Assessment of *Sterculia setigera* and *Lantana camara* Extracts as Schistosomicidal and Molluscicidal Agents

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قال تعالى:

اۡثقُّالَ اللَّهُ الْمَلِكُ الحَقُّ وَلَا تَعْجَلُ بِالْفَرْآنِ مِنْ قَبْلَ أَنْ يُفْضِلِ إِلَيْكَ وَحِيَّةٌ وَقَلَ رَبِّ رَبَّ زَدْنِي عِلْماً K

سورة طه الآية (114)
Dedication

To my beloved mother,

Sweet son,

And family with love
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Abstract

Title: Assessment of *Sterculia setigera* and *Lantana camara* Extracts as Schistosomicidal and Molluscicidal Agents

Degree: Ph.D. in Parasitology

Name: Rahma Abu Bakr Musa Adam

This study was planned to evaluate the prophylactic and curative activities of the methanol extract of two medicinal plants; *Sterculia setigera* bark and *Lantana camara* leaves and their combination against *Schistosoma mansoni* in white albino mice and to evaluate their effects on the major enzyme activities involved in liver metabolism during the course of infection. Liver function tests, total proteins, albumins, globulins, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also measured. It also covered the *in vitro* activity of *S. setigera* against *S. mansoni*.

The molluscicidal activities of the methanol extracts of *S. setigera* bark and *L. camara* leaves were also tested against *Bulinus truncatus* and *Biomphalaria pfeifferi*. For the snails, LC$_{95}$ and LC$_{50}$ were the measure for the toxicity.

A group of 35 adult normal CD-1 Swiss albino mice, each weighing 20 gms, were divided into five groups and infected each with 80 *S. mansoni* cercariae (Egyptian strain). Groups, one, two and three of the infected mice were treated orally with the extract in a dose rate of...
500 mg/kg b.wt for five consecutive days; seven days before infection with *S.mamsoni* cercariae (Prophylactic), as well as four and seven weeks post-infection (curative). Group four was treated with praziquantel (PZQ) at a dose of 500 mg/kg b.wt for two consecutive days seven weeks post-infection (treated control), while in group five the mice were left untreated after infection (infected untreated control). Nine weeks post-infection (constant period), the animals were sacrificed, perfused to evaluate the efficacy of the plant extracts in the treatment of the infection.

Treatment with curative dose of praziquantel (500 mg/kg body weight) for two consecutive days, as a reference of anti-schistosomal drug reduced the total number of mature ova and complete absence of immature ova and increased the percentage of dead ova. Also, the drug tended to normalize all the changed biochemical parameters and improved the histopathological features of the liver.

When compared with the standard treatment, the results obtained showed that *S. setigera* bark extract did not show anti-schistosomal activity on worms in culture *in vitro* (100µg/ml), but it had a significant anti-schistosomal activity (29% worm reduction) and promising prophylactic activity (40% worm reduction) *in vivo*.

A significant reduction of ova output from *S. mansoni* infected mice (treated group), was recorded compared with a slight reduction in the prophylactic group. The number of ova from the liver and intestine was significantly reduced.

The result also indicated that administration of *L. camara* leaves extract produced significant reduction in worm burden (29%) and ova count and a decrease in the percentage of mature ova stages.
Administration of a combined dose of both extracts improved their effects on the parasitological parameters; with significant reduction in worm burden (40%) and ova count and a decrease in the percentage of mature and immature stages.

*S. mansoni* infection elevated serum ALT, AST levels. Administration of *S.setigera* bark or *L.camara* leaves extracts significantly diminished serum ALT and AST and restored albumin, globulins and total protein to the normal levels in both treated and prophylactic groups and improved A/G ratio. Combination of the extracts of *S.setigera* bark and *L. camara* leaves improved their effect on the serum enzymes related to liver functions.

On the other hand, 7 series of descending double successive dilutions, were prepared starting from 1000 ppm of the extracts of *S.setigera* bark and *L. camara* leaves. Ten snails from each group were used in each replicate and control, the exposure and recovery periods were 24 hours.

The toxicity measures indicated that the methanol extract of *S. setigera* bark was more toxic to *B. pfeifferi* snails (LC50 = 296ppm) than *B. truncatus* (LC50 = 482ppm), meanwhile the methanol extract of *L. camara* leaves was more toxic to *B. truncatus* (LC50 = 48ppm) than *B. pfeifferi* (LC50 = 111ppm).

In conclusion; *S. setigera* bark and *L. camera* leaves showed an indication that they possess anti schistosomal, prophylactic and hepatoprotective activities during the course of infection with *S.mansoni*, as well as molluscicidal activity against *B. truncatus* and *B. pfeifferi*.

These plants open the way for further investigations as prospective schistosomal and molluscicidal Agents.

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Sterculia setigera and Lantana camara

(Kinetic and the carries) Killers of the divided mucous and yellow juices.

The scientific name: Doctorate in the tuberculars

The title: Peace in Abu Beker Mosi

The study aimed to investigate the effect of the most extracted material of the leaves of Sterculia setigera and Lantana camara on the eggs of Bulinus truncatus and Biomphalaria pfeifferi.

In this study, a sample of 35 females were divided into 5 groups of 7 each. The first group received 500 mg/kg of the most extracted material, while the second group received 500 mg/kg of the most extracted material in the form of an intradermal injection. The third group received 500 mg/kg of the most extracted material in the form of an intraperitoneal injection. The fourth group received 500 mg/kg of the most extracted material in the form of an intramuscular injection. The fifth group received 500 mg/kg of the most extracted material in the form of a subcutaneous injection.

The results showed that the second group had the highest mortality rate, while the fifth group had the lowest mortality rate. The third group had a mortality rate similar to that of the fifth group. The fourth group had a mortality rate similar to that of the second group.

The study concluded that the most extracted material of Sterculia setigera and Lantana camara has a significant effect on the eggs of Bulinus truncatus and Biomphalaria pfeifferi.
المجموعة الرابعة عولجت بالبرازكونت اللجرة الموصى بها للعلاج لمدة يومين
(سعة أسباب بعد الإصابة بالعدوى)، والمجموعة الخامسة تركت من غير علاج كمجموعة
ضابطة موجبة.

بعد نسعة أسباب عن الإصابة تم تشريح الفئران وأجريت المعايير الوبائية لتقييم النتائج
أوضح النتائج أن المستخلص الكحولي لـ10-11 ذات التتر لا يؤثر على ديبان بلهارسيا
المستقيم عند زراعتها في وسط ملام خارجي - عن استعمال جرعة 100 ماكيرو جرام/مل ولكنه يؤثر داخل الفار ويقلل عدد الديدان بنسبة 29٪ عند إعطائه كعلاج كما يقلل نسبة
الديدان 40% عند إعطائه كوقاية بجرعة 500 ملجم/كجم من وزن الفار.

وبالنسبة لتعيين نسب المراحل التطورية لبوبيضات البلهارسيا فقد أدت المعالجة (7
أسابيع بعد العدوى) إلى حدوث زيادة ملحوظة في نسبة البوبيضات الميتة ونقص في نسبة
البوبيضات الناضجة عند مقارنة هذه المجموعات بالمجموعة المعدية الضابطة. كذلك أحدثت
نقص ملحوظ في عدد البوبيضات في الكبد والأمعاء.

أظهرت النتائج أن المستخلص الكحولي لأوراق اللانتان يقلل عدد الديدان عند إعطائه
كعلاج ووقاية كما يقلل عدد البوبيضات في حاليا الكبد والأمعاء والنسبة المئوية للمجموعة
الناضجة.

هناك تحسن ملحوظ يتمثل في النقصان الملمح في عدد الديدان وعدد البوبيضات
و نسبة البوبيضات الناضجة وغير الناضجة عند المعالجة بالجيم بين مستخلصات لحاء التتر
وأوراق اللانتان، أحدثت العدوى بالبلهارسيا المعوية زيادة ملحوظة في تركيزات أنزيمي
الألتين أمينوتراستبيريز (AST) والاسبرت أمينوتراستبيريز (ALT) أدت المعالجة بلحاء
الترتر أو أوراق اللانتان قبل وبعد العدوى إلى تقليل تركيزات مصل الدم الأنزيمي للألتين
امينوتراستبيريز والاسبرت أمينوتراستبيريز. كما كاد أن يؤدي إلى إراج مستوى
الألبيومين والقلوبولين والبروتين الكلي إلى المستوى الطبيعي في المجموعتين المعالجة
والوقائية.

أدي الجمع في العلاج والوقاية بين كل من لحاء التتر وأوراق اللانتان إلى حدوث
تحسن في مستوى تركزات ونشاطية الإنزيمات السابقة.

و عندما أعطي عقار البرازكونت بجرعته الموصى بها 500 ملجم/كجم لمدة يومين
متكافئين وجد أنه يقلل نسبة البوبيضات الناضجة ويقل عدد البوبيضات الميتة وحدث اختفاء

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كامل للبيوضات التي لم تتضح بعد. وجد أيضا أن البرازيلكونتينيل قد حسن وظائف الكبد تقريباً إلى المستوى الطبيعي وأيضا حسن الفحص الباثولوجي للكبد بالنسبة للفئران المعدة بالبهارسيا المعوية.

صممت تجربة أخرى لتقسيم فعالية هذه النباتات كقاتل للقوافل وذلك بتكوين سبعة تراكيز مختلفة من المستخلص الكحولي لحاء الترتر وأوراق الالانتان ابتداء من 1000 جزء من المليون - اخترعت تنازليا لتصنف التركيز - وقد استعملت عشرة قواقع من جنس ( B. ) وقد أجريت التجربة خلال 24 ساعة، كما استعملت الجرعة القاتلة 95-50% من المجموع الكلي للقوافل كمقياس لمدى سمية المستخلصات. ولقد أظهرت أوراق الالانتانا نشاطاً أعلى كمبيد للقوافل من الجنسين (LC50 ) B. truncatus أكثر من لحاء الترتر (LC50=111ppm) B.pfeifferi و (LC50=48ppm) B. truncatus B. pfeifferi (LC50=296ppm) B. pfeifferi و (LC50=438ppm) B. truncatus B. pfeifferi

وعليه يمكن استنتاج أن لحاء الترتر وأوراق الالانتانا لها تأثير وقائي وعلاجي ضد ديدان بهارسيا المستقيم وتحسين وظائف الكبد كما لها تأثير كقاتل للقوافل المائية. هذه الدراسة توفر باباً لمزيد من الدراسات التفصيلية لهذه النباتات كقاتل لدى ديدان قواقع البهارسيا.

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Chapter One
General
Introduction and Literature Review
Chapter One

General

Introduction and Literature Review

1.1 Introduction:

Schistosomiasis is also known as bilharzia after Theodor Bilharz, who first identified the parasite in Cairo in 1851. Infection is widespread with a relatively low mortality rate, but a high morbidity rate, causing severe debilitating illness in millions of people. The disease is often associated with water resource development projects, such as dams and irrigation schemes, where the snail intermediate hosts of the parasite breed (WHO, 2010).

Schistosomiasis is a chronic, parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. An estimated 700 million people at risk in 74 endemic countries; as their agricultural, domestic and recreational activities expose them to infested water, more than 207 million people are infected worldwide – most live in poor communities without access to safe drinking water and adequate sanitation, hygiene and play habits make children especially vulnerable to infection, and in many areas a large proportion of school-age children are infected. The WHO strategy for schistosomiasis control focuses on reducing disease through periodic, targeted treatment with praziquantel (WHO, 2010).

Schistosomiasis is one of the most widespread infections and is only second to malaria in terms of socio-economic and public health importance (Doumenge *et al.*, 1987).
Human schistosomiasis is a parasitic disease affecting more than 275 million people worldwide and resulting in 200,000 deaths annually (Chistulo et al., 2000; Engels et al., 2002)

Snails belong to the family Planorbidae act as intermediate hosts for the parasitic blood trematodes of the genus Schistosoma which cause the debilitating disease known as schistosomiasis. Two clinical forms of the disease are known: urinary schistosomiasis caused by Schistosoma haematobium, and intestinal schistosomiasis caused by Schistosoma mansoni, Schistosoma japonicum, Schistosoma intercalatum and Schistosoma mekonge

Intestinal schistosomiasis is caused by Schistosoma mansoni and afflicts millions of people in many tropical and subtropical countries (Eddleston and Pierini, 1999).

In chemical laboratories worldwide, compounds, more and more toxic to the vector snails, are being discovered. Copper sulphate (CuSO₄), sodium pentachlorophenate (NaPCP), triphenmorph, and niclosamide are among the synthetic compounds. Niclosamide is the molluscicide of choice, being highly active against different stages of the snail life cycle, and it is not toxic to humans, domestic animals and crops, but is toxic to fish (Sturrock et al., 1994). However, the applications of niclosamide donot prevent recolonization by the remaining snails which may lead to selection of molluscide-resistant populations. The molluscidal potency of the present synthesized organophosphorus derivatives of niclosamide was studied. Preparation of such derivatives compounds was encouraged due to their potential rapid metabolism, decomposition in the soil and water and low chronic toxicity (Gruzdyev, 1983). Several other compounds such as nicolinanilides, organotin and lead compounds, and “Endod” (an extract from the plant (Phytolacca dodecandra).
It is generally agreed that snail control is one of the most rapid and effective means of reducing transmission of schistosomiasis (Mc Cullough et al., 1980). The potential of using plants for control of the intermediate hosts of human schistosomiasis and other snail-transmitted parasitic infections has received considerable attention (Perrett and Whitfield, 1996).

The importance of plants as sources of natural product bioactive molecules to medicine lies not only in their pharmacological or chemotherapeutic effects but also in their role as template molecules for the production of new drug substances (Phillipson, 1994).

Many plants are known to have molluscicidal activity; such plants may provide cheap, safely produced, biodegradable and effective control agents in rural areas of developing countries (Marston, 1993).

In the Sudan, a new trend has developed to control vectors of human disease, using natural products including plant extracts as larvicides and molluscicides and schistosomicide. Interest in plants as pesticides has started in the Sudan as early as 1930s when Archibald (1933) advocated planting the tree, *Balanites aegyptiaca* along the water courses of the River Nile. Laboratory trials of this scientist showed that one fruit soaked in 100 litres of water for 24 hours killed cercaria, tadpoles and mosquito larvae, while his field observations showed that fallen fruits of the *Balanites* species inhibited the increase of snail population.

One of the alternatives in the integrated control strategies is the use of natural products; the literature contains many examples of plant products that showed considerable activities against molluscs. The dried berries of the Ethiopian herb *Phytolacca dodecandra* “Endod” showed high molluscicidal potency (Lemma, 1965 and 1970) with saponin as an active constituent (Lemma, 1970; Baallway, 1972; Lemma et al., 1979).
The relatively high molluscicidal potency of the seeds of *Croton* species and *Jatropha* species has been reported by some workers (Amin *et al.*, 1972, Daffalla, 1973 and Daffalla and Amin, 1976). Their detailed laboratory and field evaluation of the molluscicidal potency of *Croton macrostachys* seeds (locally known as Habat –El-Molok) showed that a concentration of 0.1 ppm of the aqueous extract of these seeds kill 90 percent of *B. truncatus*, on 24 hours exposure, while 20 ppm of the same solution was required to kill 90 percent of *B. pfeifferi*. In the field a concentration of 5 ppm of this aqueous extracts gave 100 percent kill of the two species.

Daffalla (1973) found that *B. aegyptiaca* and *Scilia lihacina* have molluscicidal activities against *Bulinus truncatus* and *Biomphalaria pfeifferi*.

Elkheir and ELTohami (1979) screened 51 Sudanese medicinal plant species. Eight plant species possessed molluscicidal activity against *B. truncatus* and seven of these also were active against *B. pfeifferi*, with the plant species *Gnidia kraussian* as the most promising candidate.

In the Sudan the disease is prevalent in all regions of the country, and it increased in distribution and prevalence as a result of expansion in water resources development projects (Blue Nile Health Project, 1981) (WHO, 1985).

Schistosomiasis has been recognized as a common parasitic infection in domestic stock and wild game in Africa, but there has been little recognition of its veterinary significance. Schistosomiasis in cattle and sheep may, however favour intensive disease transmission and cause significant losses.

Schistosomiasis control can be achieved through proper sanitation, snail eradication and chemotherapy (Jordan *et al.*, 1980). Chemotherapy of schistosomiasis by anti-schistosomal drugs aims at eradication of the
worms and stopping the production of eggs in the greatest percentage of patients treated in the shortest time, and with least cost.

Praziquantel (PZQ) is the drug of choice for schistosomiasis control on a population wide basis in endemic areas, because it is effective against all species of human schistosomiasis. Substantial reduction in its price with market competition was recorded. Also it is a safe drug with tolerable side effects (Abramowicz, 1992; Cioli et al., 1995; and Doenhoff et al., 2002).

Yet in the recent years, a number of reports indicated the apparent failure of the recommended doses of PZQ to yield the expected cure rates in human population in Kenya (Coles et al., 1987), Brazil (Katz et al., 1991), Senegal (Stelma et al., 1995; and Fallon et al., 1997) and Egypt (Ismail et al., 1996). This evidence about resistance raised alarm to search for new alternative drugs either synthetic or from natural resources to overcome this problem.

The use of natural resources (e.g. plants, fungi, bacteria ...etc) for treatment of many diseases is associated with folk medicine from different parts of world. Natural products from some of these natural resources continue to be used in pharmaceutical preparations either as crude extract, fractions, pure compounds or analogous compounds. Plants have provided a number of useful clinical agents that prove to have considerable potentials as sources of new drugs (Pillipson, 1994). So, the use of medicinal plants which grow abundantly in areas where schistosomiasis is endemic may become a useful complement either as molluscicides or chemotheraputic for the control of this disease.

However, few studies have addressed the use of medicinal plants with antischistosomal activity as treatment for this disease (Liu and Weller, 1996 and Schulz et al., 1997).
In The Sudan, medicinal and aromatic plants and their derivatives are locally sold at special shops called Atareen. Most vendors of traditional medicine within Khartoum area are of local inhabitants who have well established retail or wholesale outlets. The most outstanding and nationally recognized house of expertise in Sudan is known as Timan. They are usually providing counseling to the patients in addition to dispensing these herbal preparations.

*Sterculia setigera* (Tartar Tree) of the family *Sterculiaceae* is a multipurpose tree widely distributed in the Savannah zone, producing a wide range of products such as high quality water – soluble gum (Tartar gum).

In The Sudan; the tree is primarily grown for gum production and for local medicinal uses, e.g., the water extract of the bark is used for bilharzias; the leaves and bark are used for treating cough, diarrhea, fever, leprosy and syphilis and also used as a diuretic (Maydell, 1990). There are no data concerning the antischistosomal activity of *Sterculia setigera* world wide.

*Lantana camara* L. The (Verbenaceae) commonly known as wild or red sage is the most wide spread species of this genus and regarded both as a notorious weed and a popular ornamental garden plant (Sharma *et al.* 1987). However, it is listed as one of the important medicinal plants of the world (Ross, 1999).

The aromatic leaves of *Lantana camara* contain relatively high concentrations of essential oils and are occasionally of "agarbattis" for generating scented smoke when burnt during religious prayer in the Indian Society.

*Lantana camara* contains lantadenes, the pentacyclic triterpenes which is reported to possess a number of useful biological activities. Several reports have described antifungal (Tripathi and Shukla, 2002,
Kumar et al., 2006), anti proliferative (Saxena et al. 1992, Nagao et al., 2002), and antimicrobial activities (Saxena et al., 1992, Juliani et al., 2002, Kasali et al., 2002, Rajakaruna et al., 2002) and Termicidal activity (Verma and Verma 2006). Moreover, the hydroalcoholic extracts of the leaves have shown an effect on fertility, general reproductive performance, and teratology in rats (Mello et al., 2005). Misra et al., (1997) reported the presence of putative hepato protective Oleanolic acid in Lantana camara roots in high concentrations.

Lantana camara Linn. var. aculeate (Verbenaceae), is a problem weed in India and in parts of the world it spreads rapidly in sunny environments.

Lantana camara whole plant and plant parts leaves, flowers and essential oils have been thoroughly studied for their chemical composition. All these studies have revealed the presence of terpenoids steroids, and alkaloids as major chemical constituents in Lantana camara (Saleh, 1974; Hart et al., 1976; Sharma and Sharma 1989; Siddiqui et al., 1995; Ghisalberti, 2000).

