PHARMACOLOGICAL INVESTIGATIONS OF SOLENOSTemma ARGEL LEAVES

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ABSTRACT

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 (1µg/ml) or barium chloride (0.002mg/ml). The degree 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.

INTRODUCTION

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 in 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 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 for the benefit of all (Keller, 1998; WHO, 2000).

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) Bullock Bruce, and S. triste (Nees) K. Müell. 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 Barbara and Abu Hamad (ElKamali, 1991). S. argel leaves were, at one time, used to adulterate Khartoum Senna (Tregan and Evans, 1989).

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.

Solenostemma Argel contain many pharmacologically active substances, phytochemical studies of S. argel identified two substances designated as argelin and argeloside.

In their report on S. argel, Khalid et al. (1974) showed the presence of kaempferol and steroid glycosides, while Mahran and Saber (1964), Wahba and Saber (1967), ElFishawi (1977), and Markham (1982) isolated α-amyrin, β-amyrin, β-sitosterol-containing ruixin and queceitin 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 gastrointestinal cramps and infections of the urinary tract (EFtolfami, 1996).

Moreover, protein, sugars, fibre, and vitamins are reported to be present with minerals Na+, K+, Ca++, Ni++, Mg++, and P+3 (El-Kamali et al., 1992, El-Kamali, 1994). Using thin layer chromatography, El-Kamali (1994) separated two spots which gave with Dragenroth's reagent an orange colour. Also, this physicochemical screening resulted in detection of flavonoids, sterols, tannins, alkaloids, saponins, cyanogenic glycosides and anthraquinone glycosides. Innocenti et al. (1998) studied the phytochemical constituents of Solenostemma argel.

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

All these active ingredients present in Solenostemma argel give it many pharmacological actions. This study was carried out to detect the pharmacological actions of Solenostemma argel.

MATERIALS AND METHODS

Pharmacological Methods:

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 1 μg/ml, or substituted by barium chloride, 0.002mg/ml.

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 in 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, 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 4bubles/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. When the contractions returned to approximately pre-treatment levels, the same dose of acetylcholine was repeated. At the end of the three minute 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 sub-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).

**Solenostemma argel** Alkaloids Diffusion Across Rabbit Small Intestine

The diffusion of *S. argel* alkaloids were 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 millilitre 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.

**Effects of Solenostemma argel** Extract on Isolated Rabbit Heart

A local species rabbit (weighing 1.5kg) 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 clot or blood was removed from the organ. About 0.5cm of a cannula tip of the perfused system was located in the aorta and tied firmly with a thread and mounted in 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.

**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.

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

African toads (*Rana temporaria*) 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 in 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 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 the 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 second 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.

**RESULTS AND DISCUSSION:**

**Pharmacological Methods:**

2.1.1. 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 acetycholine chloride, at a concentration 1 µg/ml, or substituted by barium chloride, 0.002mg/ml.

2.1.1.1. 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 in 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%).

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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).

2.1.1.2. *Solenostemma argel* Alkaloids Diffusion Across Rabbit Small Intestine

The diffusion of *S. argel* alkaloids were 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, 20µg/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 millilitre 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.

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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.

2.1.2. Effects of Solenostemma argel Extract on Isolated Rabbit Heart

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2.1.3. Effects of Solenostemma argel Extract on Intact African Toad Heart

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.

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

African toads (Rana temporaria) 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 in 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 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 the 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 second 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.

Figure 1: Effect of Solenostemma argel Leaves Alkaloids on Spontaneous and Acetylcholine-Induced Contractility in Rabbit Small Intestine
Figure 2: Effect of *Solenostemma argel* Alkaloids on Rabbit Small Intestine

Figure 3: Effect of *Solenostemma argel* Leaves Water Extract and Alkaloids on Barium Chloride-Induced Contractility in Guinea Pig Ileum

Figure 4: 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, 1μg/ml, or barium chloride, 0.002mg/ml, used as contractility inducers or enhancers in rabbit small intestine (CRSIM) (Figures 1, 2) or in guinea pig small intestine (GPMI) (Figure 3).

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 1, 2).

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 2).

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 3).

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 4). These findings reflected the contradicting effects of *S. argel* leaves ranging from inhibition to stimulation of rabbit small intestine.

### 3.2. Effects of *Solenostemma argel* Extracts on the Heart Models

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

![Figure 5: Effect of *Solenostemma argel* Leaves Water Extract on Contractility and Heart Rate in Isolated Rabbit Heart Model](image)

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

![Figure 6: Effects of *Solenostemma argel* Extracts of Different Parts on Contractility and Heart Rate in African Toad Heart Model](image)
Solenostemma argel leaves water extract, 12mg/ml, reduced both the contractility and the heart rate of rabbit isolated heart model (Figure 5). 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 6).

SUMMARY AND CONCLUSIONS:

ElTahir et al. (1987) studied the pharmacological activities of S. argel, including spasmylytic 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 guanethidin. The extract suppressed the activity of the uterus, and the effect was not antagonized by cinetidine, haloperidol, or propranolol. Furthermore, this extract exhibited a local anesthetic 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 cypreheptadine. It was concluded that the inhibitory activity may be due to the presence of two saline-insoluble spasmylytic compounds in the chloroform extract of S. argel leaves having spasmylytic and uterine relaxant activities. The spasmylytic 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.

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 (1μg/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 myocytotonic of different S. argel extracts, perfused continuously, leading to reduction of the heart rate and stroke.

REFERENCES:


