hospitals, namely Khartoum teaching hospital, Khartoum North teaching hospital and University of Khartoum clinic, under the supervision four physicians. One hundred patients, selected randomly, were 20-70 years old, and 40 males and 60 females. They were complaining of gastrointestinal disturbances, and diagnosed as having gastritis, indigestion, heartburn, flatulence, irritable bowel syndrome, renal colics, or colics due to menstrual pain.

<table>
<thead>
<tr>
<th>No. of Patients</th>
<th>Condition of Pain</th>
<th>No. of Patients</th>
<th>Case</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>Severe</td>
<td>40</td>
<td>Irritable Bowel Syndrome IBS</td>
</tr>
<tr>
<td>30</td>
<td>Moderate</td>
<td>25</td>
<td>Gastrointestinal Colics</td>
</tr>
<tr>
<td>45</td>
<td>Mild</td>
<td>20</td>
<td>Menstrual Disturbances</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>15</td>
<td>Renal Colics</td>
</tr>
</tbody>
</table>

*: minimum dose two leaves tablets or one-to-two extract tablets when necessary, up to two leaves tablets or one-to-two extract tablets four times a day

After being diagnosed, these patients were treated by using two tablets of Solenostemma argel leaves or extracts to be administered when necessary. The patients treated with S. argel tablets were followed up for the therapeutic effectiveness of the administered tablets as indicated by suppression of the symptoms, and relief of pain and colics, as well as being monitored for occurrence of side effects or adverse reactions. When necessary, another two-tablet dose was administered to patients when there was recurrence of pain, colics and other symptoms. In i.e., two tablets were administered three times daily, half an hour before meals.

The general observation in treatment of gastrointestinal disturbances with S. argel tablets was the quick suppression of symptoms and relief of pain occurring immediately following drug administration. However, in the case of renal colics, pain associated with menstrual disturbances, and irritable bowel syndrome, IBS, there might be a need for repetition of the treatment. The pain was relieved without any pronounced side effects or adverse reactions.
In the case of the patients (25) with gastrointestinal disturbances and spasm, the pain was relieved completely in all these patients (25, 100%); in the patients (20) with pain associated with menstrual cycle, the pain was relieved in most patients (18, 90%); in patients (15) with renal colics, pain was relieved in 11 patients (73%); while in 72% (29) of the patients (40) with IBS, pain was completely relieved. No side effects or adverse reactions were recorded, and the patients did not complain of any undesirable or intolerable toxic or adverse effects of these preparations of Solenostemma argel.

Therefore, it could be concluded that the different types (leaves, extracts or alkaloids) of Solenostemma argel tablets showed a very good therapeutic effectiveness (71%-100%), and a great margin of safety (98%-100%). These findings are very promising, but they need further confirmation in larger clinical trials involving more centres and larger numbers of clinicians and involving larger numbers of patients, as well as investigation of more experimental variables.

These S. argel preparations were well tolerated with a very low incidence of side effects, mainly mild abdominal discomfort with the powdered leaves tablets. In an experiment to investigate any possible teratogenic effects of S. argel alkaloids, ten pregnant rats were given a daily dose of 0.635g/kg of extracts or alkaloids for fifteen days. These pregnant rats gave normal birth to normal neonates (5-12), without any foetal abnormalities, desorption or deaths recorded during pregnancy, and no deformation of any organ or teratogenic effect was observed. This is an indication of the safety of Solenostemma argel extracts or alkaloids during pregnancy in rats.

Although no teratogenic effects had been recorded in the pregnant rats given S. argel extracts or alkaloids for 15 days, care must be taken when administering these preparations in early pregnancy.

4.7.3.2. Bioequivalence and Effectiveness of Solenostemma argel Leaves and Extracts Tablets in Patients with Irritable Bowel Syndrome, IBS

Forty patients with diagnosed irritable bowel syndrome, IBS, were treated with placebo tablets, generic tablets, S. argel leaves or extract tablets. Placebo tablets were prepared from lactose and starches and had a percent pain relief of 5% in IBS patients according to the findings of the present study. Solenostemma argel can be used successfully in the relief of pain of irritable bowel syndrome. The extract (88%) is more effective than the leaves (71%) tablets, and had a rapid onset of action in the pain relief.

To avoid hazards in human subjects, a convenient measure of the dose is the median effective dose (ED$_{50}$), i.e. the dose which will produce the specified therapeutic effect in 50% of all subjects. Drug potency may be of great interest to the medicinal chemist or pharmacologist who wishes to investigate the relationship between chemical structure and biological activity, but usually it is of no great importance to the clinician.

The trial was based on patients observations and comments on the relief of pain in the four groups of ten participants each, in a week rotation for one month, for each preparation. The extract tablets had an efficacy of 88%, while the leaves tablets recorded 71%, compared with the generic drug with a 90% efficacy; pain was not relieved in most patients (89%) receiving the placebo. Solenostemma argel extract tablets had a quicker onset of action and were more effective than the equivalent leaves tablets. No side effects were recorded with extract tablets, but in two patients mild stomach upset was recorded.

According to these findings, Solenostemma argel tablets, especially the extract or alkaloid formulations, would be quite useful in controlling the pain associated with IBS, by administering two tablets half an hour before meals.

It has been suggested by the clinicians to use a combination of herbal medicinal drugs including Alhargal (Solenostemma argel), Alsaikeran (Datura, Atropa belladonna) and Alna’ana (Peppermint, Mentha pipperita) for the gastrointestinal disturbance and
flatulence. Another combination of *S. argel*, *Mentha piperita* and *Ammi visnaga* (Alkhilla) has been suggested for renal colics and menstrual cycle pain. These combination products would offer patients with therapeutic advantages over any of the components of these herbal medicines alone, including possible synergistic effects due to the contribution of each component, higher margin of safety since the components would be used at lower concentrations. Therefore, these herbal combinations would be expected to have enhanced safety, effectiveness and even minimize the potential for abuse of the principal ingredients, if any.

Table 48: A Four-way Cross-over Design for Administration of Four Products to Four Groups of Patients for Four Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>LT</td>
<td>ET</td>
<td>GT</td>
<td>PT</td>
</tr>
<tr>
<td>II</td>
<td>PT</td>
<td>LT</td>
<td>ET</td>
<td>GT</td>
</tr>
<tr>
<td>III</td>
<td>GT</td>
<td>PT</td>
<td>LT</td>
<td>ET</td>
</tr>
<tr>
<td>IV</td>
<td>ET</td>
<td>GT</td>
<td>PT</td>
<td>LT</td>
</tr>
</tbody>
</table>

LT: *S. argel* Leaves Tablets; ET: Extracts Tablets
GT: Generic Tablets; PT: Placebo Tablets

The Compliance = \( \frac{N_t}{N_a} \times 100 \% \)

where \( N_a \) = the number of patients assessed in each time.
\( N_t \) = the total number of patients taking the product in the whole trial, which was 40.
Compliance of the patients calculated for each group in the four weeks was as follows:

Table 49: Compliance of the IBS Patients, Calculated for Each Group in the Four Groups, for Each Week in the Four Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1 (%)</th>
<th>Week 2 (%)</th>
<th>Week 3 (%)</th>
<th>Week 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>LT 80</td>
<td>ET 100</td>
<td>GT 100</td>
<td>PT 70</td>
</tr>
<tr>
<td>II</td>
<td>PT 90</td>
<td>LT 70</td>
<td>ET 70</td>
<td>GT 100</td>
</tr>
<tr>
<td>III</td>
<td>GT 90</td>
<td>PT 60</td>
<td>LT 60</td>
<td>ET 60</td>
</tr>
<tr>
<td>IV</td>
<td>ET 70</td>
<td>GT 60</td>
<td>PT 50</td>
<td>LT 40</td>
</tr>
</tbody>
</table>