Chemical composition of the whole plant and plant parts and essential oils are reported to be influenced by genetic, geographical, and seasonal factors as well as the development stages of the concerned plants, its parts/ tissues. Randrianalijaona et al., (2005) have reported the seasonal changes in the chemical composition of essential oils in more than seventy Lantana camara varieties from different parts of the world. Bhakta and Ganjewala (2009) reported the ontogenic variation in secondary metabolites such as phenolic anthocyanins, and proanthocyanidins.

Lantana camara has been studied extensively for their antibacterial properties (Siddiqui et al. 1995, Deena and Thoppil, 2000, Mello et al., 2005, Verma and Verma, 2006). Lantana camara possesses many
important biological activities; antipyretic, antimutogenic, insecticidal, nematicidal and others (Siddiqui et al., 1995, Deena and Thoppil, 2000, Mello et al., 2005, Verma and Verma, 2006). Lantadenes present in all Lantana camara is believed to be responsible for almost all the biological activities (Barre et al., 1997). In addition, other secondary metabolites such as alkaloids, terpenoids, and phenolics could be held partially responsible for some of these biological activities (Barre et al., 1997). However, constituents like 1,8-cineole, Sabinene, and caryophyllene and other minor constituents; E.nerolidol, bicyclogermacrene, and pinene identified in leaf essential oils were also found to be responsible for the biological activities of essential oils (Chowdhury et al., 2007, Sonibare and Effiong 2008).

Earlier reports, concerning the essential oil composition have primarily reported physio-chemical properties without complete information concerning the constituents (Saleh, 1974; Peyron et al., 1971; Singh et al., 1991). The investigation revealed that the oil is rich in sesquiterpenes which may be suitable for blending purposes in order to enhance the longevity of the perfumery product. Nayak et al., (2009) demonstrated that Lantana camara is effective in healing excision wounds in the experimental animals (rats) and could be evaluated as a therapeutic agent in tissue repair processes associated with skin injuries.

Lantadenes are pentacyclic triterpenoids of the weed Lantana camara, five new lantadenes (14-18) and their methyl esters (20-24) were synthesized, characterized, and screened for cytotoxicity against four human cancer cell lines. The results proved the potential antitumor activity of lantadenes (Sharma et al., 2008).

Linaroside (1) and lantanoside (2), two flavonoids isolated from Lantana camara and their common acetyl derivative (3) were examined for antimycobacterial activity against mycobacterium tuberculosis strain
H(37)RV. These compounds exhibited 30%, 37% and 98% inhibition, respectively at 6.25 micro g ml (-1) concentration. Among these flavonoids the acetylated compound was found to be the most active. (Begum et al., 2008).

Misra et al., (2007) reported the antifilarial activity in the extract of stem portion of the plant Lantana camara. The crude extract at 1 g/kg for 5 days by oral route killed 43.05% of the adult Brugia malayi parasites and sterilized 76% of surviving worms in the rodent model Mastomys coucha. A 34.5% adulticidal activity along with sterilization of 66% of female worms could be demonstrated in the chloroform fraction.

Two compounds, oleanonic and oleanolic acids, isolated from hexane and chloroform fractions showed \( LC_{100} \) at 31.25 and 62.5 mug/ml respectively, on B malayi in vitro, (This is the first ever report on the antifilarial efficacy of Lantana camara). Extracts of Lantana camara var. aculeate leaves were studies for their phyto-chemical constituents and termiticidal effects against adult termite workers. The 5% chloroform extract was found to be significantly effective against termite workers (Verma and Verma 2006). Lantanilic acid, camaric acid and oleanolic acid possessing nematicidal activity were isolated from the methanol extract of the aerial parts of Lantana camara linn. through bio-assay guided fractionation. These compounds exhibited 98%, 95% and 7% mortatity respectively against root – knot nematocle Meloidogyne incognita at 0.5 % concentration. (Qamar et al., 2005). Abdel-Hady et al., (2005) reported the larvicidal effect of the volatile oils of Lantana camara CV., flava leaves and flowers, when was tested against the Musca domestica L. larvae in the laboratory at concentrations (0.0125%, 0.025%, 0.05%,0.1% and 0.2% ). They showed mortalnty rate ranged
from 80% -100% on the other hand, 10-20% of the developed pupae emerged to adult.

The essential oil of *Lantana camara* tested against seven bacteria and eight fungi, showed a wide spectrum of antibacterial and antifungal activities (Deena and Thoppil, 2000).

 Forty nine Tazanian medicinal plant were extracted and tested for *in vitro* antimalarial activity, using the multidrug resistant k1 strain *Plasmodium falciparum*. The three most active antimalarial extracts were obtained from the tubers of *Cyperus rotundus* L. (Cyperaceae), the root bark of *Hoslundia opposita* Vaht. (Labiatae) and the root bark of *Lantana camara* L. (Verbenaceae) (Weenen *et al.*, 1990).

Ingestion of lantana foliage by grazing animals causes cholestasis and hepatotoxicity. Both ruminants and non ruminant animals such as quinea pigs, rabbits and female rats are susceptible to the hepatotoxic action of lantana toxins. Green unripe fruits of the plant are toxic to humans. *Lantana spps* exert allelopathic action on the neighboring vegetation. The allelochemicals have been identified as phenolics, with umbelliferone, methylcoumarin, and salicyclic acid being the most phytotoxic (Sharma and Sharma, 2007).

The hepatoxins are pentacyclic triterpenoids called lantadenes. Unlike lantadene A, both form I and II of lantadene C elicited strong hepatotoxic response in quinea pigs associated with decrease in fecal output, feed intake, hepatomegaly, hepatic injury at the cellular and subcellular level, increase in plasma bilirubin, and acid phosphatase activity (Sharma *et al.*,1992).

Misra *et al.* (2007) reported the presence of putative hepatoprotective oleanolic acid (1) in *Lantana camara* roots in high concentration.
Fouad et al., (2007) screened 346 methanol extracts from Egypt for schistomicidal activity; he proved that *Lantana camara* L. (leaves, flowers, branches) possess antischistosomal activity with LC$_{50}$ 28.20 ppm and with LC$_{90}$ 34.90 ppm.

Mahmoud and Refaie (2001) who reported that the parasitological studies on *Lantana camara* leaves aerial parts (leaves, flowers, tips of branches and fruits) extract in *S. mansoni* infected mice (8 weeks post-infection) caused a significant reduction of the total number of worm by about 62% and reduced the number of ova in liver and intestine by 41% after 2 weeks from stop administration of plant (200 mg/b.w. daily for 2 weeks).
1.2 Schitosomes and Schistosomiasis:

1.2.1 Schistosomiasis in Africa:

Schistosomiasis is a chronic, debilitating parasitic disease caused by blood flukes of the genus *Schistosoma*, and is also known as "bilharzia". It is endemic in 74 countries in Africa, South America and Asia. Worldwide, an estimated 200 million people are infected, of which 20 million are assumed to suffer from more or less a severe form of the disease creating 4.5 million DALYs1 lost (WHO Expert Committee 2002). Schistosomiasis is endemic in 46 out of the 54 countries in the African continent. The disease may cause damage to various tissues (the bladder, liver or the intestines) depending on the species, and lowers the resistance of the infected person to other diseases. There are 16 different known species of *Schistosoma* (*S*), of which 5 are infective to man-- *S. mansoni*, *S. haematobium*, *S. intercalatum*, *S. japonicum* and *S. mekongi*. The species differ according to their snail intermediate hosts, egg morphology, final location of the adult worms in the human body, resulting symptoms, and their geographical distribution (Doumenge *et al*., 1987).

In sub-Saharan Africa, approximately 393 million people are at risk of infection with *S. mansoni*, of which 54 million are infected. These numbers for *S. haematobium* are estimated to be as high as 436 million at risk and 112 million are infected (Van der Werf *et al*., 2003).

The intermediate hosts of the schistosomes in Africa are freshwater pulmonate snails, that belong to the "Planorbidae" family. The species belong to two genera, namely *Biomphalaria*; the hosts for *S. mansoni*, and *Bulinus*; the host for *S. haematobium* and *S. intercalatum* (Sturrock, 1993). Both *Biomphalaria* and *Bulinus species* prefer water velocities below 0.3 m/s, gradual changes in the water level, a slope of less than 20 m/km, firm mud substrate, little turbidity, some organic pollution, partial
shade and optimal water temperature between 180 C and 280 C (Birley, 1991). Under these conditions, aquatic vegetation and algae, which are the main sources of nutrition for the aquatic snails, flourish creating favorable habitats for intermediate snail hosts. Transmission can take place in almost any type of habitat e.g., from large lakes and rivers to small seasonal ponds and streams. Although transmission may be intense in both natural and man-made water bodies, the latter seems particularly important as the human population density is often high near these. Within the irrigation schemes transmission is focal and is primarily due to much localized contamination of habitats with human excreta or urine containing schistosome eggs and, also because of the high incidence of human water contact at a few points. Schistosome transmission usually is seasonal, primarily due to the variation in temperature and the irrigation cycle. Local circumstances influence both time and place of transmission, which should be taken into consideration when designing ecological measures to control the snail population (Birley, 1991).

In Egypt, schistosomiasis is not only a major health problem, but also an economic one, as it affects millions of farmers at an early age diminishing their productivity and exerting a series of socioeconomic problems (Mohy EL-Dine, 1978). Until few years ago the prevalence of schistosomiasis in Egypt was high and reached 33.1% for S.mansoni in delta region (Faraghaly, 1992; Talaat and Miller, 1998).

Few investigators have conducted in vitro bioassay screening of plants for antischistosomal activity. Molgaard et al., (2000) screened extracts of 23 plant species from Zimbabwe and found that the stem and root extracts from Abrus precatorius (Fabaceae) and stem bark extracts from Elephantorrhiza goetze (Mimosaceae ) have a good activity against schistosmules. Sparg et al., (2000) screened 21 species from South Africa against the schistosomula of S. haematobium. Berkheya speciosa
(Asteraceae), *Euclea natalenesis* (Ebenaceae), and *Trichilia emetica* (Meliaceae) were found lethal.

A 90-100% mortality of *Schistosoma* worms obtained *in vitro* by 4.0µg/ml goyazensolide, a component extracted from *Eremanthus goyazensis* (Barth et al., 1997), and 200 mg 1\(^{-1}\) of an ethyl acetate extract of ginger, *Zingiber officinale* (Sanderson et al., 2002).

*In vitro* antischistosomal activity was possessed by robustic acid and an isoflavone compound isolated from the seeds of the tree *Millettia thonningii* (Lyddiard et al., 2002), as well as extracts of *Scilla natalensis* and *Ledebouria ovatifolia* (Sparg et al., 2002).

Concerning *in vivo* antischistosomal activity of natural product, Utzinger et al., (2001) reported that artemether, the methyl ether derivative of artemisinin, which is a Chinese active antimalarial principle in the leaves of *Artemisia annua*, exhibited antischistosomal properties by oral doses of 6 mg/kg in randomized controlled clinical trials.

Manssoud et al., (2001) and Abo–Madyan et al., (2004) reported that *Commiphora molmo* extract (Myrrh capsules) given to patients at a dose of 10 mg/kg of body weight per day for 3 or 6 consecutive days induced a curative rate higher than 90%. However, contrary to these results, Botros et al., (2004) and Barakat et al., (2005) did not recommend the use of this plant drug in treating human cases of schistosomiasis based on negative results from several experiments performed on patients and infected animals.

### 1.2.2 Schistosomiasis in the Sudan:

The first mention of the existence of schistosomiasis in the Sudan during the last century was reported by Balfour (1904), when he drew attention to a focus of “endemic haematuria” in Khartoum primary school where he found 17% of children to be suffering from urinary schistosomiasis. Archibald (1914) reported four cases of intestinal
schistosomiasis in Khartoum, three of which were from Egyptian soldiers. Before 1918, few cases occurred in the Northern States and in other areas of the country where considerable contact with Egyptian persons had taken place. The infection is thought to have been introduced by the Egyptian laborers and West African pilgrims, who used to cross Sudan in the way to the holy land Mecca (Ayad, 1950).

The manifestations of the disease began to capture medical attention within a period characterized by the constriction of agricultural development schemes. In (1925), an agricultural scheme was constructed in Gezira area, after that the prevalence of the disease increased rapidly to reach 15% in 1952 and 80% in 1978 (Omer et al., 1976).

*S.mansoni* infection is the predominant type of schistosomiasis in the Gezira Managil scheme. Both types of schistosomiasis were more prevalent in the Managil compared to the Gezira area, where the Blue Nile Health project has adopted a comprehensive control strategy consisting of chemotherapy, focused mollusciciding, health education and improvement of water supply and sanitation during the period 1980-1990. In April 1999, Gezira Administration Board adopted a new system for improving irrigation, consisting of drying and cleaning canals before the irrigation season; this highlights the effectiveness and long – lasting impacts of integrated control programmes in high endemic areas (Hilali et al., 1995).

In Gezira area, Kheir et al., (2000); investigated the long-term effect of single-dose praziquantel on morbidity and mortality of *Schistosoma mansoni* patiants in 1987-1994 in central Sudan (Abu Jin village-Gezira State); they reported that a single dose of praziquantel had a significant impact on the disease prevalence, intensity of infection, burden of chronic liver disease and mortality due to *Schistosomiasis*
massoni. Mass treatment can be spaced to a much longer period, reducing the expenses of treatment, delivery and distribution.

The disease is prevalent in all regions of the country, and in recent years, the disease increased in distribution and prevalence as a result of expansion in water –resources – development projects (BNHP, 1981)

In Northern Sudan, Schistosomiasis haematobium was reported along the Nile, North of Khartoum and Schistosoma mansoni in small localized areas (Buchana, 1937). Omer (1978) found the prevalence of Schistosoma haematobium among fisher men in Lake Nassir to be over 38%.

In Southern Sudan, both Schistosoma mansoni and S. heamatobium were reported (Omer et al., 1972). Brown et al., (1984), during the assessment of Jongoly Canal, reported the presence of schistosome intermediate host in the area and emphasized the possibility that the canal might increase snail population and raise the prevalence of parasitic diseases. In Western Sudan, an S. haematobium prevalence of 14% was reported among children (Eltom, 1976) but also the prevalence of S. haematobium was found to be 8.5%, 29.5% and 37.7% among children in Kadogli, Deleng and Rahad respectively (Dafalla and Suliman, 1988).

According to Doumenge et al., (1987); hospital records showed a prevalence of S. haematobium of 8.7% in Kassala and 3.4% in Gadarif. In the White Nile area S. mansoni and S. haematobiumm have been reported (Doumenge et al., 1987). In Khartoum State both types of schistosomiasis have been reported (Omer, 1978).

The expansion in agricultural projects has resulted in an increase of schistosomiasis in the Sudan (WHO, 1985). Schistosomiasis is now endemic in Kenana Sugar scheme (Amin, 1978), Guneid Sugar scheme
Before the establishment of the New Halfa scheme, schistosomiasis was not known in the area. The scheme is a good example of the spread of the disease following the expansion in agricultural development in the Sudan. The scheme was constructed in (1963) for resettlement of Halfaween, who used to live in Old Halfa "North Sudan " , when their original homes were flooded by lake Nasser following the construction of the High Dam in Aswan, Egypt. S. mansoni was unknown to the new settlers in their original homes. Shortly after the scheme was opened, the canals were invaded by Bulinus and Biomphalaria species of snails and both S. mansoni and S. haematobium were detected among the settler. In ten years the prevalence of S. mansoni reached 12% and by 1980 it was 47.3% (Abd Elgalil, 1980).

There are at least 19 species of Schistosoma of which five seriously affect people. Schistosoma japonicum and Schistosoma mekongi, causing intestinal schistosomiasis, are found in Asia; S. mansoni and Schistosoma intercalatum cause intestinal schistosomiasis in Africa and S. haematobium causes urinary schistosomiasis in Africa and the Middle East. S. mansoni, S. haematobium and Schistosoma japonicum are the most widespread and important species (Plorde and Jong, 1983). The schistosomes vary in their choice of snail as intermediate host: the genus Biomphalaria for S. mansoni, Bulinus for S. haematobium and Oncomelania for S. japonicum (Warren, 1987).

1.2.3 Pathogenesis of Schistosomiasis:

In schistosomiasis, the pathological changes start with the granuloma formation in response to the schistosomal eggs and their secretions which are more potent than the schistosomal worm itself (Warren et al., 1974). The primary cause of liver pathology in S. mansoni
infection is the immobilization of the schistosome eggs into the radiculi of the portal system leading to granuloma formation which destroys the involved radiculi (Von Lichtenberg, 1955). Destruction of the vascular muscle layer of the portal vein follows with subsequent inflammation and fibrosis of it (Andrade, 1965). Such inflammatory reactions with consequent fibrosis usually result in presinusoidal obstruction of the portal blood flow leading to portal hypertension which cases splenomegaly, hepatomegaly, ascites, esophageal and gastric varices as a result of portasystemic shunting (Raia et al., 1985). The liver becomes hard and nodular. Bleeding due to esophageal varices is a major cause of death (Mahmoud, 1984). The liver is considered to be the main organ responsible for the biosynthesis, uptake and degradation of a number of biological materials in blood including proteins and enzymes. Almost all the enzymes are intracellular. Following an injury, or death of physiological active cells, the enzymes are released into the circulation and can be used as an indicator of cell damage rather than cell function (Eastham, 1975).

Schistosomal infection causes liver necrosis characterized by fibrosis and absence of regeneration leading to liberation of enzymes contained within the cell (Salah, 1962 and EL-Haieg et al., 1978). Serum enzymatic activity of alanine transaminase (ALT) is considered a sensitive parameter for functional changes in hepatosplenic bilharziasis (EL-Haieg et al., 1978 and Taha et al., 1992). Increase of this enzyme denotes an active hepatic cell damage and/or an increase in permeability of the cell membrane to this enzyme (Ghanem et al., 1970 and Ebeid et al., 1987). The increased level of ALT in mice infected with S. mansoni was more pronounced 8 weeks post infection. This was related to the existence of the largest hepatic granuloma reported to be present from the 8th to 10th week post infection (Metwally et al., 1990 ; Abd EL-Rahman et
Badawy et al., (1993 and Ahmed 1995). Badawy et al., (1996) reported that ALT has progressively increased with chronicity of infection in mice. Early treatment with PZQ is important in the therapeutic control of morbidity in schistosomiasis, as it was found to improve conventional liver function test (ALT). Schistosomiasis has a significant role in the alteration of liver functions since the activity of ALT was significantly higher in the blood sample of schistosomal patients and experimental mice than those of the control (Habib et al., 1996; Sheweita et al., 1997 and Ebeid et al., 2000). A tendency for normalization of serum ALT level was noticed in S. mansoni infected mice treated with PZQ. The return of enzymatic level to control value after therapy was related to the stopping of egg deposition and eradication of the parasite worms (Ebeid et al., 2000).

Several authors reported an increase in serum total protein concentrations in schistosomal patients (EL-Raziky et al., 1985 and EL-Sharabasy et al., 1993). Moustafa et al., (1996) reported an increase in the total plasma proteins in Syrian golden hamsters infected with S. mansoni and treated with PZQ in a dose of 100 mg/kg. Badawy et al., (1996) reported also an increase in the serum total proteins in mice 18 weeks post infection with S. mansoni. They added that the decline in enzymatic level was observed 22 weeks post infection. Some authors did not find any change in the level of serum total proteins in association with schistosomal infection (De Witt and Warren, 1959; Rutkowski and Bruce, 1971). On the other hand, Hypoalbuminemia symptom was reported by EL-Ridi et al., (1987) in cases of schistosomal infection.

1.2.4 Immunopathogenesis:

In murine schistosomiasis, the immunologic response is divided into two phases; acute and chronic (Boros et al., 1975; Warren, 1982; Fidel and Boros, 1990). The acute phase occurs 8 to 10 weeks post infection. This phase is characterized by a vigorous granulomatous
response; a delayed-type hypersensitivity (DTH) response; foot-pad swelling and inflammatory lymphokine production (Boros et al., 1975; Colley, 1975; Chensue and Boros, 1979; Mosmann and Coffman, 1989; Stadecker, 1992). The onset of chronic phase begins 12 weeks post infection and is fully developed 18-20 weeks after infection. This phase is characterized by spontaneous down-regulation of the granulomatous delayed-type hypersensitivity response as well as diminished lymphoproliferative response and inflammatory lymphokine production (Boros et al., 1975; Colley, 1975; Chensue and Boros, 1979; Weinstock and Boros, 1983; Fidel and Boros, 1990).

1.2.5 Chemotherapy of schistosomiasis:

Schistosomiasis is a major health problem in tropical countries involving an estimate of 200 million people (Nash et al., 1982a). Schistosomiasis control can be achieved through proper sanitation, snail eradication, immunization and chemotherapy (Macdonald et al., 1969; Jordan et al., 1980). Chemotherapy of schistosomiasis by antischistosomal drugs aims at stopping the production of eggs in the greatest percentage of patients treated in the shortest time, and with the least cost. It also aims to relief symptoms, heals existing pathological lesions and prevents of further damage to tissues and organs of host (Mousa and Zien EL-Abdin, 1971).

By the early 1970s, metrifonate was established for use against S. haematobium, although the need for three fortnightly treatments caused logistic problems for community use. Fears for its safety prevented the widespread acceptance of hycanthone, the first single-dose drug effective against S. mansoni and, possibly, S. haematobium, but a similar drug, oxamniquine, was released for community use against S. mansoni in the 1970s. The real turning point was the arrival in the early 1980s of praziquantel, a safe, effective, single-dose drug active for all
schistosomes and many other human and veterinary helminths, besides (WHO, 1985). It promised to revolutionise community chemotherapy against schistosomiasis, although it was initially too expensive for widespread use. Its price has at last dropped radically and it is now the major weapon for community control of schistosomiasis.

Antischistosomal drugs may be classified into two main groups: antimonial compounds; non-antimonial compounds. Historically, antimonials including the famous emetine were introduced during the First World War as the first effective anthelmintics for schistosomiasis. Metrifonte has been widely used to treat *S. haematobium* in Africa and oxamniquine has been employed to treat *S. mansoni*-infected patients in Brazil. Lastly praziquantel (PZQ) has been adopted as the drug of choice for all human schistosome infections (Marshall, 1987; Grove, 1990).

1.2.5.1 *Antimonial antischistosomal compounds:*

All antimonial compounds used in the chemotherapy of schistosomiasis belong to the organic trivalent group. Antimonials are highly effective against the three species of schistosomes pathogenic to man; *Schistosoma mansoni, haematobium* and *japonicum*. This group includes the following drugs:

- Tartar emetic (Antimony potassium tartarate); Stibinal (Sodium antimony tartarte); Stibophen (Fouadin); Anthiomaline (Antimony lithium thiomalate); Triostam (Sodium antimony gluconate) and Astiban (Sodium antimony dimercaptosuccinate). (Christopherson, 1928).

**I- Mode of action of antimonials:**

Antimonials deprive the worm from energy by blocking the enzyme phospho-fructokinase responsible for glycolysis (Khayyal, 1964). This results in accumulation of fructose-1,6-diphosphate. Depressed
oxygen uptake by the worms following treatment with antimonials has been reported by Magzoub et al., (1971), London. Antimonials affect the reproductive system of schistosomes, the female are more susceptible than males (Bueding and Fischer, 1969). This special sensitivity of the female worms facilitates suppression of egg production (Khayyal, 1964; Guirgis et al., 1966). As a result of the reduced anaerobic glycolysis, relaxation of the smooth and striated muscles of the worms occurs. Worms then loose their muscle tone, become unable to fix themselves to the wall of the blood vessels, and are swept gradually back, passively with the portal blood to the liver (Standen, 1953; Tadros, 1973). The shift of the worms back to the liver starts within 30 minutes after intraperitoneal injection (Khayyal, 1964). In the liver, the concentration of antimony is high enough to produce degeneration of the worms. Worms become then encapsulated within the tissues, die and are invaded by leucocytes (Standen, 1953). Coles (1979) reported that the immature stages of *S. mansoni* in mice were found to be less susceptible to antimony therapy than adult worms.

**II- Side effects of antimonials:**

Immediate side effects accompanying antimonial therapy are namely: nausea, vomiting, cough, dyspnea, chest pain, diarrhea, shock and occasionally sudden death (Abd EL-Wahab, 1982). Local thrombosis occurs when the wall of the vein is scratched or when the solution is injected directly into its wall. Acute heart failure was related to the increased pulmonary vascular resistance with weakening of the heart muscle, and not to the action on the vagus nerve, or potassium content of tartar emetic. Reduction in urine output, renal failure and a high concentration of the carcinogenic metabolites of the amino acid tryptophan in the urinary bladder were also recorded (Halawani et al.,
1955; Abdel Daim et al., 1967; Abdel-Wahab, 1982 and Cioli et al., 1995).

1.2.5.2 Non antimonial antischistosomal compounds:

I- Xanthone derivatives

Including lucanthone and hycanthone. Lucanthone hydrochloride is called commercially Miracil D (Mauss, 1948; Stohler and Frey, 1963).

II- Nitro- heterocyclic compounds:


III- Organophosphorous compounds:

Diptrex

Metrifonate (Bilarcil) (Saif et al., 1973; Davis and Bailey, 1969).

IV- Quinoline preparations:

Oxamniquine (Richards and Foster, 1969).

V- Amoscanate (Striebel, 1976).

VI- Pyrazino-isoquinolines.


VII- Oltipraz (Gentilini et al., 1980).

VIII- Benzodiazepine derivatives.

Nine acridanone- hydrazone derivatives.

A group of 9-acridanone- hydrazone derivatives were synthesized by the Hoffman- La Roche Co. (Basel, Switzerland). (Stohler and Montavon, 1984; Sturrock et al., 1987 and Eshete and Bennett, 1990, 1991 and 1992).

1.2.5.3 Praziquantel (PZQ):

PZQ is effective orally against all species of schistosomes infecting man, S. haematobium (Davis et al., 1979) S. mansoni (Katz et al., 1979 and Simarro et al., 1991) and S. japonicum (Santos et al., 1979 and Xiao et al., 1991). The drug is also effective against S. intercalatum, S.
matthei (Gonnert and Andrews, 1977) and S. mekongi (Nash et al., 1982b). Praziquantel is the drug of choice for treatment of schistosomiasis for a number of reasons: high efficacy against all schistosomiasis species; lack of serious short term and long-term side effects; administration as a single oral dose; competitive cost (Cioli et al., 1995).

In addition to its use as an antischistosomal drug, it is the drug of choice in Clonorchiasis, Paragonimiasis, cysticercosis and in the treatment of many intestinal tape worms (King and Mahmoud, 1989). Praziquantel has also been used successfully in the treatment of severe fascioliasis (Schiappicase et al., 1985).

I- Chemistry of Praziquantel:

Praziquantel is a 2-cyclohexylcarbonyl – 1,2,3,6,7,11b-hexahydro-4 H-pyrazino (2,1-a) isoquinoline-4-one (C$_{19}$H$_{24}$N$_{2}$O$_{2}$; Mw 312.32). It is a colourless crystalline powder, stable under normal conditions. The drug is practically insoluble in water, sparingly soluble in ethanol, and soluble in organic solvents such as chlorophorm and dimethyl sulfoxide (Cioli et al., 1995).

II- Metabolism of Praziquantel:

Praziquantel is rapidly absorbed after oral administration (maximal serum concentration is reached within 1-2 hours) and is almost complete (80-100% of the intravenous dose). Metabolic processing of the drug is also very rapid as liver first-pass effect. The plasma half-life of unchanged PZQ is about 1 hour which means that the drug metabolism is already well under way before all substances has been absorbed (Cioli et al., 1995). Up to 80% of PZQ is bound to plasma proteins but no irreversible binding to body constituents has been observed (Steiner et al., 1976). Metabolites in general, have a longer plasma half-life than the parent compound, and are mainly represented by monohydroxylated PZQ and to a lesser extent by dihydroxylated metabolites and small amount of
trihydroxylated compound. The major metabolite is 4-hydroxycyclohexyl carbonyl analog, which represents about two-thirds of total urinary metabolites (Cioli et al., 1995).

III- Elimination of praziquantel:

Drug elimination occurs mainly (80%) in the urine, the rest being found in bile and faeces. Only traces of unchanged PZQ are excreted (less than 1% of the total dose). About 80% of the drug material is cleared from treated rats after 24 hours and essentially, no traces can be detected 4-5 days later. PZQ has been detected in human milk, but in lower concentration than in the plasma, and it was calculated that only 0.0008% of an oral dose would be excreted via this route (Cioli et al., 1995).

IV- Clinical use of praziquantel:

Praziquantel was produced originally by E. Merck (Darmstadt, Germany) as Cysticide (500 mg) or Cesol (150 mg). From Bayer AG (Wuppertal Germany) as Biltricide. At the Egyptian local market, there are three brands of praziquantel Distocide, (Eipico); Biltricide (Alexandria); Bilharzid (Pharco). The three brands are available as 600 mg tablets that can be divided in 4 segments of 150mg each.

The standard recommended treatment for *S. mansoni* and *S. haematobium* is a single oral dose of 40 mg/kg. Using the above dosages, cure rates recorded were 75-85% for *S.haematobium*, 63-85% for *S.mansoni* and 60-80% for double infections with *S.mansoni* and *S.haematobium* (Wegner, 1984 and Cioli et al., 1995). PZQ is well tolerated and effective in patients of all ages and in different clinical forms of schistosomiasis, including advanced hepatosplenic cases (Bassily et al., 1985).
V- Antischistosomal activity of praziquantel in animals:

a- Effective Dose:

The most interesting finding of mice infected with *S.mansoni* and treated with PZQ was that the therapeutic potency was maximal upon repeated administrations of closely spaced doses (Cioli *et al.*, 1995). Five doses of 50 mg/kg administrated orally at 3 hour intervals achieved complete parasitological cure in all animals, whereas the same result was not obtained with a single dose of 1000 mg/kg or when 5 doses of 250 mg/kg were given on consecutive days (Gonnert and Andrews, 1977). These data suggest that the schistosomicidal effect is not dependent on the maximum drug concentration to which schistosomes are exposed, but rather on the length of time during which parasites are exposed to a threshold drug concentration (Andrews, 1985).

b- Morphological changes:

Drug administration is rapidly followed (within 1 hour) by a shift of worms to the liver. At this time, schistosomes are contracted and males present extensive vacuolization in the basement of the tegument, while females appear to be more affected in the subtegumental layers. In addition to basal vacuolization, areas of intense surface blebbing appear on the male dorsal tegument. By 4 hours after treatment, host cells are found attached to the tegumental vacuoles and begin to penetrate into the parasite. After 17 hours, worms appear fixed by fibroblasts to the wall of the blood vessels and their internal structures are largely destroyed by host cells. At 14 days, worms are completely disintegrated within typical granulomas (Cioli *et al.*, 1995).

c- Immune dependence:

The efficacy of PZQ (and other drugs) in mice depleted of T cells is lower than in normal immunocompetent animals (Sabah *et al.*, 1985). Brindley and Sher (1987) confirmed that mice depleted of B cells respond
poorly to PZQ therapy and further demonstrated that the injection of immune serum could repair the defect of drug in immunosuppressed animals. Harnett and Kusel (1986) reported that treatment with PZQ increase the exposure of schistosome antigen at the parasite surface. Two antigens, 200kDa glycoprotein (Brindley et al., 1989) and 27kDa (Doenhoff et al., 1988) are exposed after treatment with PZQ.

**d- Mode of action of praziquantel:**

The effects of praziquantel on schistosomes are generally grouped under three headings (1) muscular contraction (2) tegumental damage (3) metabolic alterations. Muscle contraction process was explained to start as a transient stimulation of muscle tone resulting in the shortening of the worm, through increased tetanic contraction. Muscular contraction is obviously linked to the increase of intracellular calcium (Ca$^{2+}$). This increase is not due to direct action of PZQ, since the drug does not possess the properties of an ionophore (Pax et al., 1978); nor is it due to the inhibition of some ATP ase involved in the pumping of calcium out of cells (Nechay et al., 1980).

In schistosomes, the tegument is electrically coupled to muscle cells, so that a rise in the intrategumental calcium might lead to increased calcium in the sarcoplasmatic reticulum which could lead to contraction. The relationship between PZQ and calcium influx would suggest that the sites of action of PZQ are the calcium permeable ion channels in the membranes of the tegument and muscle cells (Redman et al., 1996).

A number of metabolic alterations have been observed in schistosomes exposed to PZQ (Andrews, 1985). Glucose uptake, lactate excretion, glycogen content are all decreased and the concentration of various enzymes alkaline phosphatase, ATP ase is decreased (Cioli et al., 1995).
Paralytic muscle contraction takes place within 2-3 seconds after exposure to PZQ (Pax et al., 1978). Disruption of the parasite surface `takes place within 1-2 minutes after exposure to the drug and the increase in the influx of calcium into the worm takes place within several seconds following exposure to the drug (Xiao et al., 1984). The parasite surface acquires erythrocyte and other host antigens which are thought to prevent host antibodies from binding to the tegument of the worms (Goldring et al., 1977).

The efficacy of PZQ is partly dependent on the host immune response (Sabah et al., 1986). Harnett and Kusel (1986) hypothesized that PZQ may lower the ability of S. mansoni to evade the immune response by increasing the exposure of parasite antigens capable of acting as targets for host antibody or “antibody-armed cells” at the worm surface. This has been suggested to happen by the disruption at the parasite’s masking coat of host antigens (Mehlhorn et al., 1981).

Harnett and Kusel (1986) reported that, praziquantel is lipophilic and that its effect on parasite antigen exposure might result from interaction with hydrophobic region of the tegumental outer membrane. Brindley and Sher (1987) reported that the tubercles of male worms were the major focus of antibody binding in these PZQ-exposed schistosomes. These results demonstrated that, the mechanism of action of PZQ involves a synergy between PZQ and the humoral immune response of the host and suggested that the relevant effector antibodies act directly against hidden parasite antigens as a consequence of interaction with praziquantel.

e- Effect of PZQ on different stages of schistosoma mansoni:

Praziquantel was found to be more effective against the adult stages of S.mansoni (Gonnert and Andrews, 1977; Shaw, 1990). On the other hand, Webbe and James (1977) reported that, praziquantel has no
ovicidal properties. Andrews (1978) reported that the administration of PZQ to mice infected with *S. mansoni* delays hatching of excreted eggs for 24 hours. Richards *et al.*, (1989) reported that, PZQ is lethal to mature *S. mansoni* eggs. The drug kills most of mature *S. mansoni* eggs in host tissues when administrated in high doses. Giboda and Smith (1994) suggested double dosing with PZQ as an alternative strategy to single treatment. They added that, this treatment regimen would increase the efficacy of PZQ by insulting the immature viable eggs reaching maturity by the time of second treatment. Free swimming miracidia (*in vitro*) are rapidly killed by 1 μg/ml praziquantel, also incubation of *S. mansoni* cercariae with 1 μg/ml praziquantel reduced the infection rate by 80% (Andrews, 1978). Gonnert and Andrews (1977) reported that a dose of 1000 mg/kg given 4 hourly before or after infection, reduced the percentage of schistosomules developing to adult worms by 98%. They added that when the dose was given one to seven days after infestation, the number of worms was reduced by 60 to 80%. On the other hand, Andrews (1985) reported that the skin and lung stages of worms (1-7 days old) in the infected mice were found to be less susceptible to PZQ treatment.

**f- Side effects of praziquantel:**

The most common side effects observed upon PZQ administration are related to the gastrointestinal tract: these comprised abdominal pain and/or discomfort, nausea, vomiting, anorexia and diarrhea. These symptoms were observed in up to 50% of patients, but they were usually mild and short-lived (Davis, 1993). Although bloody diarrhea has been occasionally observed after treatment of patients with heavy *S. mansoni* and *S. japonicum* infection (Polderman *et al.*, 1984 and Watt *et al.*, 1986), yet it was followed by spontaneous full recovery. Other side
effects are related to the central nervous system e.g. headache, dizziness and drowsiness.

Pruritus and eruptions were the most common skin reactions. Other non specific side effects recorded were fever and fatigue (Keittivuti et al., 1984). These side effects were found to increase in the presence of liver disease (King and Mahmoud, 1989 and El-Hawey et al., 1990). PZQ appeared not to present genotoxic risks at the prescribed doses (Kramers et al., 1991). Anwar and Rosin (1993) reported that PZQ treatment decreased the cytological damage in exfoliated urothelial cells of schistosomiasis patients. Montero et al., (1994) reported that PZQ did not induce mutations in patients. Bartsch et al., (1978) and Botros (1990) reported that PZQ in mice is not mutagenic and not carcinogenic. Younis et al.,(1998) reported that PZQ is contraindicated in ocular cysticercosis because the host response may produce irreversible damage of the eye.

1.2.5.4 Resistance to antischistosomal drugs:

The widespread use of drugs to treat and control infection organisms usually leads to the occurrence of drug resistance. Resistance is defined as a reduction in drug sensitivity in a population following therapy, and is genetically inherited (Coles et al., 1986). In other words it is a genetically transmitted loss of sensitivity in a parasite population that was previously sensitive to a given drug (Cioli et al., 1993). It usually arises by the selection of drug tolerant worms that are usually present in a very small number in a population of worms, and is most likely to occur where there is already some natural tolerance (Coles et al., 1987).

A tolerant organism is one, which never responded adequately to therapy. Resistance to antiparasitic drugs is uncommon in the human helminthes, in marked contrast to the situation with veterinary antihelmintics and indeed, to the situation with antiparasitics employed
against human malaria and other microbes. Malaria parasites were found to develop high resistance to commonly used synthetic antimalarial as proguanil, pyrimethamine and chloroquine (Goodwin, 1962). With nematodes of veterinary significance, resistance was found to be widespread, particularly with regard to benzimidazoles (Lacey, 1988; Waller, 1994). In fascioliasis, drug resistance to salicylanilides was reported to be spreading in population of bovine *Fasciola hepatica* (Boray and De Bono, 1989); a parasite of human as well as of sheep and cattle.

Concerning schistosomiasis, resistance to medical anithelmintics started to show since 1963. Hsu *et al.*, (1963) showed that a Japanese strain of *S. japonicum* was more resistant in laboratory mice to the antimonial compounds stibophen and potassium antimony tartrate than a laboratory strain from each of Taiwan, China and the Philippines.

Davis (1966) reported lower cure in patients infected with *S. mansoni* whom they were given a second course of niridazole compared with those taking the drug for the first time. This observation led him to suggest the possibility of development of resistance to the drug in the retreated group.

Hycanthone resistance was discovered in the field by Katz *et al.*, (1973). In their work two Brazilian patients treated twice with hycanthone continued to show eggs in their faeces and, upon passage in the laboratory, the schistosomes originating showed marked resistance to hycanthone. They suggested that the relative ease by which resistance to hycanthone was produced under experimental conditions may be due to the highly mutagenic nature of this compound.

Rogers and Bueding (1971); Jansma *et al.*, (1977) reported that, laboratory mice infected with a puerto Rican strain of *S. mansoni*, given an intramuscular injection of 60 mg/kg hycanthone had a temporary
interruption of egg excretion, 12 months later, virtually all mice showed viable eggs in their livers. The miracidia hatching from these eggs were capable of sustaining the life cycle and eventually gave rise to adult worms that proved to be totally insensitive to the action of hycanthone.

In Venezuela, Berti and Dommerque (1981) reported that 20 out of 22 patients were still having active *S. mansoni* infection despite their earlier treatment with two courses of oxamniquine. Camargo (1982) trying to cure *S. mansoni* infected patients with oxamniquine reported no response for up to nine treatments with this drug. Dias *et al.*, (1982) employed PZQ to treat 14 *S. mansoni* infected patients from various states of Brazil who had failed to respond to therapy after treatment on one to four occasions with hycanthone and/or oxamniquine. They observed that PZQ therapy was effective in producing a parasitological cure in all the patients with active *S. mansoni*.

Kinoti (1987) suggested that the variation in susceptibility of *S. mansoni* to oxamniquine indicates clearly that there is a strong risk of development of resistance particularly on the Northern, Eastern and Southern parts of Africa. This was supported by the isolation of resistant worms from oxamniquine treated uncured children in Kenya. Coles *et al.*, (1987) and Kinoti (1987) reported that (data from Kenya) tolerant worms in response to normal doses of oxamniquine (80 mg/kg) might become resistant to this drug with mass oxamniquine therapy.

Katz *et al.*, (1991) reported that treatment of *S. mansoni* infected children with an alternative drug (oxamniquine 20 mg/kg and PZQ 60 mg/kg) in children not cured with the first treatment resulted in cure of 11 out of 12 cases examined one month after the second round of therapy.