LT: *S. argel* Leaves Tablets; ET: Extracts Tablets
GT: Generic Tablets; PT: Placebo Tablets

Complete pain relief in the patients in each group as percentage result was calculated by the following equation:

Percent pain relief by each product = Compliance X % pain relief in the group

where Percent Pain relief in the group \( (Pr) = \frac{N_r}{N_w} \times 100 \% \)

where \( P_r \) = Percent pain relief for a product in a group
\( N_r \) = Number of patients relieved of pain in a group by each product.
\( N_w \) = Number of patients participating in a group.
\( N_a \) = Number of attending patients
Table 50: Percent Pain Relief of the IBS Patients Calculated for Each Product in Each Group in the Four Groups, for Each Week in the Four Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1 (%)</th>
<th>Week 2 (%)</th>
<th>Week 3 (%)</th>
<th>Week 4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>LT 80</td>
<td>ET 100</td>
<td>GT 100</td>
<td>PT 0</td>
</tr>
<tr>
<td>II</td>
<td>PT 0</td>
<td>LT 70</td>
<td>ET 90</td>
<td>GT 100</td>
</tr>
<tr>
<td>III</td>
<td>GT 90</td>
<td>PT 10</td>
<td>LT 80</td>
<td>ET 80</td>
</tr>
<tr>
<td>IV</td>
<td>ET 80</td>
<td>GT 80</td>
<td>PT 20</td>
<td>LT 50</td>
</tr>
</tbody>
</table>

LT: S. argel Leaves Tablets; ET: Extracts Tablets
GT: Generic Tablets; PT: Placebo Tablets

Thus, it would ordinarily matter little whether the dose of a drug is 10mg, but there is an important exception to this generalization. If a drug is to be placed in a depot for slow absorption over a long time, the greater the potency the better. For them, the total amount employed can be reasonably small. In the case S. argel alkaloids tablets, the alkaloids content was nearly 4 times as that present in the dried aqueous extracts of S. argel, and therefore the dose of the alkaloids tablets is 1/4th that of the extract tablets. Usually, the smaller the tablet the easier to swallow it, and it may also be an additional advantage with oral medication as in S. argel tablets if it has gastric irritant effect, due to some properties unrelated to the therapeutic action, for the less amount that can be administered the better.

The essential attribute which may be lacking in a drug entity is efficacy at safe dose level. Ideally this means that there should be a wide range dosage between the effective and toxic dose. The ratio TD$_{50}$/ED$_{50}$ (or often, in animals LD$_{50}$/ED$_{50}$) is called the therapeutic index or ratio. A low ED$_{50}$ characterizes a potent drug, but if the TD$_{50}$ is also low, the margin of safety may be wholly inadequate. The therapeutic ratio is an initial crude indication of how safe a drug is likely to be. It is especially useful in a congeneric series, where many occlusions are likely to look after both potency and toxicity but not necessary to the same extent.

One problem in applying therapeutic ratio concept is that very few drugs are without some toxic side effect even at ordinary therapeutic dosage. The seriousness of the disease, the drug and the degree of damage likely to sustain as a result of drug toxicity, all have to be weighed. A minimum loss approach has been proposed, which would give appropriate weight to these factors. Curiously some of the most useful drugs are also among the most toxic; neither digitalis glycosides nor the opiate analgesics have favorable therapeutic ratios.
5. Summary and Conclusions

Pharmaceutical efforts aimed at utilizing medicinal herbs as formulated dosage forms are faced by three main problems when designing and formulating the appropriate dosage forms of such medicinal herbs or plants. These are: a) The type of formulation to be used to give an effective, safe and elegant dosage form by the appropriate route of administration; b) The dose and time of administration to give the required therapeutic activity for the proper period of time, without or with minimal side or toxic effects; and c) The stability of the drug in the formula during processing, storage and usage.

The principal objective of dosage form design is to achieve a predictable therapeutic response to a drug included in a formulation which is capable of large scale manufacture with reproducible product quality. Quality is the combination of attributes or characteristics of a product that determines the degree of acceptability of the product. The quality of a product is strongly related to and influenced by its design in research and development.

A questionnaire, used to investigate the practice of household usage of herbal medicines for different minor ailments, was distributed to three hundred inhabitants of AlDroshab area, Khartoum North. The data collected indicated that the medicinal herb most commonly used as a remedy for gastrointestinal disturbances was Solenostemma argel (used by 48% of the participants) compared to another four medicinal plants.

The popularity of Solenostemma argel leaves in Sudan and its very wide usage as an antispasmodic in its decoction or in powder form, were the motivation and persuasion to make a detailed multidisciplinary investigation of all aspects pertinent to the design, formulation and evaluation of a suitable and stable dosage form of Solenostemma argel leaves as powder, extracts or alkaloids.

Furthermore, surveillance of the available antispasmodic drugs showed the limitation of the high cost of some of the available products, or the common side effects and possible adverse reactions of others, e.g. dryness of the mouth and blurred vision. These observations further motivated and encouraged this work to be planned and conducted. In addition, success of this project and implementation of its findings may pave the way for other projects to be designed for further utilization of other useful medicinal plants used in folkloric practice.

The project was designed and conducted to deal with all the pertinent aspects of design, formulation and evaluation of an appropriate dosage form of Solenostemma argel leaves powders, extracts or alkaloids. Therefore, the work done was multidisciplinary and included:

The microbiological quality control of Solenostemma argel leaves and its preparations dealt with investigation of microbial contamination with specific tests for Escherichia coli and Salmonella spp. All leaves powders and products were negative for these two types of organisms. Levels of contamination in all these Solenostemma argel leaves products did not exceed 200 cfu/g.

The botanical and phytochemical experiments were aimed at the identification and classification of the herb, as well as the extraction, separation and identification of the main constituents of Solenostemma argel leaves.

In the botanical and phytochemical experiments, the general identity tests of Solenostemma argel leaves included macroscopical, microscopical, macro-chemical analysis, thin layer chromatography and column analysis for the presence of characteristic alkaloids and flavonoids. Different solvent systems were used for isolation.
of the active constituents using: a) silica gel columns eluted through with different solvent systems, and used for detection, separation, and isolation of flavonoids; b) alkaloids extraction from $S. \ argel$ homogenised leaves for complete extraction in a soxlet with different solvent systems, including methanol:water, chloroform:methanol, and chloroform to have both aqueous and chloroform extracts which were evaporated to dryness, extracted with methanol and tested for alkaloids.

The identification methods of $S. \ argel$ constituents used suitable reagents or tests for determination of the chemical identities of these active constituents, and included: ninhydrin, natural substances reagent and concentrated hydrochloric acid, coupled with UV, IR, NMR and MS spectral analysis, used for flavonoids identification; Dragendorff’s reagent, Mayer’s reagent and Mandelic acid for alkaloids identification; and foaming test for detection of the presence of saponins. For detection of flavonoids, a loin intense fluorescence was produced immediately or after 15minutes in UV spectrophotometer at $\lambda \ 365\text{nm}$. The identification tests confirmed the presence of both alkaloids and flavonoids in these extracts.

The phytochemical methods adopted in the course of this work included preparation of powdered $Solenostemma \ argel$ leaves, after inspection and identification, reduction to the required particle size, followed by sieving for powder separation and analysis. These $Solenostemma \ argel$ leaves powders were used for determination of identity, purity and quality of $S. \ argel$; for direct incorporation into tablet formulations, or for preparation of different extracts incorporated into tablet formulations. These included preparation and concentration of water extracts of $S. \ argel$ leaves, preparation of total alkaloids of $S. \ argel$ leaves, and identification of some constituents of $S. \ argel$ leaves.