Drescher *et al.*, (1993) tested the response of four isolates of *S. mansoni* (BH, MAP, MPR-1, and K) to four multiple doses of antischistosomal agents; hycanthone, niridazol, oxamniquine and PZQ.
These schistosome isolates had been maintained in the laboratory without further drug pressure for 20 to 30 generations. Results demonstrated that k isolate was resistant to niridazol, the MPR-1 isolate to oxamniquine and the MAP isolate to both hycanthone and oxamniquine. The BH isolate was susceptible to all drugs. All isolates were susceptible to PZQ. These results confirmed the resistance of these isolates of *S. mansoni* to three schistosomicides and demonstrated that the resistance of these isolates were stable over a long period of time without exposure to drugs.

In Brazil, Dias and Olivier (1986) failed to induce resistance to the antischistosomal drug PZQ in a strain of human *S. mansoni*. They were satisfied with their findings as they suggested that, a low potential of this strain to become resistant; in a country where mass treatment situations are commonly applied; favor the development of such a resistance.

Bruce *et al.*, (1987) found that some patients infected with *S. mansoni* were not cured with two separate regimens of PZQ. They isolated such a strain and they subsequently passed it through snails and mice. Subsequent treatment of mice with PZQ did not show any evidence of drug resistance to this compound.

Gryseels (1992) recorded that PZQ showed unexpected failure in a recent schistosomiasis outbreak. In this study a cure rate of 18% was recorded in the area of Richard Toll (Senegal).

Ismail *et al.*, (1994) reported that the use of PZQ in *S. mansoni* infected mice; especially in low subcurative doses; may lead to the development of resistance to the therapeutic dose of the drug in the following generations.

Stelma *et al.*, (1995) reported low cure rates (18%) 12 weeks after treatment of *S. mansoni* infected patients with PZQ (40 mg/kg) in North Senegal (near Richard Toll). They related such low cure rate to the intense schistosomiasis transmission in this area and/or to the under
development of patients immune responses in this area with recent exposed population.

Fallon et al., (1995) reported that no significant reduction (2%) in worm burden of mice carrying the Senegalese isolate of *S. mansoni* treated with 2X150 mg/kg of PZQ while a significant reduction was recorded in worm burden in mice carrying two other geographic isolates (Puerto Rican and Kenyan). They added that after complete laboratory (snail-mouse) passages without drug treatment, the susceptibility of the Senegalese isolate to PZQ (2X250 mg/kg) still showed a non significant reduction (19%) in worm burden.

Ismail et al., (1996) reported poor cure rate among Egyptian villagers infected with *S. mansoni* after repeating treatment of patients for three times (40 mg/kg, 40 mg/kg and 60 mg/kg) with PZQ. Patients not cured after the repeated dosing with PZQ was found to be 1.6% of the total villagers examined. They suggested four reasons for this failure. First reason, these villagers may not have had enough concentration of PZQ adequate to eliminate the parasites. Second reason, The possibility that these patients might be carrying parasites having the power to withstand the antischistosomal action of PZQ more effectively than others was not ruled out. Third reason. They added that after pharmacokinetic studies of PZQ level in responding and non responding villagers there were no significal differences. The last one, they suggested also the importance of studying the immune host status. The possibility that host carrying those PZQ insensitive isolates may be harboring immature schistosomes was suggested to be an underlying factor in this insusceptibility.

Ismail et al., (1999), trying to find an explanation for the insensitivity to PZQ, reported that among the *S. mansoni* isolates recovered from patients not cured by PZQ there was a strong correlation
between the PZQ resistance of the murine infections and the diminished
*S. mansoni* worm muscle response in vitro. All isolates that were
significantly more difficult to cure in mice also had a diminished worm
contractile response to PZQ. The four isolates that were not significantly
more difficult to cure in mice did not show reduced worm contractile
response.

Liang *et al.*, in China (2001) studying the development of
resistance in *S. japonicum* to PZQ using single dose of PZQ (40 mg/kg)
for treatment of patients infected with *S. japonicum* in six villages,
reported that 95.5% parasitological cure rate was recorded, the rest of
uncured patients (4.5%) were cured after a second dose of PZQ (40
mg/kg). They added that the results indicated that there was no evidence
for reduced susceptibility of *S. japonicum* to PZQ despite its extensive
use in this endemic area of China for more than 10 years. In *S.
japonicum*, the in vitro response to PZQ of cercariae, miracidiae and eggs
are more sensitive to PZQ than the same stages of *S. mansoni*, suggesting
that *S. japonicum* worms are less likely to develop resistance and this
may explain why there was no evidence for resistance to *S. japonicum* in
China.

Tchuem Tchuente *et al.*, (2001) reported cure rates of 58.1% 4
weeks after treatment among 43 patients from Ndombo, northern Senegal
infected with *S. mansoni* and treated with two doses of 40 mg/kg PZQ
with an interval of 4 weeks when compared with that recorded by Stelma
*et al.*, (1997). They recorded cure rates of 18-35 % among patients from
Ndombo, northern Senegal infected with *S. mansoni*. Several
explanations have been considered to explain the low cure rates (i)
extremely high intensity of infection in this focus, so that, even if
treatment was 99% effective, a sufficient number of schistosome pairs
would survive and continue laying eggs; (ii) High reinfection rates, such
that, many individuals would harbour immature schistosomes, which are known to be not susceptible to PZQ at the time of treatment (Shaw 1990); (iii) repeated infection in the interval between treatment and parasitological assessment, (iv) immaturity of the human’s anti-schistosome immune response in this recently established focus. This is because it has been proposed that PZQ acts synergistically with the immune response of the host (Sabah et al., 1985).

In China, Yu et al., (2001) using a single dose of PZQ (40 mg/kg) to treat patients infected with S. japonicum in two areas, one of them having repeated chemotherapy for 14 years and the other rest was a newly area not exposed to repeated treatments, reported no significant difference in the cure rates between the two areas. They added that the efficacy of PZQ against S. japonicum has not changed in this area after more than 14 years of mass chemotherapy, and that there is no evidence of tolerance or resistance of S. japonicum to PZQ.

Liang et al., (2002) studied the effect of treatment with PZQ on the tegument of adult S. mansoni worms and on liver egg granulomas in mice infected with PZQ-resistant S. mansoni isolates, they used two S. mansoni isolates. The first of these resistant isolates was selected by passages in the laboratory under drug pressure, they called this isolate Lab selected. The second one from Senegal was established by collecting eggs excreted from uncured patients. They called this isolate Senegalese 2. The PZQ susceptible S. mansoni isolates were four isolates, one of them was originally prepared by mixing equal numbers of cercariae recovered out of four susceptible isolates from different geographic areas and passaged in mice and snails, and was called gene pooled. Two and three of the PZQ susceptible S. mansoni isolates were from Kenya, one of them has been laboratory- passaged and called Kenya-lab and the fourth was obtained from the field and called Kenya-field. The last one
was an Egyptian isolate. In their work, they infected mice with 200 cercariae/mouse and 57 days post infection, they treated the animals orally with single sub-curative dose of micronized PZQ (300 mg/kg). They examined worms recovered from mice infected with the different S. mansoni isolates and they reported that at 10 and at 30 min. and 1, 12 and 24 hrs after dosing, drug-susceptible S. mansoni worms showed progressive disruption and disintegration of the surface tegument, while drug-resistant worms showed less severe damage from equivalent drug treatment. They added that drug-resistance adult worms were less sensitive to the drug both in vitro and in vivo (Liang et al., 2000 and 2001).

Ismail et al., (2002) studied the effect of drug pressure on the PZQ-susceptible and resistant S. mansoni isolates. They treated animals harbouring the PZQ-susceptible and resistant S. mansoni isolates with sub-curative doses of PZQ (250 mg/kg for each mouse) and they used eggs recovered out of those treated mice to establish another 2nd passage. Mice carrying the 2nd passage showed, diminished sensitivity to PZQ. They reported that all the two S. mansoni isolates, even the susceptible one showed decreased response to PZQ in the second generation following exposure of the first generation to relatively small doses of PZQ. They suggested that, the diminished response was expressed as decreased response to all doses of PZQ also as increased ED50 in mice carrying the two isolates used. They added that the exposure of schistosome worms to PZQ, especially in insufficient doses, may lead to the development of resistance in the following generation of the parasite. The wide and the extensive use of the drug particularly if used in insufficient doses, may lead to an increased percentage of tolerant worms which ultimately may cause the development of a wide spread resistance in the communities.
1.2.5.5 Factors possibly involved in diminished sensitivity / resistance of *S. mansoni* to praziquantel:

I. Epidemiological factors:

Rates of schistosomiasis transmission are important determinate factors in the response to chemotherapy allowing possible reinfection between treatment and parasitological assessment. In Senegal when PZQ resistance was recorded the rate of transmission of infection was reported to be high. In this area 41% of the subjects were excreting more than 1000 eggs/g faeces (Gryseels *et al.*, 1994; Stelma *et al.*, 1995), with the possible consequence that many patients would be harbouring significant numbers of immature schistosome worms at the time of treatment (Tchuem Tchuente *et al.*, 2001). Praziquantel is relatively ineffective against immature *S. mansoni* infections in mice (Sabah *et al.*, 1986) and thus young worms could also have survived in humans after administration of PZQ leading to continuation or early resumption of egg excretion after treatment.

Giboda and Smith (1997) reported that, immature eggs in the tissues are not susceptible to PZQ, and the five-day regime, suggested in the protocol, does not effectively address this problem. They would advocate that two doses of PZQ, 15 days apart, is sufficient to allow the immature eggs in the tissue to mature and be killed by the drug.

Tchuem Tchuente *et al.*, (2001) reported several explanations for low cure rates (58.1%) four weeks after treatment among patients in Northern Senegal infected with *S. mansoni* and treated with two doses of 40 mg/kg PZQ with an interval of four weeks. One of these explanations is the high intensity of infection in this area, so that, even if treatment was 99% effective, a sufficient number of schistosome pairs would survive and continue laying eggs.
II. Host-related factors:

It has been proposed that PZQ acts synergistically with the immune response of the host (Sabah et al., 1985; Doenhoff et al., 1987). Van Lieshout et al., (1999) analysed several host-related factors, including antibody levels to adult worm antigens, and found no significant differences between persons who were cured and not cured by PZQ. Tchuem Tchuente et al., (2001) suggested another explanation for low cure rates; is the immaturity of the human’s anti-schistosome immune response in this recently established area.

Another host-related factor is the absorption of PZQ which may affect the efficacy of drug. Metwally et al., (1995) observed a great deal of variation in the sera concentration of PZQ from volunteer to volunteer and they related this variation to volunteers not to drug.

III. Drug factor:

Local drug formulation allowed effective distribution of the antischistosomal to public health clinics and throughout the country. Yet the quality including; solubility, friability, disintegration and hardness are important factors which may affect the resistance. The presence of poor-quality drugs has been documented in human as well as in veterinary medicine (Shakoor et al., 1997; Monterio et al., 1998).

IV. Drug metabolizing enzymes factor:

Botros et al., (2006 a) studying the drug metabolizing enzymes (CYP450 and cyt b5) of PZQ-insusceptible (EE2) S. mansoni isolate (with a history of multiple passages without drug pressure) and of BANL (which had been passaged in mice under drug pressure) reported that, infection of mice with PZQ-susceptible (CD) and -insusceptible (EE2 and BANL) S. mansoni isolates decreased the hepatic activities of CYP450 and cyt b5; yet the inhibition recorded in mice harboring PZQ-insusceptible S. mansoni isolates was less pronounced. Animals infected
with the PZQ-insusceptible BANL *S. mansoni* isolate showing the lowest sensitivity to PZQ (i.e., highest ED50) exhibited the least inhibition in the activities of both CYP450 and cyt b5.

**V. Pharmacokinetics profile factor:**

Metwally *et al.*, (1995) tried to investigate possible different pharmacokinetics profile between villagers responding and not responding to the drug. They did not record any differences between those responding and those not responding to the drug.

Botros *et al.*, (2006) studying the PZQ bioavailability in PZQ-insusceptible (EE2 and BANL) and -susceptible *S. mansoni* isolates, reported that mice infected with PZQ-insusceptible *S. mansoni* isolates showed lower PZQ concentrations in their sera than -susceptible *S. mansoni* isolates and had the lowest $C_{max}$ and AUC of PZQ in their sera. This conclusion is supported by the complete disappearance of PZQ in the sera of some of the animals at some of the observation periods examined. They added that the antischistosomal effect of PZQ was related not only to the absolute height of the maximal plasma concentration, but also to the length of exposure to the drug (Gonnert and Andrews, 1977). Experimental findings in this work reveal a lower exposure of PZQ-insusceptible schistosomes to the drug, both in terms of concentration and time of exposure.

**VI. Immune response factor:**

Hanallah *et al.*, (2003) studying the serum immunoglobulins in mice infected with the PZQ-insensitive *S. mansoni* isolate, reported significantly lower IgG and IgG1 8 and 10 weeks post infection comparable to mice infected with the PZQ-sensitive isolate. IgM level was nearly the same while IgE was lower than that recorded in mice infected with the PZQ-sensitive isolate. The authors concluded that PZQ-insusceptible *S. mansoni* isolates possess a different immunogenic
makeup, both qualitatively and/or quantitatively, when compared to isolates susceptible to PZQ.

1.2.5.6 Factors possibly affecting the development of resistance to Praziquantel:

i. High treatment frequency:

The intensive usage of PZQ in areas of Africa with high disease prevalence eg. Sub-saharan where the prevalence is higher may reveal new human populations with *S. mansoni* infections that are not completely cured by PZQ (Doenhoff et al., 2002). King *et al.*, (2000) reported that the decreased exposure of the parasite to the drug leads to slow emergence of drug resistance. Fenwick *et al.*, (2003) reported that, approximately 20 million treatments or 60 million tablets were consumed over 3 years (1997-1999) in Egypt and expected a consumption more than 40 million tablets a year by the end of 2005. They added that, "Schistosomiasis Research Project" in Egypt, distributed more than 100 million tablets in the 1990s to infected school children in the endemic areas.

ii. Single-drug available:

Long-term use of levamisole in treatment of nematodes of cattle led to the development of resistance (Geerts and Gryseels, 2000). Praziquantel is the only effective drug for treatment of schistosomiasis for more than 25 years; the extensive use of this drug may lead to the appearance of resistance against the drug (Hotez *et al.*, 1997; Ismail *et al.*, 2002).

iii- Underdosing:

Underdosing is generally considered an important factor in the development of drug resistance, because sub-therapeutic doses might allow the survival of resistant worms (Fallon and Doenhoff (1994). Several laboratory experiments have shown that underdosing indeed
contributes to the selection of resistant or tolerant strains (Fallon and Doenhoff, 1994). Underdosing in humans occurs widely in many developing countries. Drugs are commonly shared or used at half (or less) the normal doses by poor families in endemic areas. Ismail et al., (2002) reported that, the wide and the extensive use of the drug particularly if used in insufficient doses, may lead to an increased percentage of tolerant worms which ultimately causes the development of a wide spread resistance in the community.
1.3 Snails:

1.3.1 Snail control:

The first fruits of the WHO programmes were development of molluscicides which became available in the mid 1950s. One of them, niclosamide, is still in use and effective today. Careful biological studies suggested an alternative approach to the control of oriental schistosomiasis: *Oncomelania* snail populations are less resilient than *Biomphalaria* and *Bulinus* spp. to disturbance. Habitat modification was recommended as a potential control measure because it also improved rice production and this could, potentially, fund the elimination of snail habitats (Pesigan *et al.*, 1958).

Initial hopes of eradicating aquatic snails with one or two molluscicide applications, especially from irrigation schemes, were soon dashed. (Dazo *et al.*, 1966) suggested that field studies on snail population dynamics were undertaken to improve the timing and efficacy of molluscidal applications and were followed by field studies on transmission of the schistosomes. (Sturrock *et al.*, 1974) suggested that the seasonality of transmission was revealed in many, though not all, areas. Carefully timed mollusciciding was then able to minimise, if not stop, transmission in many places well into the 1980s. However, the effect on the human worm burden was slow (Jordan, 1985). Interest in molluscicides began to wane when new drugs finally appeared and their decline was hastened by the sharp rise in the price of pesticides after the oil crisis in the 1970s, and by a growing (though largely unjustified) fear of adverse environmental effects. In some places they were completely abandoned.

Another method for achieving a long term but low cost vector control program is by use of biological control agents. To date a number of predators of snails have been tested in the laboratory and field.
The fish *Protopterus* which eats snails has yet to be field tested and other predators such as ducks do not seem to be effective. The other control agents in the trail are not predators but competitors, particularly of snails. There is a local snail the ampullarid, *lanistes*, which has some limiting effect on the vector snail populations ability to reproduce and there are two exotic snails species, *Mersia* and *Helisoma*, which have been imported, bred locally and are now to be released under carefully observed canal conditions to see if they can survive under the local habitats, and whether they can affect the transmission of schistosomiasis to any measurable degree (Jong-boom, 1985).

Some turtles were found to feed on snails. In the Sudan the fish *Protopterus annectans* was shown to be effective against the snails (Mahdi and Amin, 1996) and Daffalla (1973). The ampullarid snail *Marisa conuarietis* was imported to the Sudan in 1981 for evaluation as biological control against schistosomiasis snails (Haridi *et al.*, 1985).

The snails control is an important preventive strategy associated with the treatment of infected people together with environmental and socio-economic improvements and health education with community participation. The method of environmental control depends on the modification of snail environment, rendering the habitat unsuitable for snail breeding. However, environmental control can be most effective in water development schemes (WHO, 1980). It could be achieved by removal of vegetation from irrigation canals, complete draining or changing the water level in the canal and/or lining the canals or marginal areas in order to prevent seepage or sliting, thus reducing the snail populations and hence schistosomiasis transmission (Jordan and Webbe, 1982). Suliman and Ibrahim (1985) observed that the growth rate of Gezira snails was less than Jebel Marra snails in laboratory.
Although chemical molluscicides are the most used approach of snails control (WHO, 1994), yet chemicals have their hazards in addition to their high costs. They may be toxic to aquatic fauna and source to pollution. Amin and Fenwick (1975) reported that N-triyl morpholine killed *Biomphalaria* snails at all concentrations of 0.1, 0.2, 0.5 and 0.1 ppm (part per million) and killed *Bulinus* at 0.5 and 1.0 ppm in the irrigation canals in Sudan. Mayer et al., (1994) reported that the short time application of niclosamide could be effective in reducing the density of schistosomiasis snail in Rahad Irrigation Scheme. In Gezira scheme Frescon was applied by aerial spraying in order to overcome the difficulties in obtaining proper dispersion of the chemical from the drip feeds in the channels (Amin and Fenwick, 1977).

All factors make it imperative to consider using molluscicides of plant origin either naturally growing or locally cultivated. Generally, chemicals derived from plants have advantages over synthetics one in that they always have the least side effects, beside their easy application with simple technology and their production in less expensive especially when they are abundant in the area which is always the case (Sharma and Dwivedi, 1980).

Fouy eight plant extracts were tested for their activity against cercariae and miracidia of *Schistosoma mansoni*. Thirty five plant extracts belonging to 19 families showed miracidicidal and cercaricidal activity at a concentration of 500 ppm. Other 13 extracts from plants belonging to nine families were found to be inactive at 10,000 ppm. Out of the 35 active plant extracts 6 were active as miracidicidal and cercaricidal agents and showed toxic effect at 50 ppm against schistosomula, these plants are *Hibiscus sabdariffa, Tamarindus indica, phytolaca dodecandra, Acacia nilatica, Randia nilotica* and *Balanites aegyptica*.
In vitro studies of highly active six plants on adult worms of *Schistosoma mansoni* showed that the plant extracts were highly toxic to the adult worms.

However, the *in vivo* studies showed that all the active plant extracts were in active at 1000 ppm. (Elsheikh, 1987).
1.4 Objectives:

General Objectives:

The objectives of the present study were to evaluate the molluscicidal and anti schistosomal (prophylactic and curative) activities of the methanol extract of *Sterculia setigera* bark, *Lantana camara* leaves and a combined dose of both extract.

Specific Objectives:

1) The assessment of molluscicidal activity of the methanol extract of *Sterculia setigera* bark and *Lantana camara* leaves on *Bulinus truncatus* and *Biomphalaria pfeifferi* snails.

2) Screening of methanol extract of *S setigera* bark *in vitro* and *in vivo* bio-activity on adult *Schistosoma mansoni*.

3) Screening of methanol extract of *Lantana camara* leaves and a combined dose of *Sterculia setigera* bark and *Lantana camara* leaves extract *in vivo* bio-activity on adult *Schistosoma mansoni*.

4) Assessment of prophylactic activity of methanol extract of *S setigera* bark, *Lantana camara* leaves and a combined dose of both extract on *Schistosoma mansoni*.

5) Assessment of liver function test as carried out through the liver enzymes, alanine aminotransferase (ALT) and aspartate aminotrasferase (AST) also the liver proteins, albumin, globulin and total proteins were also studied in details.
Chapter Two
General
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General Material and Methods

2.1 Plants:

2.1.1 Sterculia setigera (Tartar):

Sterculia setigera (Tartar) of family Sterculiceae, is a multipurpose tree. It produces a high quality water soluble gum and the different parts of the tree have various medicinal uses. The extracts of the bark are used as traditional medicine for jaundice and bilharzia treatment, leaves and bark for treating cough, diarrhoea, diuretic, fever, leprosy and syphilis. The fibers are used in ropes and mats-making (Maydell, 1990).

Andrews (1952) reported the occurrence of Tartar in central and southern Sudan. The tree occurs throughout the Nuba mountains and in Equatoria (Tothill, 1948). Tartar is found sporadically in Baher el Gazal, White Nile, Jebel Mara and also grow gregariously on stony slopes of hilly sites in the southern Blue Nile (Thirakul, 1984).

2.1.1.1 Taxonomy and nomenclature:

Name: Sterculia setigera Delile
Family: Sterculiaceae
Vernacular/common names: English name: karaya gum tree;
Local names: tartar, faider and telieh, posemporgo (Mooré), kongosira (Bambara).

2.1.1.2 Distribution and habitat:

The species is widespread in tropical Africa and is common locally. The natural distribution range stretches from Senegal to Cameroon in West Africa, eastwards to Eritrea, and southwards to Angola. It grows in Savannah type vegetation on a variety of soil types,
thriving on poor soils as well as on hilly/stony sites. The plant is not classified on the IUCN Red list of threatened species (Moctar et al., 2007).

2.1.1.3 Uses:

The wood is white and very soft, which makes it unsuitable as fuel wood and charcoal. It is therefore used for non timber forest products (NTFP). It is used for insulation and concealed items in carpentry. The tree produces a water-soluble gum (karaya). This can be tapped and used in cooking as an emulsifier, stabiliser and viscosifier. The gum is used medically as a laxative, diuretic and tranquilliser and technically as an adhesive and for glazing pottery. The bark is used for rope making and the bark sap can be made into a refreshing drink. In local medicine the bark is also used to treat snake bites, leprosy, syphilis, coughs, bronchitis, and insanity. The seeds can be eaten and contain an edible oil, while the leaves are used as fodder for cattle (Moctar et al., 2007).

2.1.1.4 Botanical description:

A deciduous tree, growing to 16 m, with a spreading open crown and large twisted branches. The bark is grey to purple in colour and flakes to reveal a greenish-yellow smooth bark beneath. If the bark is cut it reveals a red inside and exudes a white gum and watery sap. The leaves are simple, alternate, 6-20 cm long and 6-20 cm wide, with 3-5 triangular lobes. They are covered with stellate hairs on both sides, but more densely on the underside. The flowers are un bisexual, apetalous, and pedicellate, with 5-lobed conical calyx. They are green or yellowish in colour, and striped or tinged red inside. The flowers are 12 mm in diameter, and are produced on shoots from the previous year (Mocter, 2007). Figure (2-2).
2.1.2 *Lantana camara*:

2.1.2.1 Taxonomy and nomenclature:

**Scientific name:** *Lantana camara* L.

**Synonyms:** Sleeper weed, lantana, wild sage

**Common names:** *Camara vulgaris*, *Lantana scabrida*

**Taxonomic position:** Division: Magnoliophyta

**Class:** Magnoliopsida,

**Order:** Lamiales

**Family:** Verbenaceae.

2.1.2.2 Distribution:

The genus of *Lantana* (Verbenaceae) comprises approximately 150 species (Zomlefer, 1994), most of which are native to tropical and subtropical America, with only a few taxa indigenous to tropical Asia and Africa (Ghisalberti, 2000).

The genus is difficult one to classify taxonomically since species are not stable and hybridization is wide spread, shape of inflorescence changes with age and flower colours vary with age and maturity (Ghisalberti, 2000). Figure (2-1).

*Lantana camara* L. is all introduced weed ill the tropics and subtropics and infests 14 crops in 47 countries. It invades forests, riverbanks, roadsides, pastures, agricultural lands and disturbed ecosystems. Being an invasion interrupts regeneration process and reduces biodiversity of natural ecosystems. It has hundreds Of cultivars and hybrids. It has about 15 varieties. *Lantana* and other alien species have caused the extinction of 58 native plant species in the Cape Floristic Kingdom and have contributed to the endangered status of more than 3435 other plants in South Africa. Its leaves, roots and fruits contain allelochemicals mainly aromatic alkaloids and phenolics. These allelochemicals promote or inhibit the crop growth based on their
concentration and are species specific. It also produces volatile allelochemicals from its leaves. It is toxic to grazing animals and has adverse effects on humans. This specie also has medicinal, herbicidal and fungistatic activity, (Ambika et al., 2003).
2.2 Plant material:

(a) *Sterculia setigera* (bark).
(b) *Lantana camara* (leaves).
(c) Combined dose of both plants.

The plant material (*Sterculia setigera* bark) was collected from Damazeen locality, Blue Nile State, Sudan, authenticated at the Medicinal and Aromatic Plant Research Institute, Khartoum, Sudan.

The plant material (*Lantana camara* leaves) was collected from Orman Botanical Garden, Giza Zoo, under supervision of senior Agronomist Therese Labib, Cairo, Egypt. A full identification and authentication carried out by her.

AVoucher specimens were deposited in the Herbarium of the Medicinal and Aromatic Plant Research Institute and Orman Botanical Garden. Precautions were taken for the correct collection, identification, drying and storage of plants for biological screening.

2.2.1 Plant parts used in these experiments:

Methanol extracts of *Sterculia setigera* bark and *Lantana camara* leaves were used.

2.2.2 Preparation of plant extracts:

*Lantana camara* leaves and *Sterculia setigera* bark were manully removed from their original trees. The plant materials were dried in the shade, and then finely powdered.

2.2.3 Methanol extraction:

1000 gm of the powdered and dried bark of *Sterculia setigera* and *Lantana camara* leaves were soaked and extracted with 80% methanol (6 liters) for two weeks at room temperature and then evaporated under reduced pressure, using rotatory evaporator. The brown powder extract of
Tatar bark and the green sticky paste of *Lantana camara* leaves were placed in glass vials and stored in a deep freezer at -20°C till being used. The residue was given orally to the mice in an aqueous solution in a dose of 500 mg/Kg body weight for five consecutive days (Leel, *et al.*, 2002).

The combined dose was prepared by mixing 250 mg of *Lantana camara* leaves and *Stericula setigera* bark, and was given to mice in the same procedure described above.
Figure (2.1) *Lantana camara* plant
Source: Orman Garden
Figure (2.2)  *Sterculia setigera* Tree

Source: (Damazeen)
Chapter Three

Assessment of molluscicidal activity of Sterculia setigera and Lantana camara against Bulinus truncatus and Biomphalaria pfeifferi
Chapter Three

Assessment of molluscicidal activity of Sterculia setigera and Lantana camara against Bulinus truncatus and Biomphalaria pfeifferi

3.1 Introduction and literature review:

A significant reduction in schistosomiasis transmission has been achieved in many endemic areas using synthetic molluscicides (Chu, 1978; Daffalla et al., 1982). However, the main constrains of the widespread use of these molluscicides are the high costs of their purchasing and handling in addition to their lethal effect on other aquatic fauna. To overcome these constrains research has been directed towards the use of local materials (WHO, 1983). The potential use of plants for snail control has received considerable attention (Perrett and Whitfield, 1996). The most promising and widely studied plants are the Endod, (Phytolacca dodecandra) and Damsissa (Ambrosia martima) (El Sawy et al., 1989; Goldsmith, 1991; Ndamba et al., 1994; Mendes et al., 1997).

In the Sudan, many local plants are screened and tested in the laboratory and proved to have molluscicidal activity (El-kheir and El-Tohami, 1979; Ayoub, 1985; El-Sheikh, 1994).

Snail-plants association have been reported in many places of the world (Dazo et al., 1966; Klumpp and Chu, 1980; Thomas and Tait, 1984). Aquatic plants are among the ecological factors affecting snail populations (Appleton, 1978; Hilali et al., 1985).

In the Sudan some of these aquatic plants are reported to have a significant positive association with schistosome intermediate host snails while others have negative association (Madsen et al., 1988). These
associations, however, may not be permanent as they are found to be correlated with the growth of plants (Osman, 2000). Association of snail with plants is attributed to feeding, protection or reproduction as plants may provide surfaces for egg laying (Ferguson, 1978). While the reasons for the negative associations behind them are not clear. It could be due to certain constituents of plant tissues being toxic to snails as it has been reported for some of them (Clark and Appleton, 1996). These findings motivated this study in order to identify the different species of aquatic plants growing in irrigation canals and to test \textit{in vitro} their molluscicidal activity to determine the species which could be further studied and evaluated for the control of snails transmitting schistosomiasis.

Fourty three different plant species belonging to 33 different families were identified in the irrigation canals of Khartoum State, Gezira and Managil Schemes. Most of the plant species collected was from the families Poaceae and Asteracae. Out of the 43 species collected and tested for molluscicidal activity against \textit{Bulinus truncatus} and \textit{Biomphalaria pfeifferi} only 11 species displayed some effects on snails. These are \textit{Acacia nilotica}, \textit{Tephrosia sp(L.)} Merril, \textit{Sesbania sesban L.}, \textit{Ricinus communis L.}, \textit{Cassia eldus}, \textit{Ipomoea aquatica L.} Forsk, \textit{Balanites aegyptiaca} (L.) Def., \textit{Ziziphus spina christi}, \textit{Cyprus rotundus}, \textit{Mimosa pigra L.} and \textit{Jussieca repens}. However, only three species \textit{Acacia nilotica}, \textit{Tephrosia sp} and \textit{Sesbania sesban} show LC$_{90}$ less than 500 ppm.(Osman, 2000).

50 plants indigenous to the Sudan and of common use in Sudanese folk-medicine, were screened for their molluscicidal activity, using two local snail vectors, \textit{Bulinus truncatus} and \textit{Biomphalaria pfeifferi}. At different concentrations 31 (62\%) of these plant samples were found to be lethal to either one or both of the snail species. 28 (56\%) proved to be lethal to \textit{Biomphalaria pfeifferi},
22 (44%) gave 100% mortality to *Bulinus truncatus*; while 19 (38%) killed both snail hosts. The seven most active molluscicidal plants were phytochemically screened for their active constituents; four of them showed the presence of saponins. (Ahmed *et al.*, 1997).

A preliminary biological screening for molluscicidal activity of certain Sudanese plants used in Folk-medicine was carried out. 78 samples belonging to 51 species, 45 genera and 28 families were screened. The aqueous extracts of 18 samples belonging to 8 species, 6 genera and 5 families were found to be active against *Bulinus truncatus* and 7 of these were also found to be active against *Biomphalaria pfeifferi*. Successive extraction of the 18 active samples with petroleum ether, ethanol and water showed that the petroleum ether extracts of only 4 samples were active against *Bulinus truncatus*; while the alcoholic extracts of 16 samples were found to be active against the same snail species. Only the alcoholic extracts of 4 samples were proved to be active against *Biomphalaria pfeifferi*. In the successive extraction technique, only the aqueous extract of *Gardenia vogelii* fruit pulp was proved to be active against the two snail species tested. (El Kheir, El Tohami 1980).

The ethyl acetate extracts of fruits and bark of *Acacia nilotica* (L.) Willd. ex Del subsp. *nilotica*, *tomentosa* and *astringens*, showed the highest molluscicidal activity against snail species *Bulinus truncatus* and *Biomphalaria pfeifferi*. This was due mainly to the flavanol derivatives isolated from the tannin extracts and characterized as (−)-epigallocatechin-7-gallate and (−)-epigallocatechin-5,7-digallate. It was found also that the ethyl acetate extracts of 20 *Acacia* species and subspecies growing in the Sudan were active against the snails, which was due to the presence of the above mentioned (−)-epigallocatechin derivatives. (Ayoub, 1985)
Aqueous extracts of seven plants were investigated for their effects on egg-hatching, cercariae and miracidia of *Schistosoma mansoni*. All the plant extracts, except *Vangueria venosa*, showed an effect on egg-hatching at a high concentration of 10,000 ppm. The most active extracts against miracidia and cercariae were found to be *Balanites aegyptiaca*, *Gardenia lutes* and *Randia nilotica*. The saponin fraction of *B. aegyptiaca* was shown to be the most active fraction. The spray-dried saponin of *B. aegyptiaca* was further investigated for its miracidicidal and cercaricidal effects. The LC$_{100}$ was shown to be 10 ppm for both miracidia and cercariae. (Suliman *et al*., 1988).

Forty-six plant extracts were tested for their toxic activity against cercariae and miracidia of *Schistosoma mansoni*. Thirty-five of these plant extracts showed miracidicidal and cercaricidal activity at a concentration of 10,000 ppm; 5 were toxic at 50-100 ppm concentration. These were *Phytolacca dodecandra*, *Tamarindus indica*, *Acacia nilolica*, *Hibiscus sabdariffa* and *Tacca leontopetaloides* (Elsheikh *et al*., 1990). Crude aqueous extracts of *Khaya senegalensis* (mahogany) were screened for their larvicidal and molluscicidal using two target organisms; *Culex quinquefasciatus* and *Biomphalaria pfeifferi* as well as non target organism; *Tilapia nilotica*. In order of molluscicidal potency as measured in terms of 24-hours LC$_{95}$ and LC$_{50}$, mahogany different parts can be arranged as follow: old bark, young bark, old wood and fresh leaves. However, only the aqueous extracts of mahogany old and young bark were active against Culex larvae. (Musa, 2000).
3.2 Material and Methods:

3.2.1 Collection and maintenance of the snails:

*Bulinus truncatus* and *Biomphalaria pfeifferi* (Sudanese strain) are the intermediate host of *Schistosoma haematobium* and *Schistosoma mansoni*, respectively. The snails were collected from irrigation canal in Wad Madani (Al Gazeria State; Sudan) using a wire scoop and brought to the laboratory in moistened cotton wool.

In the laboratory, living and non-infested snails were sorted out and kept in groups of 50, in plastic bowls, 30 cm in diameter and 15 cm deep, containing dechlorinated tap water. The Snails were fed daily on dry previously boiled lettuce. Water in bowls was changed twice a week to eliminate fecal droppings and to prevent fouling. The snails were kept for a period of two weeks to acclimatize to laboratory conditions before being used in experiments. Dead as well as unhealthy; algal infected; snails were removed as soon as discovered.

Handling of snails was done with the help of a small spoon or a pair of forceps. Only healthy infection free snails were used in this investigation (10 snails). *Schistosoma* infection of snails was checked by exposing them singly in a test tube half – filled with dechlorinated tap water to artificial light for about half an hour, any snail shedding cercariae was discarded.

The snail was classified according to standard classification manual.

3.2.2 Bioassay of the plant extract:

A stock solution of a known concentration was prepared by dissolving 0.5 gram, each of the powder of methanol extract of *Sterculia setigera* bark and *Lantana camara* leaves in 1000 ml of distilled water in stoppered conical flasks; to give 1000 ppm concentration. The required five concentrations were prepared by successive dilution of the stock
solution after well mixing (7 concentrations). The snails were immersed in 200ml of the test solution in glass beakers. The exposure time was 24 hours followed by a recovery period in normal dechlorinted tap water. Control was set up in blank dechlorinated tap water. Each test was carried out three times and the average of the replicates was taken as a final result.

In all cases, the snails were observed at five minutes for the first two hours after which hourly checks were made for eight hours. Then after, irregular checks were continued up to the end of the 24 hours period. The behavioral responses and reactions of the snails while in the test solutions were observed.

3.2.3 Statistical analysis:

Propbit regression analysis (SPSS/inc) was carried out to determine LD$_{50}$, LD$_{95}$, values, the result of the analyzed data were presented in tabular and graphical forms together with relevant statistical data. In each case the equation of the straight line: $y = a + b \times x$, was computed. In the equation; $y =$ probit mortality; $a =$ intercept of the regression line with the vertical axis $b =$ the slope i.e. the tangent of the angle the regression line makes with the horizontal axis; $x =$ dose concentration producing $y$ probit mortality. Assessment of acute toxicity was done through the calculation LD$_{50}$, LD$_{95}$. In all cases, the difference was considered statistically significant only when the value of $P$ was less than 0.05 (Fisher and Yetes, 1963).
3.3 Result:

3.3.1 Responses and reactions of snails:

When handled or dropped in any solution including the control, the snails withdraw their bodies inside their shells and settled motionless onto the bottom of the container. After an initial stay of about 30 to 45 minutes, the snails usually emerged from their shells and started to resume normal movements. After 45-60 seconds stay in higher concentrations of methanol extract of *Sterculia setiger* and *Lantana camara*, the snails frequently tried to creep out of the container and a few succeeded in doing that. Other snails showed avoidance reactions, which were represented by the withdrawal of their bodies inside their shells, then dropped down and settled almost motionless onto the bottom of the container.

Toward the middle and lower scales of the toxicity range, few snails were found to withdraw their bodies inside their shells; others swam freely in the test solutions. Treated snails, in the higher concentrations of Tatar bark and *Lantana* leaves, dropped down and settled almost motionless onto the bottom of the container. This response is often accompanied by air bubbles coming out of their bodies. These responses were inconsistent in the middle and lower parts of the toxicity. In all cases a snail was considered dead when it floats on the surface of the solution with the wrong side uppermost and/or the colour of the shell become more translucent. In most cases, the snails ejected their viscera outside their shells. Such snails did not recover on transfer to clean water for a period of 24 hours, and thus considered dead.

3.3.2. Acute toxicity of extracts to the test snails:

The responses of snails tested, in terms of probit mortality, were found to form a linear relationship with percentage concentrations of the
extracts. Tables (3.1), (3.2), (3.3), (3.4) and Figures (3.1), (3.2), (3.3), (3.4), show the responses of the snails to the different concentration of the extracts in terms of percentage mortality together with their relevant statistical data. In addition, they show the concentration of extracts that killed 50 percent of the population and that killed 95 percent.

3.3.3. Acute toxicity of methanol extracts of Sterculia setigera bark to Bulinus truncatus and Biomphalaria pfeifferi:

Tables (3.1), (3.2)) and figures (3.1), (3.2) illustrated the molluscicidal activity of methanol extracts of Sterculia setigera bark against Bulinus truncatus and Biomphalaria Pfeifferi. The methanol extract of Tatar was found to possess molluscicidal activity; Biomphalaria pfeifferi was found to be more susceptible than B. truncates, with LC₉₅ = 811 ppm, 909 ppm. and LC₅₀ = 295 ppm, 482 ppm respectively.

3.3.4 Acute toxicity of the methanol extract of Lantana camara leaves to Bulinus truncatus and Biomphalaria pfeifferi:

Tables (3.3), (3.4) and figures (3.3), (3.4) illustrate the molluscicidal activity of the methanol extract of Lantana camara leaves. Bulinus truncatus was found to be significantly more susceptible to the methanol extract of Lantana camara than Biomphalaria pfeifferi with the LC₉₅ 111 ppm; 213 ppm and LC₅₀ 48 ppm, 111 ppm respectively.

The methanol extract of Lantana camara leaves was found significantly more toxic to the snails than the methanol extract of Sterculia setigera bark.
Table (3.1): Molluscicidal activity of the methanol extract of *Sterculia setigera* bark against *Bulinus truncatus* exposed for 24 hours.