Also these phytochemical experiments included detection, separation and identification of flavonoid compounds in the water or methanol extracts of $S. \ argel$ by using paper chromatography (PC) or thin layer chromatography (TLC, silica gel) run in B:A:W system (40:10:50). The chromatograms were sprayed with natural substances reagent or ninhydrin. Separation of the different flavonoid compounds was carried out by using an accelerated eluted solvent passage through a polyamide chromatographic column using different solvent systems. The pharmacological activities of the separated portion were checked on contracting rabbit’s small intestine. In addition, detection and identification of kaempferol-3-glucoside was carried out as a preliminary study of flavonoids of $S. \ argel$ extracts with light petroleum, chloroform, ethyl acetate, methanol, methanol-water, and water, and the extract was fractionated and finally the constituent compounds were purified and characterized by HPLC, UV, IR, NMR and MS spectra.

Furthermore, the phytochemical investigations included detection and identification of $Solenostemma \ argel$ alkaloids applied to TLC silica gel eluted in B:A:W system, detected by spraying with Dragendorff’s reagent, and color fixation by using 2M sulphuric acid ethanol solution. Spectrophotometer scan was carried for the highest pick of the most available alkaloidal constituents in $Solenostemma \ argel$ prepared extracts.

The pharmacological experiments were aimed at investigation of the biological activities of $Solenostemma \ argel$ leaves powder, extracts and alkaloids on different experimental models, and confirmation of the effectiveness of $S. \ argel$ preparations. $Solenostemma \ argel$ extracts showed inhibitory effects upon the spontaneous contractions as well as the induced contractions in contracting rabbit small intestine and guinea pig small intestine model by sub-maximal doses of acetylcholine (1$\mu$g/ml) or barium chloride (0.002mg/ml). The degrees of relaxation that occurred at these sub-maximal doses were estimated and compared with those produced by reference drugs such as hyoscine or papaverine. Other experimental models, including isolated rabbit heart and intact African toad heart, showed depressant effects on the myocardium of different $S. \ argel$ extracts, perfused continuously, leading to reduction of the heart rate and stroke.
Furthermore, the local anaesthetic effects of *S. argel* extracts were studied on African toad’s foot withdrawal reflex and compared with kaempferol, quercetin, rutin. Lignocaine was taken as a reference local anaesthetic used to calculate the relative potencies of the other tested drug substances. The relative potency of *S. argel* alkaloids was 50% of that of lignocaine and equivalent to that of kaempferol. *Solenostemma argel* alkaloids diffusion across rabbit small intestine was studied with and without the addition of 0.01g of sodium fluoride. Sodium fluoride resulted in inhibitory effects (70 to 90%) on *S. argel* alkaloids diffusion rate across the rabbit small intestine preparation. This indicated that an active transport mechanism was involved in the diffusion of *S. argel* alkaloids across the small intestine tissue, and which was blocked by sodium fluoride.

Furthermore, the median effective doses (ED₅₀) of *Solenostemma argel* alkaloids and hyoscine were calculated using the MedUSA system by recording the inhibitory effects of these two drug substances to reduce the contractions of guinea pig ileum model induced by 1 µg/ml of acetylcholine, at a sub-maximal dose. The means of reductions of the stimulated contractions recorded were calculated for each concentration and used to plot the relevant graphs. The median effective doses, ED₅₀, were calculated for both hyoscine (0.035mg) and *S. argel* alkaloids (0.275mg) to compare their antispasmodic effects; the activity of *S. argel* alkaloids was one-eighth that of hyoscine.

Toxicological experiments, using various experimental models, were aimed at further investigations of the possible toxic effects of *Solenostemma argel* preparations in different dosage regimens to ascertain the safety of these *S. argel* products. The toxicological investigations included evaluation of acute toxicity of *Solenostemma argel* by determination of the lethal doses of *Solenostemma argel* in Albino Canadian rats and local species rabbits. Intra-peritoneal doses of *S. argel* were carefully given every five minutes, without damaging a vein or a nerve, and starting from 0.025mg/kg dose and doubling it successively till the animal death occurred. The mean lethal dose was calculated for each group, and was found to be 6.35g/kg for the rabbits and 5.49g/kg for the rats (n=4).

Another toxicological parameter determined was the median lethal dose (LD₅₀) of *Solenostemma argel* alkaloids in sixty Albino rats assigned to six groups of ten animals each. Starting from the recorded lethal dose of 5.49g/kg, each group was given a different dose by intra-peritoneal injection of: 25%, 37.5%, 50%, 75%, 87.5%, and 100% of the lethal dose. The animals were observed for twenty four hours. The number of deaths in each group was recorded as percentage of the whole number (sixty). The log dose was plotted versus the mean deaths; the LD₅₀ obtained from the graph was 5.0g/kg in Albino rats.

Toxicological investigations also included evaluation of acute toxicity and determination of the toxic effects of *Solenostemma argel* in a group of seven young Nubian goats as compared to a control group of seven goats. Individual animals in the test group were given a 5g/kg dose of *S. argel* syrup once daily for 45 days. Then animals in the two groups were sacrificed and different investigations were carried out. There were no significant (P<0.05) changes in the weights of the vital organs of the treated animals compared to those of the control group. However, histopathological examination of some of the vital organs of the treated goats revealed some tissue abnormalities mainly including: congested heart; hyperemia of the intestinal tissues revealing catarrhal inflammation with lymphocyte infiltration; liver tissue necrosis of centrilobular hepatocytes, fatty cytoplasm vacuolation and slight congestion of the sinusoids; kidneys tissue necrosis of the renal tubules, pyknosis, karyolysis of tubular epithelial cells, and interstitial mononuclear cells infiltration.
Also there were some changes in the serum constituents of the treated animals including: phosphorus, creatinine, calcium, total protein and albumin concentrations; alkaline phosphatase and aspartate aminotransferase had high levels in the treated animals. Furthermore, RBCs count, MCV, and PCV were higher in treated animals compared to the normal goats. Similar studies of the toxic effects of S. argel alkaloids were conducted in white Albino Canadian rats, without significant alterations occurring in their vital organs, but there were significant increases in urea (at level P<0.05), and in creatinine, calcium, phosphorous, alkaline phosphatase, and aspartate aminotransferase (at level P<0.01). There were no significant changes recorded in both total protein and albumin concentrations. Phosphorus, creatinine, calcium, total protein and albumin concentrations, and alkaline phosphatase and aspartate aminotransferase activities were at high levels in animals given the high dose of 0.64gm/kg of S. argel alkaloids. Changes in haematological parameters in these Albino rats included significant decreases in haemoglobin and packed cell volume with the maximum dose of 640mg/kg and the effective dose of 160 mg/kg.

Following all the phytochemical, pharmacological and toxicological experimental findings verifying and confirming the efficacy and safety of Solenostemma argel leaves powder, extracts and alkaloids, it was necessary to design, formulate and evaluate the dosage form suitable to deliver these drug substances effectively and safely.

In the pharmaceutical experiments of the present work, Solenostemma argel leaves preparations were designed and formulated in tablets as an oral dosage form similar to its oral use in folkloric medicine. Therefore, the powdered leaves, powdered dried extracts, and dried alkaloids extracts were used after being incorporated into tablet dosage forms. Tablets were selected as a dosage form since they represent the preferred drug delivery system because of their several advantages including: improved dosage accuracy and reliable delivery of the drug; ease of their preparation, evaluation, control, storage, and dispensing; convenience of use and compliance of patients, and ease of product identification; their inherently higher chemical stability, compared to other dosage forms, and having the longest shelf life; the cost of fabricating the entire compressed tablet, particularly if a simple tablet granulation process is employed, is lesser than that of filling capsules and other operations; and they are the preferred dosage form by pharmaceutical manufacturers because they best lend themselves to rapid mass production and are the least expensive of the solid dosage forms.