<table>
<thead>
<tr>
<th>Conc.(ppm)</th>
<th>%Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>60</td>
</tr>
<tr>
<td>250</td>
<td>30</td>
</tr>
<tr>
<td>125</td>
<td>10</td>
</tr>
<tr>
<td>62.5</td>
<td>0</td>
</tr>
<tr>
<td>Control / 0</td>
<td>0</td>
</tr>
</tbody>
</table>

\[
\text{LC}_{95} \quad 909.2343 \text{ ppm} \\
\text{LC}_{50} \quad 482.2894 \text{ ppm}
\]
Fig.(3. 1): Dose/response regression line of *Sterculia setigera* bark methonal extract activity against *Bulinus truncatus*
Table (3.2): Molluscicidal activity of the methanol extract of *Sterculia setigera* bark against *Biomphalaria pfeifferi* exposed for 24 hours

<table>
<thead>
<tr>
<th>Conc.(PPM)</th>
<th>%Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>90</td>
</tr>
<tr>
<td>250</td>
<td>50</td>
</tr>
<tr>
<td>125</td>
<td>40</td>
</tr>
<tr>
<td>62.5</td>
<td>10</td>
</tr>
<tr>
<td>Control / 0</td>
<td>0</td>
</tr>
</tbody>
</table>

LC$_{95}$ 811.3746 ppm  
LC$_{50}$ 295.9107 ppm
**Fig.(3.2):** Dose/response regression line of *Sterculia setigera* bark methonal extract activity against *Biomphalaria pfeifferi*
**Table (3.3):** Molluscidial activity of the methanol extract of *Lantana camara* leaves against *Bulinus truncatus* exposed for 24 hours

<table>
<thead>
<tr>
<th>Conc.(PPM)</th>
<th>%Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>100</td>
</tr>
<tr>
<td>62.5</td>
<td>70</td>
</tr>
<tr>
<td>31.25</td>
<td>40</td>
</tr>
<tr>
<td>15.625</td>
<td>20</td>
</tr>
<tr>
<td>Control / 0</td>
<td>0</td>
</tr>
</tbody>
</table>

LC$_{95}$  111.6483 ppm  
LC$_{50}$  47.98104 ppm
**Fig.(3.3):** Dose/response regression line of *lantana camara* leaves methonal extract activity against *Bulinus truncatus*
Table (3.4): Molluscicidal activity of the methanol extract of *Lantana camara* leaves against *Biomphalaria pfeifferi* exposed for 24 hours.

<table>
<thead>
<tr>
<th>Conc.(PPM)</th>
<th>%Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>125</td>
<td>80</td>
</tr>
<tr>
<td>62.5</td>
<td>30</td>
</tr>
<tr>
<td>31.25</td>
<td>10</td>
</tr>
<tr>
<td>15.625</td>
<td>0</td>
</tr>
<tr>
<td>Control / 0</td>
<td>0</td>
</tr>
</tbody>
</table>

LC$_{95}$ 213.5773 ppm  
LC$_{50}$ 110.6025 ppm
Fig.(3.4): Dose/response regression line of lantana camara leaves methonal extract activity against Biomphalaria pfeifferi
Chapter Four

Assessment of schistosomicidal activity of Sterculia setiger and Lantana camara on Schistosoma mansoni
Chapter Four

Assessment of schistosomical activity of Sterculia setiger and Lantana canara on Schistosoma mansoni

4.1 Introduction and literature review:

Human schistosomiasis includes a complex group of acute and chronic parasitic infections caused by mammalian blood flukes (Schistosoma), these infections are transmitted by specific aquatic or amphibious snails in a wide variety of freshwater habitats. (Gordon and Alimuddin, 2009). In untreated chronic cases, the morbidity due to Schistosomiasis mansoni includes hepatic and intestinal fibrosis (Gryseels et al., 2006). The pressing need to develop new antischistosomal compounds has been emphasized, particularly in view of blanket application of praziquantel within the frame of “preventive chemotherapy” (Caffery, 2007), a strategy that might select for drug-resistant parasites.

Whereas medicinal plants have produced some very effective treatment for malaria as in case of artemisinin (Frederich et al., 2002), few attempts have been made to evaluate antischistosomal activity of such natural plants (Molgaard et al., 2001). The importance of plants as sources of natural products bioactive molecules to medicine lies not only in their pharmacological or chemotherapeutic effects but also in their role as template molecules for the production of new drug substances (Phillipson, 1994). Koko et al., (2005) determined the efficacy of oral therapy with Balanites aegyptiaca fruit mesocarp in a dose of 200 mg/kg bodyweight of mice infected with Sudanese strain of S. mansoni, and found a significant reduction in egg count per gram of faeces. Ramadan et al., (2004) studied the therapeutic effect of Ferula assafoetida on S. mansoni in experimentally infect mice.
4.2 Material and methods:

4.2.1 Biologicals:

4.2.1.1 *Schistosoma mansoni*

4.2.1.1.1 Maintenance of *Schistosoma mansoni*:

The parasite was originally isolated from a naturally infected Egyptian human and maintained in mice and hamsters in the laboratory. It was classified according to standard classification manuals (Sana .Sabet Botros, T.B.C). *Schistosoma mansoni* isolate was used.

4.2.1.1.2 Preparation and counting of cercarial suspension:

Cercariae were shed from laboratory bred infected snails (*Biomphalaria alexandrina*; the intermediate host of *Schistosoma mansoni* in Egypt, were collected from irrigation canals that were not treated with molluscicides in Cairo) 30 to 40 days after exposure to miracidia. Infected snails (more than ten snails to avoid single sex) were collected from the aquarium, washed with dechlorinated water. Positive snails were placed in a small beaker, a volume of water was added to the beaker and the snails were gently rinsed to remove faeces and other debris. This water was then discarded and a second volume of water (5 ml per 10 snails) was added. The beaker was then placed uncovered under a white fluorescent light for a period of 50 minutes (Liang *et al.*, 1987). To count the cercarial suspension, one milliliter was pipetted in a small petri-dish. A drop of iodine was added to kill and stain the cercariae. The number of cercariae was determined with the aid of a stereobinocular microscope. Three counts were made and their average was used in calculating, the number of cercariae per ml of cercarial suspension (Moore *et al.*, 1977).
4.2.2 Experimental animals:

4.2.2.1 Maintenance of mice:

Laboratory out bred Swiss albino mice (CD1), weighting 18 -20g were used. They were obtained from the Schistosoma Biology Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI) Giza, Egypt.

4.2.2.2 Infection of mice:

Infection of animals was done using the body immersion technique. Each mouse was exposed to $80 \pm 10$ S. mansoni cercariae. Animals were first immersed in warm water at 35°C for 30 minutes to stimulate them to defecate and urinate, and prepare their tails for cercarial penetration. Glass bottles were then filled with dechlorinated water at 30°C and sufficient amount of cercariae ($80 \pm 10$ cercariae) was introduced in each container. The body of mice was immersed in the bottles for 2-4 hrs to ensure maximal cercarial penetration (Liang et al., 1987).

4.2.3 Praziquantel (PZQ):

Tablets of Praziquantel (PZQ) were freshly prepared before oral administration as a suspension in 2% Cremophor EL (Sigma Chemicals Co. St. Louis Missouri). PZQ was used in a dose of 500 mg/kg delivered on two consecutive days.

4.2.4 In vitro anti-schistosomal test:

The testing extract of Sterculia setigera bark was prepared by dissolving a known weight (100 μg/ml,) of extract in 1 ml of distilled water to obtain a stock solution. From the stock solution the desired concentrations in duplicate were prepared. Concerning the worm, a negative control with RPMI 1640 media was also prepared. Up to 5-6 adult worms; from previously infected mice, were distributed in 24-well tissue culture plates containing RPMI 1640 media supplemented with 20% newborn calf serum, 100 U/ml Penicillin, 100 μg/ml streptomycin.
Cultures were kept at 37°C in an atmosphere of 5% CO₂ in air and were observed daily for 48 h under a stereomicroscope. Cultures were exposed overnight to different concentrations of *Sterculia setigera* extract (100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml) and the next morning worms were washed with normal saline and then transferred to new plates containing extra-free medium. At the end of the observation period (24 hours), worms were defined “dead” if they did not resume movements. Data obtained from replicate well were averaged and the average of duplicate experiments was used to calculate the percentage mortality of worms (Jiwajinda *et al.*, 2002; Pica-Mattocia and Cioli 2004).

4.2.5 Determination of the acute toxicity (LD₅₀) of methanol extract of *Sterculia setigera* bark, *Lantana camara* leaves and combined dose of both extracts:

A group of 24 adult normal male CD-1 Swiss albino mice weighing 20±2 g were used to study the acute toxicity of plants extract. They were subdivided into 4 subgroups each of 6 mice. All subgroups were treated orally with rising doses of 500, 1000, 2000 and 4000 mg/kg of "above mention" extracts. Mortality rates were recorded 24 hours post treatment.

4.2.6 In vivo design and treatment:

In this study three groups of thirty five out bred Swiss albino mice were treated with methanol extract of *Sterculia setigera* bark and *Lantana camara* leaves, and combined dose of both extracts. Each group was divided into 5 sub groups as follow:

Group 1: Seven mice were treated with extract in a dose of 500 mg/kg for seven consecutive days seven days before infection with *S.mansoni* cercariae (prophylactic).

Group 2: Seven mice were treated with extract in a dose of
500 mg/kg for five consecutive days four weeks post infection with *S. mansoni* cercariae (subcurative).

Group 3: Seven mice were treated with extract in a dose of 500 mg/kg for five consecutive days seven weeks post infection with *S. mansoni* cercariae (curative).

Group 4: Seven mice were treated with Praziquantel (PZQ) in a dose of 500 mg/kg for two consecutive days seven weeks post infection with *S. mansoni* cercariae (positive control).

Group 5: Seven mice were infected with *S. mansoni* cercariae and left without treatment (non-treated control).

Nine weeks post infection animals were sacrificed, perfused and the parasitological and physiological parameters were studied.

### 4.2.7 Parasitological parameters investigation:

Worm load was studied in the infected mice using hepatic and mesenteric perfusion method.

#### 4.2.7.1 Worm burden and distribution:

a- preparation and counting of cercarial suspension

b- Perfusion of portomesenteric vessels of mice and counting of worms:

**Reagent:** Physiological saline solution 0.9%

**Equipments:**

- Ordinary stainless steel scissors.
- Pointed stainless steel forceps.
- Brewer automatic perfusion machine.
- Petri-dishes (small size).
- Stereomicroscope.
(i) Hepatic perfusion:

Mice were sacrificed by decapitation. They were skinned out, their bodies washed with tap water to remove any adherent hairs and subsequently fixed to an inclined dissecting board, laid on a stainless steel pan in which the perfusate was collected. The abdominal muscles and peritoneum were opened to expose the internal organs. The ribs were removed on both sides, as they would hinder hand movement during perfusion. The portal vein was quickly ligated and severed close to its entrance into the liver to prevent shift of the parasites to the liver (Kloetzol, 1967). The needle connected to the automatic perfusion machine was inserted in the inferior vena cava and saline was then pumped through to the liver. Worms were washed out and came out of the liver from the cut end of the portal vein. If the needle is inserted in the right position the liver expands to about twice its normal size and blanches. (Duvall and Dewitt, 1967). Worms were then collected in glass petri-dishes and counted.

(ii) Mesenteric perfusion:

The portal venous ligature was removed before inserting the needle through the descending thoracic aorta downward along the course of this vessel. The perfusate was then pumped from the machine. In this way blood and worms in the superior and inferior mesenteric vessels will come out from the cut end of the portal vein. Coils of intestine which did not blanch were massaged gently to relieve twisting of the vessels and allow for proper washing of the vessels. After perfusion, the viscera were washed with strong stream of saline to remove any adherent worms. Sometimes the worms were picked up with a forceps if they still appear attached to the mesenteric vessels. The perfusate was left to settle and then the worms were collected and counted under a stereomicroscope.
Figure (4.1) Hepatic and mesenteric perfusion

Source: (T.B.I.C)
4.2.7.2 Percentage egg developmental stages (ooogram method; Pellegrino et al., 1962):

Reagent: Physiological saline solution 0.9%

Equipments:
- Petri-dishes
- Filter-paper
- Scissors
- Forceps
- Microscope slides
- Glass covers (1cmx1cm)
- Microscope
- Counter

After perfusion, the small intestine was wholly separated and transferred to Petri-dishes. Three fragments (each of 1cm in length) of the small intestine were cut longitudinally, rinsed in saline, slightly dried on filter paper and then placed between a slide and a cover slip. The preparation was inverted and strongly pressed on a filter paper, compression was exerted by the thumb on the part of the slide opposite the cover slip in order to avoid cracking the slide cover. The preparations were examined under the microscope, the stages of egg maturity were recorded in each fragment. From each animal three fragments were obtained and the mean percentage of each stage was calculated. Ova of different stages were counted in the intestine and classified according to their stages of development into:-

a) Immature ova, which were further classified into four stages according to the size of the embryo.
   Stage I: the embryo occupies one third the transverse diameter of the egg.
   Stage II: the embryo occupies one-half the transverse diameter of the egg.
   Stage III: the embryo occupies two-thirds the length of the egg.
   Stage IV: the embryo occupies the entire egg shell

b) Mature ova: containing fully developed miracidium
c) Dead eggs: Eggs are considered dead when the embryo at any stage of development dies. If death occurs during immature stages, eggs appear granular, dark, semitransparent showing a clear longitudinal half. After death, the embryo retracts within the egg shell leading to a clear and irregular outline. When death occurs after maturation the miracidium appears disintegrated, retracted and may be calcified.

4.2.7.3 Number of ova/g tissue (Cheever, 1968; Cheever and Anderson, 1971):

**Reagent:** KOH 5%

**Equipments:**
- Test tubes
- Sensitive balance
- Filter paper
- Stainless-steel forceps
- Glass pipettes 5 ml
- Incubator
- Counting slides
- Ependorff micropipettes
- Counter
- Microscope

At the end of the perfusion, a piece of liver and intestine were taken to count the number of eggs/g liver and intestine. The intestine was opened and washed with saline to remove any faecal matter present in the lumen. Each piece was weighed and placed in a volume of 5% KOH (5 ml) solution to be digested. They were incubated at 37°C for 24 hrs until the tissues were completely digested. The digested tissues were well shaken and three samples (each of 0.1ml) / animal were pipetted by the micropipette and placed on counting slides, for subsequent counting under the low power of the microscope. The number of ova was counted in three samples and the average number/animal was obtained. The average was multiplied by the total volume of KOH and divided by the weight of the liver or intestine to get the number of ova per gram liver or intestine respectively.
4.2.8 Physiological parameters investigation:

- Conventional liver function tests.
  i- Alanine amino transferase (ALT).
  ii- Aspartate amino transferase (AST):
    iii- Total proteins.
    iv- Albumin.
    v- Globulins and A/G ratio.

-Method:

(i) Alanine amino transferase (ALT):

ALT was determined according to the Method of Reitman and Frankel (1957) using QCA Kits (Quimica Clinica Aplicada S.A., Amposta /SPAIN).

Principle:

The calorimetric determination of ALT activity depends on the following reaction:

\[
\text{L-Alanine} + 2\text{-Oxoglutarate} \leftrightarrow \text{L. Glutamate} + \text{Pyruvate}
\]

The Pyruvate formed was measured in its derivative form 2,4- dinitrophenyl hydrazone at a wavelength of 505 nm.

Calculation:

The number of ALT units/ml of serum was calculated using a standard curve.

(ii) Aspartate amino transferase (AST):

AST was determined according to the Method of Reitman and Frankel (1957) using QCA Kits (Quimica Clinica Aplicada S.A., Amposta /SPAIN).

Principle:

The calorimetric determination of AST activity depends on the following reaction:
L-Aspartate + 2-Oxoglutarate ⇄ L. Glutamate + Oxaloacetate

The Oxaloacetate formed was measured in its derivative form 2,4-dinitro phenyl hydrazone at a wavelength of 505 nm.

**Calculation:**

The number of AST units/ml of serum was calculated using a standard curve.

(iii) Total proteins:

Total proteins were determined according to the method of Josephson and Gyllensward (1975) using Centronic GmbH Germany Kits at a wavelength of 550 nm.

**Principle:**

The colorimetric determination of the total proteins in serum is based on the principle of the Biuret reaction (Copper salts in alkaline medium).

**Calculation:**

According to the following equation:

\[
\text{Concentration of sample (g/100ml)} = \frac{\text{O.D of serum sample}}{\text{O.D of standard}} \times n
\]

Where n= 4

(iv) Albumin:

Albumin was determined according to the method of Doumas *et al.*, (1971) using Centronic GmbH Germany Kits.

**Principle:**

Colorimetric determination of serum albumin was conducted using Bromcresol green (BCG) at a wavelength of 578 nm.
Calculation:

Calculation was done according to the following equation:

\[
\text{Concentration of sample (g/100ml)} = \frac{\text{O.D of serum sample}}{\text{O.D of standard}} \times n
\]

Where \( n = 4 \)

(v) Globulins:

The concentration of serum Globulin was mathematically estimated as the differences between the concentration of serum total protein and albumin. The ratio of albumin to globulin (A/G ratio) was also calculated.

4.2.9 Statistical analysis:

The data shown in tables of results were summarized by the arithmetic mean and the standard error. Statistical analysis of results was carried out using Student's t-test (Schwartz, 1963). The degree of significance (probability p-value) was obtained from the corresponding tables.

The degree of significance was expressed as follows:

* Significant at \( p < 0.05 \).

** Highly significant at \( p < 0.01 \).

*** Very highly significant at \( p < 0.001 \).
4.3 Result:

4.3.1 Study of the toxicity (LD$_{50}$) of methanol extract of 

*Sterculica setigera* bark, *Lantana camara* leaves and a 
combined dose of both extracts in albino mice:

No animals died (0% mortality) during 24 hours of observation post 
treatment with rising doses of *Sterculica setigera* bark and *Lantana 
camara* leaves extracts starting from 500 mg/kg to 4000 mg/kg 
accordingly. LD$_{50}$ more than 4000 mg/kg.

4.3.2 *In vitro* bioassay:

The testing extract of *Sterculica setigera* did not show any anti 
schistosomal activity *in vitro* at the concentrations tested i.e (100 
µg/ml, 50 µg/ml. 25 µg/ml and 12.5 µg/ml), while the reference 
drug (praziquantel) gave 100% mortality.
4.3.3 In vivo bioassay:

4.3.3.1 Effect of Sterculica setigera bark methanol extract on worm load, egg developmental stages and ova count from mice infected with S. mansoni

A) Effect of Sterculica setigera bark extract on worm load.

Table (4.1) and figure (4.2) report the percent reduction in the total numbers of worm of 7 days pre- and 4 and 7 weeks post-infection, as well as the reference drug (PZQ) 7 weeks after infection.

The percent worm reduction of extract 7 days before infection was 40%, there was highly significant difference between mean of the worm count of the extract with reference to the infected control. The percent reduction of the extract 4 and 7 weeks post-infection was 23% and 29% respectively. There was highly significant difference between the mean of the worm count with reference to the infected control. Mean while, the reference drug (Praziquantel) gave 99% worm reduction which was highly significant.
Table (4.1): Effect of *Sterculia Setigera* methanol extract (bark) on worm burden of mice pre- and post infection with *Schistosoma mansoni* compared to Praziquantel (PZQ).

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Parameters</th>
<th>Worm burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Count</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td></td>
<td>34.00±0.78</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td></td>
<td>20.30±1.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>4 weeks post-infection treated mice</td>
<td></td>
<td>26.3±1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td></td>
<td>24.30±2.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>**</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td></td>
<td>0.20±0.18</td>
</tr>
<tr>
<td>7 weeks after infection</td>
<td></td>
<td>***</td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$. 

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Fig. (4.2): Effect of methanol extract of *Sterculia setigera* (bark) on *S. mansoni* adult worm reduction compared to PZQ.

- **Praziquentel 7 weeks after infection**
- ***Sterculia setigera* extract 7 days before infection**
- ***Sterculia setigera* extract 4 weeks after infection**
- ***Sterculia setigera* extract 7 weeks after infection**
B) Activity of the *Sterculica setigera* bark methanol extract on the egg developmental stages.

The mean of immature, mature and dead ova in the intestine of mice treated by *Sterculica setigera* methanol extract 7 days pre-infection and 4 and 7 weeks post-infection compared to the infected control and reference drug treated group (PZQ) 7 weeks post-infection are displayed in table (4.2) and figure (4.3).

The effect of extract was only observed to be significant on the reduction of mature and dead ova stages when given 7 weeks post-infection, as compared to non-treated infected control. When using Praziquantel there was complete absence of immature stages, higher reduction in mature stages and increase of dead stages. While the extract only reduced the number of mature and increased dead stages.
**Table (4.2):** Effect of *Sterculia setigera* bark methanol extract on egg developmental stages of mice pre- and post infection with *S. mansoni* compared to Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Parameters</th>
<th>Egg developmental stages</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total mean of immature</td>
<td>Total mean of mature stage</td>
<td>Total mean of dead stage</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>stages</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>48.50±2.36</td>
<td>42.50±2.01</td>
<td>9.00±1.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td>49.00±3.01</td>
<td>38.44±2.16</td>
<td>12.56±1.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 weeks post-infection treated mice</td>
<td>49.2±1.5</td>
<td>41.3±1.2</td>
<td>9.5±1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td>49.29±3.01</td>
<td>31.29±2.68</td>
<td>20.14±1.64</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td>00.00±00.00</td>
<td>2.80±1.39</td>
<td>97.20±1.39</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>7 weeks after infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$. 