The pharmaceutical experiments were aimed at optimization of all the physicochemical characteristics of S. argel tablets prepared from the powdered leaves, extracts or alkaloids in a wide range of pharmaceutical formulations with different types and concentrations of diluents, binders and disintegrants, in addition to the optimization of drug particle size, and process conditions, such as the state and time of binder or disintegrant additions; the optimized formulations were subjected to a wide range of quality control tests including physical and chemical parameters;

Alkaloids contents of Solenostemma argel leaves preparations and tablets were determined using UV spectrophotometer at λ 294.5nm and the standard calibration curve of Solenostemma argel alkaloids. The prepared Solenostemma argel tablets of the different formulae were given codes and included three principal formulae A, B and C with many modifications mainly depending on the types of binders used. The S. argel leaves tablets prepared were either 250mg, using a 9mm-diameter dies, and containing 175mg of S. argel extract in each tablet, or 500mg, using a 12-mm diameter dies, and the alkaloid content in each tablet was 80mg. The codes for the chosen bases were as follows: 1) SPC, for PVP, formula C; 2) SSC, for Sorghum + PVP, formula C; 3) SMC, for Maize starch + PVP, formula C; 4) SZC, for Zora Starch+ PVP, formula C; 5) SGC, for Guar starch + PVP, formula C; 6) SEB, for S. argel Water Extract, with PVP, formula B; and 7) SAA, for S. argel alkaloids, with PVP, formula A, without MCC.

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Solenostemma argel leaves pharmaceutical preparations were optimized for drug substance particle size due to its significant effect on the physical and therapeutic properties of drug product; the strength of the granular materials increases with decreasing particle size or to an effect of particle size during the strength measurement. Solenostemma argel leaves were powdered using a hammer mill to a particle size and distribution that would flow freely; small particles would pass through the screen almost as fast as they were formed, and thus a hammer mill tends to yield a relatively narrow size distribution. Sieve analysis and separation of S. argel leaves powder was applicable to particles in the size range of $\leq 160$ to $\geq 250$ microns, and used both as a method of particle size analysis and separation of a sample of powder into various sizes ranges, in order to produce materials within a specified range, and screen out very fine particles or over-size materials. The amount of powder that passed through the 160-micron mesh was found to be 63%, and this fraction of powdered S. argel leaves was mainly used in the formulation of optimized S. argel tablets.

Granulation, the generic name used for particle size enlargement, is seen as an answer to different flow problems to ensure better results when mixing different powders to reduce dust hazard problems, to insure uniform fill in a tablet machine, and to produce tablets conforming to the monograph requirements and specifications. Wet granulation method was used as the method of tablet preparation or production, and involved wetting of the mixed powders with a suitable binding agent and screening the moist mass, drying, and sizing of the dried granules. It has the advantages of improving the physical properties of the drug substances, such as flowability, compressibility, wettability, and solubility, but it is not suitable for thermolabile or moisture-sensitive drug substances. Its chief disadvantages are the number of separate steps involved as well as the time and labour necessary to carry out the procedure especially on large scale production. In addition, the wet granulation method was used because of its popularity in local pharmaceutical firms, and the increased probability that the granulation would meet all the physico-chemical requirements for the compression of good tablets.

Following granules sizing, the granules were mixed with the other formulation ingredients, such disintegrants, lubricants and glidants, and compressed into tablets using a single-punch tableting machine. Tablets were made by compressing unit volumes of particles; plain flat-face or convex punches were used, and the tablets produced by the convex punches were more friable and produced more powder.

From these optimization data of S. argel tablets, it was concluded that: a) a combination of binding agents was needed and a reduction of binder concentration was a must to optimize these formulae; b) the use of a disintegrating agent, before and after granulation, in S. argel leaves tablets also optimized these formulae; c) PVP preparations showed lower friability, followed by gum, starch and guar; and d) PVP preparations had the best dissolution rate properties, followed by gum and guar, while starch had the least rate.

The compressed tablet specifications evaluated included general appearance, diameter size, shape thickness, weight, hardness, friability, disintegration and dissolution. Average tablet weight, weight variation test, thickness and diameter, and other physicochemical properties of the different tablets were evaluated with reference to the BP or USP monographs requirements. In tablet hardness, tablets require a certain degree of hardness to withstand mechanical shocks of handling in manufacture, packaging and shipping. The strength of tablets was determined by breaking it by the Monsanto tester. Another measure of tablet strength is the term ‘friability’ which is often measured particularly for tablets that tend to powder, chip and fragment when handled, and in the friability test, a maximum weight loss of not more than 1% is considered acceptable for most products. With regard to tablet disintegration time, the average disintegration time of six uncoated tablets intended to disintegrate in the stomach is in
10 to 120 minutes maximum; for herbal tablets, the disintegration time is one hour (Ind. P). Tablet dissolution test was applied to confirm the quality of Solenostemma argel tablets and their content uniformity; determination of the percentage dissolved of S. argel constituents of the different tablets, under UV spectra λ294 nm, was carried out.

Stability experiments were aimed at the investigation and determination of the possible effects of different storage conditions, including on-going stability conditions and accelerated stability conditions, on the physicochemical properties of optimized S. argel tablets. In quality assurance studies, stability is defined as the extent to which a product retains, within specified limits, and through its period of storage and use, i.e. its shelf-life, the same properties and characteristics that it possessed at the time of its manufacture. Types of stability generally recognized as criteria for acceptable levels of stability are: a) Chemical Stability: a condition maintained throughout the shelf life of the drug product, as each active ingredient retains its chemical integrity and labeled potency, within the specified limits; and b) Physical Stability: with the original physical properties including appearance, palatability, uniformity, dissolution and suspendability being retained.

Stability testings are a series of tests designed to obtain information on the stability of pharmaceutical products for definition of shelf life and utilization period under specific packaging and storage conditions. At appropriate time intervals, samples of the products were assayed for potency by use of stability indicated methods, and observed for physical changes. Accelerated stability testings are studies designed to increase the rate of chemical degradation and physical change of drug by using exaggerated storage conditions, and container/closure systems. In general, the quality control tests, together with the real-time study for stability, and in the development phase, accelerated stability tests, were carried out to compare in short-term experiments alternative formulations, packaging and processing.

These stability testings were carried out by placing 100 tablets of each formula at room temperature, at 30°C±15°C, and RH 60%±15%; in an incubator at 37°C±2°C and RH 70%±5%; and in another incubator at 50°C±5°C and RH 70%±5%, for intervals extending from 6 months to 48 months. At appropriate time intervals, samples of the products were assayed for potency by using stability indicated methods, and observed for physicochemical characters and properties changes including: uniformity of tablet weight, diameters, thickness, microbial growth, and other organoleptic observations, e.g. odor, color, texture and hygroscopicity of tablets, tablet hardness, friability, disintegration time, dissolution rate of tablet constituents carried out by a UV spectrophotometer, powder contents estimation with time, and mottling, capping, and lamination, if any, were recorded. In the tested tablets, only in certain formulae, there were changes in the hardness, odor, friability and powder contents.

In the stability studies of tablets, stable tablets would retain their original physical properties, such as size, shape, weight, and color, under normal handling and storage conditions throughout their shelf-life; in addition the in vitro availability of the active ingredient should not change appreciably with time. Excessive powder or solid at the bottom of the container, cracks or chips on the surface of the tablets, or appearance of crystals on the surface of the tablets or container walls are indication of instability of uncoated tablets. All these instability features were not detected in all S. argel tablets of the different formulae during different storage conditions, except for zone in some formulae and the powders in others.

Clinical investigations were aimed at determination of any possible side effects, adverse reactions or toxic manifestations at therapeutic dosage regimen in ten healthy male volunteers, followed by evaluation of the therapeutic activities and side effects of S. argel tablets in a group of 100 patients but with different clinical complains mainly in the gastrointestinal tract, including irritable bowel syndrome and colics, or in the
urinogenital tract, including renal spasms and menstrual pain. These trials were performed for the evaluation of the safety and effectiveness of *Solenostemma argel* tablets and the assurance of safety and protection of the human subjects participating in these investigations.

Questionnaire (II) was distributed among individuals who had the practice of using herbal medicines, and the following findings were recorded: the effectiveness of the medicinal herb used was indicated by 60% of the participants who felt completely relieved of pain after a single administration of the herbal medicine compared to 26% who used the drug several times due to repeated attacks of pain, 4% had mild pain relief, and 15% did not feel relieved of their colics and pains. With regard to the quantity or dose of herbal medicine to be used, 85% used the preparation just by guessing or used an arbitrary quantity, while 15% either measured or weighed specified amount of the herbal medicine.

Ten healthy male subjects participated in these clinical trials after signing a written consent indicating their participation in this study at their own will and choice. Each of these healthy subjects was subjected to a medical examination by a clinician; blood pressure and heart beats were measured before and after treatment, and all of them proved to be in very good health. These volunteers were monitored or followed up for the occurrence of any side effects or adverse reactions following the administration of the specified doses of *S. argel* tablets, started with low doses, e.g. one tablet of one of *S. argel* products, three times daily, and gradually increased till the highest dose used was attained, which was four tablets three times a day, for each of the leaves, extracts or alkaloids tablets. None of the volunteers complained of blurred vision, dryness of the mouth, change in heart beats (tachycardia or bradycardia), palpitation, headache or vomiting during the period of the trial. Only one of these subjects complained of dryness of the mouth and another patient complained of stomach upset felt at the time of administration of the leaves tablets.

Clinical trials were organized in three teaching hospitals under the supervision four physicians. One hundred patients, selected randomly, 20-70 years old and 40 males and 60 females, were complaining of gastrointestinal disturbances, irritable bowel syndrome, renal colics, or colics due to menstrual disturbances. After being diagnosed, these patients were treated by using two tablets of *Solenostemma argel* leaves or extracts administered when necessary. The patients were followed up for the therapeutic effectiveness of the administered tablets as indicated by relief of pain and colics, and were monitored for occurrence of side effects or adverse reactions.

In 25 patients with gastrointestinal disturbances and spasm, the pain was relieved completely in all these patients (25, 100%); in 20 patients with pain associated with menstrual cycle, the pain was relieved in most patients (18, 90%); in 15 patients with renal colics, pain was relieved in 11 patients (73%); while in 72% (29) of the 40 patients with IBS, pain was completely relieved. None of the patients complained of any undesirable or adverse effects of these preparations of *Solenostemma argel*.

Forty patients with diagnosed irritable bowel syndrome were treated with placebo tablets, generic antispasmodic tablets, *S. argel* leaves or extract tablets. Placebo tablets, prepared from lactose and starches, had a 5% pain relief, the extract (88%) is more effective than the leaves (71%) tablets, and had a rapid onset of action in the pain relief, compared to the generic tablets (90% pain relief) as the reference product. According to these findings, *Solenostemma argel* tablets, especially the extract or alkaloid formulations, would be quite useful in controlling the pain associated with IBS.

Therefore, it could be concluded that the different types (leaves, extracts or alkaloids) of *Solenostemma argel* tablets showed a very good therapeutic effectiveness (71%-100%), and a great margin of safety (98%-100%). These findings are very promising, but they need further confirmation in larger clinical trials involving more
centres and larger numbers of clinicians and involving larger numbers of patients, as well as investigation of more experimental variables.

6. Recommendations

The following aspects and subjects are recommended for further future studies and investigations:

a) Detailed studies for separation and identification of Solenostemma argel Alkaloids using different phytochemical methods, and thorough pharmacological and toxicological investigations of these isolated and identified alkaloids.

b) Study of the anti-arrhythmic effects of Solenostemma argel and its effects on the heart functions and activities.

c) Investigation of the anti-cholesterolemia effects of Solenostemma argel and identification of the constituents responsible for these effects.

d) Investigation of the effects of foods, types and quantities, on Solenostemma argel absorption, especially milk and acidic beverages.

e) Study of the effects of Solenostemma argel on iron absorption.

f) Detailed studies of the anti-cholinergic, local anaesthetic and hypotensive actions of Solenostemma argel and isolation of its constituents responsible for these actions to be used as lead compounds for development of new drug entities.

g) The use of rabbit’s small intestine model to study and identify the types of diffusion of different drug substances, and evaluation of the factors that will affect drug diffusion, e.g. Diffusion of Solenostemma argel tablets and the effects of different types of acids, alkalis and minerals, such as calcium, iron, or aluminium compounds.

h) Detailed toxicological studies of medicinal herbs and plants preparations particularly those intended for long-duration administration.

i) The use of Medusa Computer System for comparative evaluation and studies of medicinal herbs and reference therapeutic drugs to determine their relative potencies and ED50.

j) Determination of ED50 and LD50 of herbal medicines in different animal models and determination of their therapeutic window and range.

k) The use of contractile rabbit’s small intestine model (CRSIM) to identify and evaluate the antispasmodic activities of different plants constituents.

l) Detailed studies of the use of Guar with PVP as binding and disintegrating agents in crude plant tablets formulations.
7. REFERENCES

Abd-Alim, MHM and El-Deen, EZ (1987).
Influence of the Physical Properties of Binders and Powders on
the Characteristics of Granules.

Abdou, HH (1989).
In: Dissolution, Bioavailability and Bioequivalence, H.H. Abdou, (ed).

Adam, SEI (1972).
A Review of Drug Hepatotoxicity in Animals.

Effect of Impomea carnea on the Liver and on Serum Enzymes in
Young Ruminants.
Journal of Comparative Pathology, 83: 351.

Effect of Magnesium Stearate on the Compactability of Microcrystalline Cellulose.
Journal of Pharmacy and Pharmacology, 40: 72P.

Airth, JM, Brady, DF and Radecka, C (1967).
Variability of Uniformity of Weight Test as an Indicator of the Amount of
Active Ingredient in Tablets.

Alam, AS and Parrot, EL (1971a).
Effect of Aging on Some Physical Properties of Hydrochlorothiazide Tablets.
Journal of Pharmaceutical Sciences, 60: 263.

Alam, AS and Parrot, EL (1971b).
Effect of Dissolution Media on the Disintegration and Dissolution of
Hydrochlorothiazide Tablets.

Modification of Arachidonic Acid Metabolism by Flavonoids.

Alcaraz, MJ and Hoult, JRS (1985).
Action Flavonoids and the Novel Anti-inflammatory Flavon, Hypolactin-8-glycoside
On Prostaglandin Biosynthesis and Inactivation.
Biochemical Pharmacology, 34: 2477-2482.

Alcaraz, MJ and Hoult, JRS (1986).
Effect of Hypolactin-8-glycoside and Related Flavonoids on
Soyabean Lipo-oxygenase And Snake Venom Phospholipase A.
Archives Internationals de Pharmacodynamic et de Therapie, 206: 4-12.

Flavonoids as Anti-inflammatory Agents.