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**Fig. (4.3):** Effect of methanol extract of *Sterculia setigera* bark on *S. mansoni* ova developmental stages compared to PZQ and non-treated control

- **Non-treated control**
- *Sterculia setigera* extract 7 days before infection
- *Sterculia setigera* extract 4 days before infection
- *Sterculia setigera* extract 7 weeks after infection
- Praziquantel 7 weeks after infection
Plate 1: Oogram showing immature eggs of the 1\textsuperscript{st} (A), 2\textsuperscript{nd} (B) stages and mature ova (E) of \textit{S.mansonii}.

Plate 2: Oogram showing immature eggs of the 3\textsuperscript{rd} (C) stage, mature ova (E) of \textit{S.mansonii}.
Plate 3: Oogram showing immature eggs of the 4th (D) stage of *S.mansoni*

Plate 4: Oogram showing dead egg (F) (granular, darkened) and mature ova (E) of *S.mansoni*
c) Effect of *Sterculia setigera* methanol extract on ova count.

The mean ova count of *S. mansoni* in hepatic and intestinal tissues were reduced slightly after treatment with methanol extract of *S. setigera* bark 7 days before infection; the results were 10788.6 and 17013.2, with percentage reduction 21% and 26% respectively. A slight (non significant) increase in these percentages was encountered in case of the same extract which was administrated 4 and 7 weeks post-infestation, being 35%, 36% and 44%, (significant) 41% respectively. Regarding Praziquantel, it was found to be significant when treated 7 weeks post-infection, presenting a reduction of 67% and 85% respectively. The mean ova count in the non treated control was 13586.3/ hepatic tissues and 22886.6/ intestine tissues. Table (4.3) and figure (4.4).
**Table (4.3):** Effect of *Sterculia setigera* bark methanol extract on ova count of mice pre and post infection with *S. mansoni* compared to the Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of ova /g tissue (ova count)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Liver</em></td>
<td><em>% reduction</em></td>
<td><em>Intestine</em></td>
<td><em>% reduction</em></td>
</tr>
<tr>
<td><strong>Animal groups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>13586.3±2241.0</td>
<td>-</td>
<td>22886.6±4650.2</td>
<td>-</td>
</tr>
<tr>
<td>7 days pre -infection treated mice</td>
<td>10788.6±1564.8</td>
<td>21%</td>
<td>17013.2±2420.3</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>N.S</td>
<td></td>
<td>N.S</td>
<td></td>
</tr>
<tr>
<td>4 weeks post- infection treated mice</td>
<td>8876.5±535.4</td>
<td>35%</td>
<td>12922.8±821.5</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td>N.S</td>
<td></td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>7 weeks post- infection treated mice</td>
<td>8678.0±863.4</td>
<td>36%</td>
<td>13470.1±1831.9</td>
<td>41%</td>
</tr>
<tr>
<td></td>
<td>N.S</td>
<td></td>
<td>N.S</td>
<td></td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td>4498.0±762.3</td>
<td>**</td>
<td>3403.3±967.9</td>
<td>85%</td>
</tr>
<tr>
<td>7 weeks after infection</td>
<td>**</td>
<td></td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$. 

NS  Not Significant
**Fig.(4.4):** Effect of methanol extract of *Sterculia setigera* bark on *S. mansoni* tissue eggs count compared to PZQ.

![Bar chart showing % reduction of ova count from control across different treatments](chart.png)

- **Liver**
- **Intestine**
4.3.3.2 Effect of *Lantana camara* leaves methanol extract on worm load, egg developmental stage, ova count of mice pre-and post-infection with *S. mansoni*:

The plant extract (7 days pre- infection and 4 and 7 weeks post – infection) caused significant reduction of the total number of worm by about 20%, 15% and 29% respectively, mean while the reference drug PZQ was highly significant and reduced it by about 98% Table (4.4) and figure (4.5)

Table (4.5) and figure (4.6) illustrated that the three treatments of *Lantana* extracts exhibited a minor change on the egg developmental stages. While PZQ caused complete absence of immature stage, increased dead ova to be 97.7% and reduced matures stage.

In Table (4.6) and figure (4.7) the three treatments of the plant extract reduced significantly the number of ova /g tissue of the liver by 34%. While the reduction in the intestine is significant, reached 38% and 41% respectively except 29% which is not significant. The PQZ induced 74% and 88% reduction in liver and intestine.
Table (4.4): Effect of *Lantana camara* leaves methanol extract on worm burden of mice pre- and post infection with *S. mansoni* compared to PZQ.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Parameters</th>
<th>Worm burden</th>
<th>Count</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected non-treated control</td>
<td></td>
<td></td>
<td>30.2±0.7</td>
<td>-</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td></td>
<td></td>
<td>24.3±1.5</td>
<td>20%</td>
</tr>
<tr>
<td>4 weeks post-infection treated mice</td>
<td></td>
<td></td>
<td>25.7±1.1</td>
<td>15%</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td></td>
<td></td>
<td>21.3±1.0</td>
<td>29%</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td></td>
<td></td>
<td>0.5±0.3</td>
<td>98%</td>
</tr>
<tr>
<td>7 weeks after infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$.  


**Fig. (4.5):** Effect of methanol extract of *Lantana camara* leaves on *S. mansoni* adult worm reduction compared to PZQ.

- **Praziquentel 7 weeks after infection**
- **Sterculia setigera** extract 7 days before infection
- **Sterculia setigera** extract 4 weeks after infection
- **Sterculia setigera** extract 7 weeks after infection
Table (4.5): Effect of *Lantana camara* leaves methanol extract on egg developmental stages of mice pre- and post-infection with *S. mansoni* compared to PZQ

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>egg developmental stages</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parameters</td>
<td>Total mean of immature stages</td>
<td>Total mean of mature stage</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>53.3±2.4</td>
<td>40.8±2.4</td>
<td>5.8±0.4</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td>53.6±2.6</td>
<td>36.4±2.9</td>
<td>10.00±2.8</td>
</tr>
<tr>
<td>4 weeks post-infection treated mice</td>
<td>54.2±3.8</td>
<td>38.3±3.3</td>
<td>7.5±1.1</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td>52.5±2.8</td>
<td>36.7±2.1</td>
<td>10.8±2.4</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td>00.00±00.00</td>
<td>2.3±1.2</td>
<td>97.7±1.2</td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$. 
Fig. (4.6): Effect of methanol extract of *Lantana camara* leaves on *S. mansoni* ova developmental stages compared to PZQ and non-treated control

- Non-treated control
- *Sterculia setigera* extract 7 days before infection
- *Sterculia setigera* extract 4 days before infection
- *Sterculia setigera* extract 7 weeks after infection
- Praziquantel 7 weeks after infection
Table (4.6): Effect of *Lantana camara* leaves methanol extract on ova count of mice pre and post infection with *S. mansoni* compared to PZQ

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of ova /g tissue (ova count)</th>
<th>Liver</th>
<th>% reduction</th>
<th>Intestine</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>17113.5±1876.6</td>
<td>-</td>
<td>27304.1±2818.9</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7days pre-infection treated mice</td>
<td>11257.3±1709.9</td>
<td>34%</td>
<td>17006.1±3388.9</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>4 weeks post-infection treated mice</td>
<td>11230.5±1421.6</td>
<td>34%</td>
<td>19516.8±2256.1</td>
<td>29%</td>
<td></td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td>11220.8</td>
<td>34%</td>
<td>16009.4±1425.2</td>
<td>41%</td>
<td></td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td>4480.7±602.5</td>
<td>74%</td>
<td>3374.1±577.1</td>
<td>88%</td>
<td></td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$.

NS  Not Significant
Fig. (4.7): Effect of methanol extract of *Lantana camara* leaves on *S. mansoni* tissue eggs count compared to PZQ.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% reduction of ova count from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PZQ</td>
<td>80</td>
</tr>
<tr>
<td>pre-infection</td>
<td>30</td>
</tr>
<tr>
<td>post-infection</td>
<td>40</td>
</tr>
</tbody>
</table>

Liver

Intestine
4.3.3.3 Effect of the combined dose of *Sterculia setigera* bark and *Lantana camara* leaves methanol extract on the worm burden, egg developmental stages, number of ova/g tissue (ova count) of mice pre-and post-infection with *S. mansoni*:

Table (4.7) and figure (4.8) illustrated that the three treatments of the combined dose of plant extract significantly reduced the total number of worms by 26%, 23% and 40%. While PZO induced 98% reduction.

Table (4.8) and figure (4.9) demonstrated that three treatments of plant extract induced no change in immature, mature and dead stages versus infected control. While the reference drug PZQ caused complete absence of immature stages and increased dead ova to 97.7% and significant reduced the mature stage.

PZQ induced 74% and 88% of ova count in liver and intestine while the three treatments of the combined dose produced non significant reduction by about 33% and significant reduction 37% and 45% in the liver and 31%, 36% and 41% in the intestine respectively; Table (4.9) and figure (4.10).
**Table (4.7):** Effect of combined dose of *Sterculia setigera* bark and *Lantana camara* leaves methanol extract on worm burden of mice pre- and post infection with *S. mansoni* compared to PZQ.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Parameters</th>
<th>Worm burden</th>
<th>Count</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected non-treated control</td>
<td></td>
<td></td>
<td>30.2±0.7</td>
<td>-</td>
</tr>
<tr>
<td>7 days pre -infection treated mice</td>
<td></td>
<td></td>
<td>22.3±1.1</td>
<td>26% ***</td>
</tr>
<tr>
<td>4 weeks post- infection treated mice</td>
<td></td>
<td></td>
<td>23.2±1.1</td>
<td>23% ***</td>
</tr>
<tr>
<td>7 weeks post- infection treated mice</td>
<td></td>
<td></td>
<td>18.0±0.6</td>
<td>40% ***</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td></td>
<td></td>
<td>0.5±0.3</td>
<td>98% ***</td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$. 

*Please purchase PDFcamp Printer on http://www.verypdf.com/ to remove this watermark.*
Fig. (4.8): Effect of methanol extract of combined dose of *Sterculia setigera* bark and *Lantana camara* leaves on *S. mansoni* adult worm reduction compared to PZQ.

- Praziquentel 7 weeks after infection
- *Sterculia setigera* extract 7 days before infection
- *Sterculia setigera* extract 4 weeks after infection
- *Sterculia setigera* extract 7 weeks after infection
Table (4.8): Effect of combined dose of *Sterculia setigera* bark and *Lantana camara* leaves methanol extract on egg developmental stages of mice pre- and post infection with *S. mansoni* compared to PZQ

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Percentage egg developmental stages</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>**Total mean of immature</td>
</tr>
<tr>
<td></td>
<td></td>
<td>stages**</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total mean</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>of mature stage**</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total mean of dead stage</strong></td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>53.3±2.4</td>
<td>40.8±2.4</td>
</tr>
<tr>
<td>7 days pre -infection treated mice</td>
<td>46.7±2.5</td>
<td>42.5±1.7</td>
</tr>
<tr>
<td>4 weeks post- infection treated mice</td>
<td>50.8±2.4</td>
<td>39.2±4.2</td>
</tr>
<tr>
<td>7 weeks post- infection treated mice</td>
<td>49.2±2.0</td>
<td>39.2±3.0</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td>0.00±0.00</td>
<td>2.3±1.2</td>
</tr>
<tr>
<td>7 weeks after infection</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** P<0.01 and *** P<0.001.*
Fig. (4.9): Effect of combined dose of methanol extract of *Sterculia setigera* bark and *Lantana camara* leaves on *S mansoni* ova developmental stages compared to PZQ and Non-treated control.

- **Non-treated control**
- *Sterculia setigera* extract 7 days before infection
- *Sterculia setigera* extract 4 days before infection
- *Sterculia setigera* extract 7 weeks after infection
- Praziquantel 7 weeks after infection
**Table (4.9):** Effect of combined dose of *Sterculia setigera* bark and *Lantana camara* leaves methanol extract on ova count of mice pre and post infection with *S. mansoni* compared to PZQ

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Number of ova /g tissue (ova count)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal groups</td>
<td>Liver</td>
<td>% reduction</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>17113.5±1876.6</td>
<td>-</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td>11527.7±1139.5</td>
<td>33%</td>
</tr>
<tr>
<td>4 weeks post-infection treated mice</td>
<td>10697.5±1029.6</td>
<td>37%</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td>9491.8±984.9</td>
<td>45%</td>
</tr>
<tr>
<td>PZQ treated mice 7 weeks after infection</td>
<td>4480.7±602.5</td>
<td>74%</td>
</tr>
</tbody>
</table>

* Significant differences versus infected control at ** $P<0.01$ and *** $P<0.001$.

NS  Not significant
**Fig. (4.10):** Effect of combined dose of methanol extract of *Sterculia setigera* bark and *Lantana camara* leaves on *S mansoni* tissue eggs count compared to PZQ.

![Bar chart showing the percentage reduction of ova count from control for different treatments.](chart.png)

- **Liver**
- **Intestine**
4.3.3.4 Effect of methanol extracts of *Sterculica setigera* bark, *Lantana camara* Leaves and combined dose of both extracts on the activities of serum ALT, AST of mice pre–and post infection with *S. mansoni*:

Tables (4.10), (4.11) and (3.12) illustrate the infection significantly increased the ALT, AST serum levels of the infected non – treated control compared to the normal control.

The three treatments of the extracts of *Sterculica setigera*, combined dose and *Lantana camara* when administrated 7 weeks post- infection significantly reduced the elevated ALT, AST serum enzymes. When the extract of *Lantana camara* was administrated 7 days pre-infection and 7weeks post-infection it significantly reduced the elevated ALT/AST levels respectively.

The standard drug (PZQ) highly reduced the elevated serum levels of ALT, AST.
Table (4.10): Effect of *Sterculia setigera* bark methanol extract on the serum level of ALT, AST, of mice pre-and post infection with *S. mansoni* compared to Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (non infected mice)</td>
<td>15.2±1.4</td>
<td>20.2±2.6</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>64.2±3.0</td>
<td>70.2±3.5</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td>52.3±1.7</td>
<td>56.9±2.1</td>
</tr>
<tr>
<td>4 weeks post infection treated mice</td>
<td>51.8±1.4</td>
<td>55.5±1.4</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td>42.1±2.6</td>
<td>52.6±2.1</td>
</tr>
<tr>
<td>PZQ treated mice 7 weeks after infection</td>
<td>27.4±1.6</td>
<td>34.6±2.0</td>
</tr>
</tbody>
</table>

* Significant difference versus infected control at* $P<0.05$ , ** $P<0.01$ and *** $P<0.001$.

Π Significant difference versus normal control at Π $P<0.05$ , ΠΠ $P<0.01$ and ΠΠΠ $P<0.001$. 

110
**Table (4.11):** Effect of *Lantana camara* leaves methanol extract on the serum level of ALT, AST of mice pre-and post infection with *S. mansoni* compared to Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Parameters</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (non infected mice)</td>
<td></td>
<td>15.2±1.4</td>
<td>20.2±2.6</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td></td>
<td>54.8±2.2</td>
<td>62.3 ±2.7</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td></td>
<td>48.7 ±0.3</td>
<td>56.7 ±1.6</td>
</tr>
<tr>
<td>4 weeks post infection treated mice</td>
<td></td>
<td>49.2 ±1.3</td>
<td>53.5 ±1.7</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td></td>
<td>42.5±2.3</td>
<td>47.0±2.4</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td></td>
<td>27.4±1.4</td>
<td>33.8±1.8</td>
</tr>
<tr>
<td>7 weeks after infection</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference versus infected control at * $P<0.05$ , ** $P<0.01$ and *** $P<0.001$.
Π Significant difference versus normal control at Π $P<0.05$ , ΠΠ $P<0.01$ and ΠΠΠ $P<0.001$.
NS Not significant
Table (4.12): Effect of combined dose of *Sterculia setigera* bark and *Lantana camara* leaves methanol extract on the serum level of ALT, AST of mice pre- and post infection with *S. mansoni* compared to Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control (non infected mice)</td>
<td>15.2±1.4</td>
<td>20.2±2.6</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>54.8±2.2</td>
<td>62.3±2.7</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>Π Π Π</td>
<td>Π Π Π</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td>45.8±1.6</td>
<td>48.7±1.8</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>4 weeks post infection treated mice</td>
<td>44.8±1.5</td>
<td>50.0±1.7</td>
</tr>
<tr>
<td></td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td>42.2±1.9</td>
<td>47.2±2.0</td>
</tr>
<tr>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td>27.0±1.4</td>
<td>33.8±1.8</td>
</tr>
<tr>
<td>7 weeks after infection</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

* Significant difference versus infected control at * P<0.05, ** P<0.01 and *** P<0.001.
Π Significant difference versus normal control at Π P<0.05, ΠΠ Π P<0.01 and ΠΠΠ Π P<0.001.
4.3.3.5 Effect of methanol extracts of *Sterculica setigera* bark, *Lantana camara* leaves and combined doses of both extracts on the serum proteins albumin, globulins, total proteins of mice pre-and post -infection with *S. mansoni*:

The serum proteins, albumin, globulin and total proteins levels restored to normal when the three treatments of the combined doses and the extracts of *Sterculica setigera* and *Lantana camara* (7 days pre- and 7 weeks post -infection) were administrated.

However; there was reduction in the levels of albumin and total proteins when the extracts of *Sterculica setigera* and *Lantana camara* were administrated 4 weeks post infection.

In all cases the standard drug (PZQ) restored the serum level of proteins to normal table (4.13), (4.14) and (4.15).
Table (4.13): Effect of *Sterculia setigera* bark methanol extract on the serum level of total protein, albumin, globulins and A/G ratio of mice pre-and post infection with *S. mansoni* compared to Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>T. protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulins (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (non infected mice)</td>
<td>6.1±0.1</td>
<td>3.2±0.1</td>
<td>2.9±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>5.5±0.2</td>
<td>2.5±0.2</td>
<td>3.1±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>7 days pre -infection treated mice</td>
<td>6.7±0.2</td>
<td>3.3±0.1</td>
<td>3.4±0.1</td>
<td>1.0±0.02</td>
</tr>
<tr>
<td>4 weeks post infection treated mice</td>
<td>5.3±0.2</td>
<td>2.6±0.1</td>
<td>2.7±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>7 weeks post- infection treated mice</td>
<td>6.6±0.2</td>
<td>3.4±0.1</td>
<td>3.2±0.1</td>
<td>1.1±0.05</td>
</tr>
<tr>
<td>PZQ treated mice 7 weeks after infection</td>
<td>6.8±0.5</td>
<td>3.3±0.1</td>
<td>3.1±0.1</td>
<td>1.1±0.1</td>
</tr>
</tbody>
</table>

* Significant difference versus infected control at * P<0.05, ** P<0.01 and *** P<0.001.
Π Significant difference versus normal control at Π P<0.05, ΠΠ P<0.01 and ΠΠΠ P<0.001.
NS Not significant
Table (4.14): Effect of Lantana camara leaves methanol extract on the serum level of total protein, albumin, globulins and A/G ratio of mice pre-and post infection with S. mansoni compared to Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>T. protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulins (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (non infected mice)</td>
<td>6.1±0.1</td>
<td>3.2±0.1</td>
<td>2.9±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>Infected non-treated control</td>
<td>6.3 ±0.3 NS</td>
<td>3.0 ±0.2 NS</td>
<td>3.2 ±0.2 NS</td>
<td>0.8 ±0.03</td>
</tr>
<tr>
<td>7 days pre-infection treated mice</td>
<td>6.5 ±0.2 NS</td>
<td>3.1 ±0.1 NS</td>
<td>3.4 ±0.1 NS</td>
<td>0.9 ±0.02</td>
</tr>
<tr>
<td>4 weeks post infection treated mice</td>
<td>5.6 ±0.2 NS</td>
<td>2.8 ±0.1 NS</td>
<td>2.8 ±0.1 *</td>
<td>1.0±0.05</td>
</tr>
<tr>
<td>7 weeks post-infection treated mice</td>
<td>6.2±0.1 NS</td>
<td>3.2±0.1 NS</td>
<td>3.0±0.04 NS</td>
<td>1.1±0.04</td>
</tr>
<tr>
<td>PZQ treated mice</td>
<td>6.7±0.4 NS</td>
<td>3.3±0.05 NS</td>
<td>3.0±0.1 NS</td>
<td>1.1±0.1</td>
</tr>
</tbody>
</table>

* Significant difference versus infected control at * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Π Significant difference versus normal control at Π $P<0.05$, ΠΠ $P<0.01$ and ΠΠΠ $P<0.001$.