Effect to de Flavonoideis, 7,3, Hidroxilades Sorbo la Secretion Enzimatica de
PMNS ; Humanos. Reunion National de la Asociacion Espaiola de Farmacologs
Valencia.

Allen, T (1975).
pp113-125.

Artuson, G and Jonsson, GE (1975).
   *Stimulation and Inhibition of Biosynthesis of Prostaglandins in Human Skin by Some Hydroxyethylated Intosides.*
   *Prostaglandins*, 10: 941-948.


Augsberger, LL and Shangraw, RF (1966).
   *Effect of Glidants in Tableting.*

   Levelling off the Effect of Particle Size on Dissolution in Spironolactone Tablets.
   (Unpublished Article), * JPM, Co, Nour Jordan; * Faculty of pharmacy, University of Jordan, Amman, Jordan.

Banker, GS (1974).

Banker, GS and Anderson, NR (1986).

   Effect of Particle Size and Source of Variables of Youngus Modulus of Microcrystalline Cellulose.

   Structure–Activity Study on the Influence of Phenolic Compounds and Bioflavonoids on Rat Renal Prostaglandins Synthetase.
   *Naunyn Schmiedeberg’s Archives of Pharmacology*, 307: 73-74.

Baumann, J, Bruchhausen, FV and Wurm, G (1980).
   Flavonoids and Related Compounds as Inhibitors of Arachidonic Acid Peroxidation.
   *Prostaglandins*, 20: 627-639.

   Dihydrocaffeic Acid, a Metabolite of Bioflavonoid as a Cofactor of Prostaglandins Synthetase.

   *Compaction Mechanism*, In : *Compaction of Particulate Solids*, Residential Continuing Course Education, School of Powder Technology, University of Bradford, Chapter 5.

   *Inhibitory Effects of Natural Flavonoids on Secretion from Mast Cell Neutrophils.*

   Inhibition of Aggregation and Secretion of Human Platelets by Quercetin and Other Flavonoids: Structure-Activity Relationship.
   *Agents and Actions*, 12: 382-387.

B. P., Her Majesty’s Stationary Office, University Press, Cambridge,


B. P., Her Majesty’s Stationary Office, University Press, Cambridge, vol. II,
p A 108, Appendix XI (C) and (D).

B. P., Her Majesty’s Stationary Office, University Press, Cambridge, vol. II,
Appendix XII A, pp A 113.

B. P., Her Majesty’s Stationary Office, University Press, Cambridge, vol. II,
Appendix XII B, pp A 114, Appendix XIIe.

B. P., Her Majesty’s Stationary Office, University Press, Cambridge, vol. II,
Appendix XVI A, pp 191.

B.P., Her Majesty’s Stationary Office, University Press, Cambridge, vol. II,
Appendix XVII, pp A 195.


B.P., Her Majesty’s Stationary Office, University Press, Cambridge,

Biological Comparison of Local Anaesthetics.
J. Pharmacology Experimental Therapeutics, 85: 78-84.

Cabrera, C (2000).
Delivery Systems and Dosage Strategies in Herbal Medicine
Accessed at the website: http://www.gaiagarden.com/articles/dosagestrategies

Castello, RA and Mottocks, AM (1962).
Discolouration of Tablets Containing Amines and Lactose.

Modification rates of gastrointestinal absorption of drugs.
11. Quaternary ammonium salts.

Pharmaceutical composition for enhanced absorption of the
therapeutically active quaternary ammonium salt ingredient.

Peroxidant States and Tumor Promotion.
Science (Washington), 227: 375-381.

Chowhan, ZT (1988).
Pharmaceutical Technology, 12: 46.
Punch Geometry and Formulation Consideration in Reducing Tablet Friability and their Effect on in vitro Dissolution.

Factors Influencing Comparative Bioavailability of Spironolactone Tablets.

Cliff, M (1988).
New Concepts in Pharmaceutical Technology.

Herbal Dosage Forms.
Accessed at the website http://www.cami.usip.edu/qacs/herbaldosage.htm

Cooper, J and Rees, JE (1972).
Tableting Research and Technology.

Cordell, AG (1981).
Introduction to Alkaloids: A Biogenic Approach of the Alkaloids in Relation to the Common Amino Acids, AG Cordell (ed.), J. Wiley & Son, New York, USA.


Danish, FQ and Parrot, EL (1971).
Effect of Concentration of and Size of Lubricant on the Flow Rate of Granules.

Evaluation of Sorghum Starch as a Tablet Disintegrant and Binder.

The Effect of Some Binding Agents on the Mechanical Properties of Granules and their Compression Characteristics.

Antihistaminic Effects of Galphimia glauca Gallic Acid and Quercetin.

Promising Phytomedicals J Janick and JE Simon.

Peak Offset Times as an Indication of Stress Relaxation During Tableting on Rotary Tablet Press.

WHO/Pharm./94/565.

The Anti-inflammatory and Analgesic Activity of Certain Sudanese Plants.
Master Degree Thesis, University of Khartoum.

Phytochemical Study of Solenostemma argel Growing in Egypt.
*Journal of African Medicinal Plants, 1.*

Medicinal Plants in Sudan: Medicinal Plants in Khartoum Province.
National Center for Research, Khartoum, Sudan pp 61-62.

Studies for Determining Antimicrobial Activity of Solenostemma argel (Del) Hyne.
Extraction with Methanol/Water in Different Portions.
*Science Journal, 14,* 138.

Botanical and Chemical Studies on Solenostemma argel (Del) Hayne Grown in Khartoum.
A Thesis Submitted to the University of Khartoum.

Botanical and Phytochemical Studies on S. argel (Del) Hayne.

The Most Common Herbal Remedies in Central Sudan.
*Fitoterapia 4,* 301.

The Most Common Herbal Remedies in Dongla Province, Northern Sudan
*Fitoterapia 2,* 118.

ElKheir, YM (1966).
Investigation of Certain Plants Used in Sudanese Local Medicine.
M. Pharm. Thesis, University of Khartoum.

Elkheir, YM and Salih, GH (1980).
Investigation on Medicinal Plants of Sudan.
*Fitoterapia,* 51: 143.

Pharmacological Actions of the Leaves of Solenostemma argel (Del) Hayne: Spasmolytic and Uterine Relaxant Activities.

ElTohami, MS (1996).
Medicinal and Aromatic Plants in Sudan.

Chemical, Physical and Lubricant Properties of Magnesium Stearate.


Some Formulation Factors Affecting the Tensile Strength, Disintegration and Dissolution of Uncoated Oxytetracycline Tablets.
*Journal of Pharmacy and Pharmacology,* 28: 8-16.

Evans, WC (1989).
Fawcetty, JK and Scott, JC (1960).
The Use of Urease in the Determination of Urea.

Feinstein, W and Bartilucci, J (1966).

Powders, In: Remington’s Pharmaceutical Science, 14th ed.,
Mack publishing Co., Easton Pa., pp 1626-1648.

Effect of Flavones Inhibitors on Transport ATPase and Histamine Secretion from Rat Mast Cells.

Influence of Formulation on Dissolution Rate, In : Dissolution Technology,
L. J. Lesson and J. T. Carstensen (eds.), Academy of Pharmaceutical Sciences,

Finnery, DJ (1964).

Ford, EJH, Adam, SEI and Gopinath, C (1972).
Hepatic Aminopyrine-N-Demethylase Activity in the Calf.
Journal of Comparative Pathology 83, 355.

The First World Congress on Medicinal and Aromatic Plants for Human Welfare,
Maastricht, Netherlands, JULY 19 – 1992
Accessed at the website  http://www.actahort.org/books/333/Index.htm

Garrett, ER (1962).
Selection and Evaluation of the Assay of Pharmaceutical Product:
Reproducibility of Assay and Drug Recovery from Dosage Forms.


Evaluation of Tablet Breaking Strength Testers.

Gray, C (1997)
Growing Popularity of Complementary Medicine Leads to National Organization for MDs.

On the Mechanism of Anti-thrombotic Action of Flavonoids.
Biochemical Pharmacology, 36: 317-322.

Antioxidative Substances in Leaves of Polygonum hydropiple.

Flavonoids, In : “Phytochemistry of Organic Metabolites”.

Hawely, GG (1971).  

Powder Mixing in the Tableting of Fenfluramine HCl: Evaluation of a Mixer.  

Pharmacology of Radicals, Recent Views on their Relation in Inflammatory Mechanisms.  
Life Sciences, 34: 713-720

Hiestand, EN (1966).  
Powders: Particle - Particle Interactions.  

Hiestand, EN (1978).  

Hiestand, EN and Wells, JE (1977).  
Powder and Bulk Solids Handling and Bulk Solids Handling and Processing.  

The Physical Processes of Tableting.  

Higgs, GA and Vane, JR (1983).  
Inhibition of Cyclooxygenase and Lipoxygenase.  
British Medical Bulletin, 39: 265-270.

Higuchi, T, Nelson, E and Busse, LW (1954).  
The Physics of Tablet Compression.  

Ho, AYK and Jones, TM (1988).  
Rise Time: New Index of Tablet Compression.  

Role of Dietary Flavonoids in Protection against Cancer and Coronary Heart Disease.  

In Vitro Inhibition of the Biosynthesis of Slow Reacting Substance, Anaphylaxis (SRSA) and Lipoxygenase Activity by Quercetin.  
Biochemical Pharmacology, 32: 367-370.

Utilization of Hydrophilic Gums for the Control of Drug Release From Tablet Formulation. I: Disintegration and Dissolution Behavior.  
Journal of Pharmacy and Pharmacology, 25: 71 P.

Hunter, BM and Garnderton, D (1973)  
The Influence on Pharmaceutical Granulation of the Type and Capacity of Mixers.  
Journal of Pharmacy and Pharmacology, 25: 71 P.

Studies on Metronidazole Tablet Formulation.

Jacob, T and Plein, N (1968).
Factors Affecting Dissolution Rate of Medicaments from Tablets II.
Journal of Pharmaceutical Sciences, 57:

Jaiyeoba, KT and Spring, MS (1980).
The Granulation of Ternary Mixtures: The Effect of the Wettability of the Powders.

Occupational Allergic Rhinitis from Guar Gum.

Kaning, JL (1965).
Communication, In: Remington’s Pharmaceutical Sciences, E. W. Martin (ed.),

Inhibition of 12-o-tetradecanoylphorbol-13-actate by Quercetin;
Possible Involvement of Lipoxygenase Inhibition.
Carcinogenesis, 4: 1301-1305.

Homeopathic Medicinal Products in Germany and Europe:
Legal Requirements for Registration and Marketing Authorization.

Investigation on Peucedanum ostruthium L. and Solenostemma argel (Del) Hayne.
Doctorate Thesis Submitted to the University of Szeged, Hungary.

Sudanese Plants. 1. Solenostemma argel.
Herba Hung., 13: 33-35

Khan, K.A. and Rhodes, CT (1971).
Evaluation of Five Commercially Available Tablet Disintegrants for Possible Use
in Insoluble Direct Compression Systems.
Journal of Pharmacy and Pharmacology, 23: 216 S.

New Concept in Pharmaceutical Technology.

Kind, PRN and King, EJ (1954).
The Two Power Colorimetric Assay of Alkaline Phosphatase.
Journal of Clinical Pathology, 7: 322.

Oral Dosage Forms In: Remington’s Pharmaceutical Sciences, A.R.Gennaro (ed.),

Textbook In Vitro Practical Pharmacology (Miscellaneous Experiments, Exp. 91:
Local Anaesthetics and the Foot Withdrawal Reflex of the Frog, p135,

Lachman, L and Cooper, J (1963).
A Programmed Automated Film Coating Process.
Modification of Platelet Function and Arachidonic Acid Metabolism by Bioflavonoids.  
*Biochemical Pharmacology*, 33: 1525-1530.

Last, M and Chavundka, G (1986).  
Manchester, Manchester University Press.

Laychock, SG (1986).  
The Biochemistry of Cell Activation as Related to Putative Actions of Flavonoids.  

Lec, TF, Mattelino, ML and Middleton, EJr (1982).  
Effect of Quercetin on Human PMNL Lysosomal Enzyme Release and Phospholipids Metabolism.  
*Life Sciences*, 31: 2765-2774.

Lees, KA (1963).  
Fine Powders in Pharmaceutical Practice, Chemical and Physical Aspect.  
*Journal of Pharmacy and Pharmacology*, 15: 43 T.

Herbal Medicine and Health Promotion: A Comparative Study of Herbal Drugs in Primary Health Care.  
Amsterdam, Royal Tropical Institute.

Levine, RM and Blair, BB (1955).  
Factors influencing the intestinal absorption of certain mono-quaternary anti-cholinergic compounds with especial reference to benzomethamine.  

Effect of Certain Tablet Formulation Factors on Dissolution Rate of the Active Ingredient. III: Lubricants.  

Lewis, RA (1986)  
Mast Cell Dependant Immediate Hypersensitivity Responses.  
*Progress in Chemical and Biological Research*, 213: 457-470.

Linter, CJ (1985).  

Mechanism of Size Enlargement, In : Compaction of Particulate Solids.  
Residential Continuing Course Education, School of Powder Technology, University of Bradford. Chapter 3.

Lowenthal, W (1972).  
Distintegration of Tablets.  
*Journal of Pharmaceutical Sciences*, 61(11):

Mechanism of Action of Starch as a Tablet Disintegrant. II: Location and Structure of Starch in Tablet.  

Luduena, FP (1960).  
Responses of Smooth Muscle of Gastrointestinal and Genitourinary Tracts.  
Madani, BA (2000).
Toxicological Studies of Sudanese Medicinal Plants.
Master Degree Thesis Submitted to Khartoum University.

Formulation of Tablets from Sudanese Senna Pods.
Master Degree Thesis Submitted to Khartoum University.

Phytochemical Study of Solenostemma argel Hayne Leaves.

Mahran, GH and Saber, AH (1964).
A Contribution to the Pharmacognostical Study of Leaves and Stems of Solenostemma argel Hayne Growing in Egypt.

Mahran, GH, Wahba, SK and Saber, AH (1976).
Phytochemical Study of Solenostemma argel Leaves.
*Bull Fac. Pharm. Cairo*.

Mariana, MXL, Mamdouh, AZ and Jaime, T (1994).
Quercetin Glycosides in Psidium guajava L. Leaves and Determination of the Spasmolytic Principle.

Marlowe, E and Shangraw, RF (1967).
Dissolution of Sodium Salicylate from Tablet Matrices Prepared By Wet Granulation and Direct Compression.


McIntyre, A (2003)
Herbs at the Forefront of Modern Medicine.
Accessed at the website www.positivehealth.com/permit/Articles/Herbal/Herbal

Middleton, EJr (1986).
Effect of Flavonoid on Basophile Histamine Release and Other Secretary Systems.

Flavonoid Inhibition of Human Basophiles Histamine Release Stimulated by Various Agents.
Biochemical Pharmacology, 33: 3333-3338.

Inhibition of *in vitro* Platelet Aggregation and Arachidonic Acid Metabolism by Flavone.
*Biochemical Pharmacology*, 33: 357-363.

Muray, RK, Granner, DK, Mayes, PA and Rodwell, PA (1988).
Harper's Biochemistry, Norwalk, California, U.S.A.

Differences of Apparent Fracture Stress on Tablets on Test Methods.
*Journal of Pharmacy and Pharmacology*, 29: 41P.
Noyes, AA and Whitney, WR (1897).  
The Rate of Solution of Solid Substances in their Own Solution.  

Orcutt, JA (1956).  
Antispasmodics Assay in vitro:11. Improved Design of an Assay  
Based on a New Empirical Function Concentration and Time.  

Comparative Evaluation of Mean Particle Size of Bulk Drug Powder in  
Pharmaceutical Preparations by Fourier-Transformed Powder Diffuse  
Reflectance Infra-Red Spectroscopy and Dissolution Kinetics.  

The Determination of Creatinine in Plasma or Serum and Urine:  
A Critical Examination.

Parrot, EL (1986).  
Milling; In: Theory and Practice of Industrial Pharmacy, L Lachman, A Lieberman,  

Parrot, EL and Saski, W (1971).  
Compressed Tablets; In: Experimental Pharmaceutical Technology, Eugene, L.P.  

Mechanism of Starch as Disintegrating Agent in Aspirin Tablets.  

Specific Suppression of Human T Lymphocyte Function by Leukotriene B4.  
Journal of Immunology, 131: 551-553.

Factors Affecting the Disintegration and Dissolution of  
Chloroquine Phosphate / Starch Tablets.  

Novel Pregnane Glycosides from Solenostemma argel, Presented at  
“The Changing Face of Natural Products Chemistry”, The 44th Annual Meeting of the American Society of Pharmacognosy, July 12-16, 2003; Chapel Hill, NC

Factors Influencing Bioavaialability: Factors Influencing Drug Absorption from GIT,  
Churchill Livingstone, Edinburgh, Chap. 9, pp 135-173.

Herbal Remedies – Global Market,  


Reynolds, JEF (1982).  
The Evaluation of Drug Toxicity in Lower Animals.
*Drug Toxicity*, 376-387.


Ripple, EG (1986).
Mixing: In : *The Theory and Practice of Industrial Pharmacy*, I. Lachman, H.A.

Ripple, EG (1985).
*Powders*, In: *Remington’s Pharmaceutical Sciences*, A.R.Gennaro (ed.),

Leukotriene B4 Induced Human Suppressor Lymphocytes.
*Biochemical and Biophysical Research Communications*, 108: 1531-1537.

Diversity in Structure and Function of Neuronal Nicotinic Acetylcholine
Receptor Channels.

Ross, SA, Megalla, SE, Dishay, DW and Awad, AH (1980).
Study for Determining Antibiotic Substances in Some Egyptian Plants.

Tablets; In: *Pharmaceutics, the Science of Dosage Form Design*,

*Journal of Pharmacy and Pharmacology*, 26: 104 P.

Generated Surface Area Measurement of Disintegrating Tablets.
*Journal of Pharmacy and Pharmacology*, 29: 263-266.

Oral Dosage Forms; In: *Remington’s Pharmaceutical Sciences*, A.R.Gennaro

Rukangira, E (2001)
The African Herbal Industry: Constraints and Challenges.
A paper presented at “The natural Products and Cosmeceuticals 2001 conference”

An Evaluation of the Relative Importance of Formulation and Process Variables
Using a Factorial Design.

Schioter, LC and Wagner, JC (1962).
Automated Dissolution Rate of Capsules and Tablets.

Optimization Techniques in Pharmaceutical Formulation and Processing; in:

Scott, MHP (1977).
*Journal of Pharmacy and Pharmacology*, 29: 66 P.

The Properties of Tablets Containing Microcrystalline Cellulose.  


Stanely-Wood, NG and Shubair, MS (1979).  
The Influence of Binder Concentration on the Bond Formation of Pharmaceutical Granules.  

Granulation; In: *Pharmaceutics: the Science of Dosage Form Design*,  

Anti-Aggregatory Effects of Flavonoids *in vitro* and their Influence on Lipoxygenase and Cycloxygenase *in vitro*.  
*British journal of Pharmacology and Pharmacy*, 36: 455-463.

Swinyard, EA (1985a).  
Gastrointestinal Drugs; In: *Remington’s Pharmaceutical Sciences*,  

Swinyard, EA (1985b).  
Introduction of New Drugs; In: *Remington’s Pharmaceutical Sciences*,  

Availability Study on Microcrystalline Cellulose in Making Phenobarbitone Tablets,  

A Preliminary Investigation of the Potential Antimicrobial Activity of *Solenostemma argel*.  


Thief, WJ, Nguyen, IT and Sbekna, FJ (1986).  
Content Uniformity of Microdose Tablet Dosage Form (1µg-10mg) Produced by Fluid-bed Granulation of Interactive Mixture.  

Tableting Characteristics of Micro-aggregated Egg-albumen Particles Containing Paracetamol.  

Train, D (1957).  
Transmission of Forces Through a Powder Mass During the Process of Pelleting.  

Trease, EG and Evans, WC (1989).  
Tutadhar, MD, Carless, JE and Ummers, MP (1983).  
The Effect of Polymorphism, Particle Size and Compression on the Dissolution Rate of Phenylbutazone Tablets.  
Journal of Pharmacy and Pharmacology, 35: 269-274.

Tyler, VE (1996).  
What Pharmacists Should Know About Herbal Remedies.  

Influence of Various Starches on the Dissolution Rate of Salicylic Acid from Tablets.  

United States Pharmacopoeia and National Formulary (1980), USP X/NF. U.S.A.  

United States Pharmacopoeia and National Formulary (1980), USP X/NF. U.S.A.  

United States Pharmacopoeia and National Formulary (1980), USP X/NF. U.S.A.  

United States Pharmacopoeia and National Formulary (1985), USP X/NF. U.S.A.  

Vane, JR (1957).  
A Sensitive Method for the Assay of 5-Hydroxytryptamine.  

Some Aspects of Inhibitory Activity of Hypolattin-8-Glucoside in Acute Inflammation.  

Biopharmaceutics: Absorption Aspects.  

Biopharmaceutics: 19. Rate of Dissolution: I.V.  
Measuring in vivo Rates of Dissolution from Capsules and Tablets.  

Williams, TJ (1983).  
Interactions between Prostaglandins, Leucotrienes and Other Mediators of Inflammation.  


Regulatory Situation of Herbal Medicines: A Worldwide Review.  
Document WHO/TRM/98.1; Traditional Medicine, Department of Essential Drugs and Medicines Policy, World Health Organization, 1211 Geneva 27, Switzerland.

General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine.  
Document WHO/EDM/TRM/2000.1; Traditional Medicine, Department of Essential Drugs and Medicines Policy, World Health Organization, 1211 Geneva 27, Switzerland.

Traditional Medicine, Department of Essential Drugs and Medicines Policy, World Health Organization, 1211 Geneva 27, Switzerland.

WHO Traditional Medicine Strategy 2002–2005  
Document WHO/EDM/TRM/2002.1; Traditional Medicine, Department of Essential Drugs and Medicines Policy, World Health Organization, Geneva,

**Beein flusscung des Arachidonsto Hevechsels durch flavonoide.**  
*Deutsche Apotheker Zeitung*, 122: 2062-2068.

The Design of Dosage Forms; In: Pharmaceutics, the Science of Dosage Form Design,  

**Determination of Total Flavonoids in Leaves of Ginkgo (Ginkgo bilaba) and Studies on its Extraction Processes.**  
*Zhongcaoyao*, 23(3): 12.