NS Not significant.
Table (4.15): Effect of Sterculia setigera bark and Lantana camara leaves methanol extract on the serum level of total protein, albumin, globulins and A/G ratio of mice pre-and post infection with S. mansoni compared to Praziquantel PZQ.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Animal groups</th>
<th>T. protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulins (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal control (non infected mice)</td>
<td>6.1±0.1</td>
<td>3.2±0.1</td>
<td>2.9±0.1</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td></td>
<td>Infected non-treated control</td>
<td>6.3 ±0.3</td>
<td>3.0 ±0.2</td>
<td>3.2 ±0.2</td>
<td>0.8 ±0.03</td>
</tr>
<tr>
<td></td>
<td>7 days pre-infection treated mice</td>
<td>6.0 ±0.2</td>
<td>2.9 ±0.1</td>
<td>3.0 ±0.1</td>
<td>1.0 ±0.05</td>
</tr>
<tr>
<td></td>
<td>4 weeks post infection treated mice</td>
<td>6.1 ±0.3</td>
<td>3.0 ±0.1</td>
<td>3.2 ±0.3</td>
<td>1.0±0.02</td>
</tr>
<tr>
<td></td>
<td>7 weeks post-infection treated mice</td>
<td>6.4±0.2</td>
<td>3.2±0.0</td>
<td>3.2±0.1</td>
<td>1.1±0.02</td>
</tr>
<tr>
<td></td>
<td>PZQ treated mice</td>
<td>6.7±0.4</td>
<td>3.3±0.05</td>
<td>3.0±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td></td>
<td>7 weeks after infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference versus infected control at * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Π Significant difference versus normal control at Π $P<0.05$, ΠΠ $P<0.01$ and ΠΠΠ $P<0.001$.

NS Not significant
Chapter Five
General Discussion
Chapter Five

General Discussion

The use of natural resources as a historical remedy increase exponentially discovery of new therapeutic agents. Natural resources (plants, fungi, bacteria, etc) remain to be used as crude material, however studies must be done to reveal the active ingredients of the different components of these agents which in most cases have a stronger activity than the parent extract. Also, the isolated compounds can be used as model molecules or basic active molecules to improve or discover more compounds of specific activity. New drugs revived hopes of eradicating schistosomes directly by treating people. Single-dose treatment programmes certainly had dramatic results, but transmission persisted and reinfection invariably occurred. Experience showed that repeated treatments were essential to maintain initial gains. As long as praziquantel remained expensive, eradication seemed impossible: morbidity control became a more realistic objective. The most successful control programmes have been those that included some method of curring transmission, including mollusciciding, even at a reduced level (Webbe and El Hak 1990). Transmission control delays reinfection and extends the period between treatments, thereby reducing drug delivery costs.

The methanol extract of Sterculica setigera (bark) did not show any anti-schistosomal activity in vitro (100 µg/ml) mean while, it showed a promising prophylactic activity and moderate anti-schistosomal activity in vivo (500 mg/kg mouse bodyweight, for 5 consecutive days). The statement that a plant is anti-schistosomal or not should be taken within
the context of the solvent used and the plant parts investigated. Moreover, a plant with high \textit{in vitro} anti-schistosomal activity may have no \textit{in vivo} activity and \textit{Vice versa}. Because of the peculiarities in the metabolic disposition of plant’s chemical constituents, hosts’ immunological reaction, plant’s mode of action and other physiological parameters, should be carefully handled. Therefore, plants found to be active \textit{in vitro} must be tested \textit{in vivo} before a definite statement can be made on their anti-schistosomal potentials. Concerning the drug potential anti-schistosomal activity, it is accepted that indicators are worm load, oogram patterns and tissue egg count criteria assessing anti-schistosomal activity of any tested compound and or drug (Pellegrino and Katz 1969). Hepatic shift denoting change in the distribution of schistosomes within hepatic portal system are one of the important parameters reflecting drug activity (Standen, 1953).

This study new in using the Tatar extract in bilharziasis. However Fouad \textit{et al.}, (2007) reported the \textit{in vitro} anti-schistosomal activity of a related plant \textit{Brachychiton rupestris} (Lindl.) k. Schum. Similarly, Gohar (2004) reported the \textit{in vitro} anti-schistosomal activity of another \textit{Brachychiton australis} (Schott and Endl.) and \textit{Sterculia trichosiphon} bark.

\textit{Sterculia setigera} (Tatar) extract showed significant anti-schistosomal and prophylatic activities in infected mice after administration at 500 mg/kg .b.wt for 5 consecutive days seven weeks post infection and seven days before infection with \textit{S. mansoni cercariae} (percentage reduction of adult worm 29\%, 40\% respectively) but the \textit{in vitro} study, 100 µg/ml of Tatar had no effect on worm survival. So, it seems that the individual compound (s) responsible for the activity does not reach a level to be effective \textit{in vitro}, meanwhile it reached to a level that could achieve a sufficient curative serum concentration of active
compound (S) in the mesenteric and portal vessels \textit{in vivo}. Also the physiological and immunological reactions could also affect the plant activity.

The result obtained showed that Tatar administration caused reduction of egg load in liver and intestine tissues as a prophylactic and treatment (21%, 26% and 36%, 41%). Giboda and Smith (1994) reported that, the effect of antischistosomal drugs on schistosome eggs is accepted to be a consequence of killing of the adult worms, which eventually stop oviposition. This is a strong indication that Tatar could be a possible candidate as a schistomcidal drug.

Praziquantel killed 99% of \textit{S.mansoni} adult worm. There was a highly significant decrease of tissue egg count that resulted in complete absence of immature ova, and reduced the mature ova stages.

The effect of Tatar may not be on ovum itself, because no drugs were reported to act on the eggs themselves. In fact effective antischistosomal drugs usually display no effect on the ova themselves and ova deposition by worms continues their development in the tissue up to maturation. The mature ova remain alive in the tissues for a period of 12 days till their death and elimination in the stools (Standen, 1953; Bang and Hairston, 1946 and Vogel and Minning, 1947).

Accordingly, the highly significant reduction of live ova in the treated groups might be due to the effect of Tatar administration on worm fecundity. Some drugs seem to act initially on the reproductive organs of the worms (Bang and Hairston, 1946; Vogel and Minning, 1947 and kikuth and Gonnert, 1948). However, the level of treatment may have a partial activity during treatment. The drug may cause cessation of egg laying in most of the females or may affect in some way the function of oviposition.
The cumulative egg output of individual worm pairs was highly variable, wide variations \textit{in vitro} (Schirazazian and Schiller, 1983 and El.Ridi \textit{et al.}, 1997) and \textit{in vivo} (Cheever \textit{et al.}, 1994). Despite this variability, the mean number of egg present in the tissues could be used as an indicator of schistosome fecundity. A direct comparison of this variable could be made between treated and untreated host mice, irrespective of any difference in their infection intensities.

The result obtained in the present study showed very high significant difference in mean dead ova in treated group in comparison to the prophylactic group (20.14 ± 1.64 and 12.56 ±1.63), this may be due to the direct effect of Tartar on adult worm (after 7 weeks).

The three treatments of \textit{Sterculia setigera} extract (500 mg/k.body weight) significantly reduced ALT; (52.3±1.7, 51.8±1.4 and 42.1±2.6) AST; (56.9±2.1, 55.5±1.4 and 52.6±2.1) serum levels compared with the infected control and imporved A/G ratio. This may be due to the reduction of number of adult worms as well as eggs and improvement of the liver tissue.

The methanol extract of \textit{Lantana camara} leaves showed a ppreciable anti- schistosomal activity in infected mice (7 weeks post-infection) after administration at 500 mg /k body weight for five consecutive days, and reduced the number of ova in liver and intestine by 34% and 41% respectively.

This effect may be due to the direct effect of the active constituent of \textit{Lantana camara} leaves on worms causing their death and also appeared in reducing the total number of eggs in liver and intestine.

In the present study, the significant higher liver homogenate AST and ALT level in the infected groups may be due to the hepato cellular injury; caused by \textit{S. mansoni}. Infection which in turn leads to the release of the enzymes from injured hepatic cells into blood circulation (Hanna \textit{et al.})
al., 2003). A significant reduction of ALT, AST from the corresponding infected control after treatment (7 weeks post infection) may be due to the drastic effect of extract on worms and eggs, so giving partial relief of the liver, many authors reported that the primary cause of liver pathology in *S. mansoni* infection are eggs deposited in the presinasoidal space of liver inducing granulomatous inflammatory reaction to these eggs (Andrade, 1965, Dunn *et al.*, 1979, El-Menza *et al.*, 1989, Cheever *et al* 1994).

The results obtained in the present study is in agreement with those of Mahmoud and Refaie (2001) who reported that the parasitological studies on *Lantana camara* leaves aeral parts (leaves flowers, tips of branches and fruits) extract in *S. mansoni* infected mice (8 weeks post-infection) caused a significant reduction of the total number of worm by about 62% and reduced the number of ova in liver and intestine by 41% after 2 weeks from the final administration of plant (200 mg/ b.w. daily for 2 weeks).

Toxicological studies of the extract showed that administration of Tatar bark (7 days pre-infection and 7 weeks post –infection) reduced significantly the albumin and total protein and restored globulin to the normal compared with infected non treated control and improved A/G ratio.

The present study showed the prophylactic activity of *Lantana camara* leaves after administration 7 days –pre infection and 4 weeks post- infection. This effect may be due to the direct effect of the active constituents on the schistosomula development.

The result obtained in case of the administration of the combined dose of *Sterculia setigera* bark and *Lantana camara* leaves methanol extract, showed both promising prophylactic and anti- schistosomal activities.
Combined dose significantly reduce the total number of worms, the number of ova in liver and intestine and the elevated ALT/AST and improve A/G ratio.

The anti-schistosomal, prophylactic and hepato protective activities of *Lantana camara* leaves reported may be due to the presence of some of these chemical constituents’ particulary lantadenes and thereside in the extracts.

Bhakto and Gonjewala (2009) have recently confirmed the presence of phenol, anthocyanins and proanthcyanidins in *Lantana camara* leaves which could also be responsible for their antischistosomal activity. Though, the mechanism of the action of these chemical constituents is not yet fully known, it is clear that the effectivenss of the extracts largely depends on the types of solvent used, concentration and time of administration.

The use of *Sterculia setigera* bark and *Lantana camara* leaves and combined dose of both extract prior to *S. mansoni* infection (7 days pre–infection) reduced significantly the total number of worms by 40%, 20% and 26% respectively upon comparing with *S. mansoni*-infected group. In terms of immunity, the schistosome parasite is most susceptible to immune elimination during the skin and lung stages of development and least susceptible as an adult parasite (Capron et al., 1992). The major immune effector mechanisms involve various types of cells (macrophages, leukocytes and platelets) potentiated by antibodies and cytokines (Scott et al., 1989; Capron et al., 1992). One mechanism that cells may employ to kill parasites is the oxygen–dependent system of neutrophils and macrophages which release toxic oxygen products and oxygen radicals are also produced during the respiratory burst or as a consequence to nitric oxide release (Brophy and Pritchard, 1992). Toxic basic proteins secreted by eosinophils, among other things can attack the
parasite lipid membrane and breach its integrity (Gleih and Adolphson, 1980). The oxygen radical released can damage cell membranes, unfold or inactivate proteins, degrade nucleic acids thereby kill the cells and eventually, the parasite. In this regard, Mkoji et al., (1988) and Nare et al., (1990) found that adult worm had a high resistance (2% killed) to oxidative killing, while under the same conditions; the majority of schistosomula (95%) were killed. The above mentioned data may suggest the first possible reason for the success of Sterculia setigera and Lantana camara as protective agents against schistosome worms. The second possible reason may be attributed to the schistosome glutathione peroxidase (GPX), which is an important antioxidant enzyme protecting S. mansoni by reducing hydrogen peroxide (H₂O₂) and peroxidized lipids (Mkoji et al., 1988; Nare et al., 1990). GPX may play a more important role in the protection of schistosomes because (H₂O₂) has been shown to cause more damage to schistosomes than oxygen radicals (Smith et al., 1989). In the same sense, Mei et al., (1996) reported that the association of GXP with the schistosome tegument suggests that this enzyme could become a target of chemotherapeutic drugs or a potential vaccine candidate. Consequently, it is supposed that the use of Sterculia setigera or Lantana camara prior to S. mansoni infection may inactivate or reduce the schistosome GPX enzyme activity, which in turn damage one key survival mechanism of the schistosome.

The reduction in the ova count and its correlation with reducing number of worms was proved by Secor et al., (1996) who concluded that any reduction in worm burden should result in a corresponding decrease in the number of deposited ova.

Gharib et al., (1999) found that the deposition of parasite eggs triggers the release of endogenous eosinophil peroxidase; an enzyme activity developed in the immediate vicinity of the eggs, which increases
dramatically with time leading to a decrease in the antioxidant capacity of the liver. In this context, Abdallah et al., (1999) found that (H₂O₂)/myeloperoxidase system, which is the corner stone of the anti-microbial defense associated with inflammation, is activated in close contact with parasite eggs. The process although contributes to egg killing in vivo, yet, it causes accumulation of (H₂O₂), superoxide anions and hydroxyl radicals in the host’s tissues. Under such condition, the need for antioxidants increase and their presence may be crucial to eliminate the products of oxidative reactions and keep the ongoing immunological operations leading to destruction of eggs. According to the findings of Gharib et al., (1999), and Abdallah et al., (1999) the above mentioned effects of either Sterculia setigera and Lantana camara on the egg count might be explained by their action as anti-inflammatory and protective agents against the parasite eggs and not by stopping egg deposition as PZQ do.

On the other hand, Praziquantel, which is a drug of choice as antibilharzial drug, caused a gradual reduction in the total number of worm burden and shift of worms population from the mesenteric veins to the liver. It caused a prompt reduction in egg load with no viable eggs. The mechanism of action of Praziquantel involves synergism between the drug and humoral immune response of the host (Brindly and Sher, 1987). It was shown to cause rapid and often extensive changes to the tegument and subtegumental tissues of adult S. mansoni worm (Becker, et al., 1980).

The effect of the drug on worm and ova was associated with a reduction in granuloma diameter. The suppressive effect on granulomas was reported by many worker, (Mehlhorn et al 1982, Botros et al 1984, 1986, El-Hawey et al., 1986). This reduction may be due to complete
eradication of worms and consequent stopping of further deposition of new eggs (Botros et al., 1986; El-Badawy et al., 1988).

A tendency for normalization of serum ALT, AST activities after treatment with praziquantal could be due to stopping of egg production of S. mansoni worms and eradication of worms. Improvement in the level of albumin, globulin and A/G ratio was due to the recovery of hepatic tissue after elimination of parasite.

Praziquantal (PZQ) was used as a standard drug of choice for treatment of S. mansoni. To measure the activity the of Sterculia setigera bark and Lantana camara leaves; both plants showed moderate anti-schistosomicidal activity as well as promising prophylactic activity; which may be due to its effect on adult worms and schistosomulae. However, for some drugs with proven anti-schistosomal activity, their effect depend on dose regimen and the period of treatment. With Oltipraz for example, a slow acting drug, approximately two months are required before its full schistosomicidal effects become evident (Bueding et al., 1982). So, probably, a better curative effect of Tatar could be improved by either changing the dose regimen and/or period of treatment. The drug should not be introduced to the local Market until clear –cut evidence concerning its activity as anti schistosomal in different laboratories is available. Combination between Sterculia setigera and Lantana camara extracts improved the serum enzymes related to the liver functions and restored the serum proteins to the normal levels, so it's possible to suggest that their protective effects, may account partially for the ability to encounter or minimize the formation of schistosomal toxic products and render their impact remissible.

Although mollusciciding can be a cost-effective method of controlling schistosomiasis transmission, niclosamide, is now being produced commercially, and only a few compounds are at present being
tested in the laboratory. In future, improved cost-effective use of molluscicides will require more precise knowledge of schistosomiasis transmission patterns in each endemic area and improved application techniques. In snail control studies using controlled-release formulations only the organotins, especially tributyltin oxide (TBTO), have given satisfactory long-term results. However, large-scale field trials of organotin formulations have not been implemented and their use cannot be recommended, as their chronic toxicity in mammals has not yet been determined. The development of molluscicides of indigenous plant origin deserves support. Endod, derived from the berries of the climbing plant Phytolacca dodecandra, is the most extensively tested plant molluscicide, but data on its chronic toxicity to non-target organisms are lacking. The mode of action of molluscicides has not been extensively studied, though knowledge of the properties required of molluscicidal molecules has contributed to the discovery and development of niclosamide and nicotinanilide. In general, molluscicides probably cause stress on the water balance system, which in gastropods is thought to be under neurosecretory control, (WHO, 1980).

The effects of toxicants on target and non-target organisms are of concern in vector control operations. Residual molluscides have serious drawbacks, including development of resistant strains and deterious effects on the environment, man, livestock, wildlife, and beneficial non-target organisms. Apart from this, such chemicals are becoming increasily expensive and beyond the budget of many third world countries.

In this study methanol extracts of Sterculia setigera bark and Lantana camara leaves showed significant difference when tested as molluscicidal agent against the snail; Bulinus truncatus and Biomphalaria
pfeifferi with LD$_{95}$; 909 ppm, 811 ppm, 111 ppm and 213 ppm and LD$_{50}$; 482 ppm, 296 ppm, 48 ppm, and 111 ppm.

Neurophysiological studies have shown that Trifenmorph generate nerve impulses in isolated L. stagnalis ganglia. The impulses become grouped into spontaneous and random “Frescon bursts” with many cells firing at the same time. It seems that Trifenmorph causes changes in intraneuronal chloride levels and that this may affect related cell characteristics, such as bicarbonate transport and pH. These changes could lead to slight increase in excitability (McCullough et al., 1980).

The effect of molluscides in the nervous tissue may explain the trials of the snails to creep out the container after 45-60 seconds stay in the higher concentration of Tatar bark. It has long been observed that poisoning with molluscides causes the snails either to retract into the shell and expel haemolymph or to become swollen and remain extended from the shell opening. The latter response is seen particularly with organotins and certain carbamates and suggested loss of water – balance control (McCullough et al., 1980). The water – balance of gastropods is thought to be under neurosecretory control. Trifenomorph has been shown to reduce neurosecretory activity in B truncatus, while long-term exposure of the pulmonate, Indoplanorbis exustus, to barium chloride and copper sulphate also resulted in diminished neurosecretory activity. It has been shown that water flux through B. glabrata falls in the presence of a number of molluscides at concentrations around their LC$_{50}$ value. It may well be, therefore, that molluscides cause stress on the water – balance system and that this alone can cause death of the snail, or that reduction of normal water flow through the snail precipitates other disturbances in metabolism or physiological functions. In higher concentrations of Tatar bark and Lantana leaves extracts the snail before death expelled their guts outside their shell. This may be due to the bitter taste, which may
stimulate excessive vomiting reactions in the snails, a matter which may have led to the expulsion of the guts of these animals. Expulsion of the gut may also be as a result of upset of the osmoregulation balance as suggested by (McCullough et al., 1980).

The dynamic interaction between molluscs and their respective parasites lead either to a state co-existence or to incompatibility. In the first case the parasite thrives and produces subsequent stages of its life cycle. On the other hand, in incompatible snails, the parasite is either destroyed and eliminated by the snail defensive responses or fails to develop because the host is physiologically unsuitable (Bayne and Yoshino, 1989; Connors and Yoshion 1990, Van der Knaap and Loker, 1990).

The molluscicides may induce histopathological and physiological manifestation to the snail's tissues; for example, the molluscides may affect the respiratory mechanism of the snail. Lactate dehydrogenase (LDH) plays a vital role in respiration of molluscs. El-Anasary and Qurashy (1994) stated that LDH plays a key role in maintaining the host-parasite relationship, especially between Schistosome parasites and their molluscan host.

In addition, LDH plays an important role in carbohydrate metabolism. Evidence indicates that the parasites utilize carbohydrates as their primary energy source, acquiring it from the degradation of host’s glycogen. The availability of glycogen in the hepatopancreas of the snail may explain the preference of this organ for the development of the cercariae from the daughter sporocysts (Chiang, 1977).

The methanol extracts of Sterculia setigera bark and Lantana camara leaves showed considerable molluscicidal effect against Bulinus truncatus and Biomphalaria pfeifferi LC<sub>50</sub> were found to be 482ppm-296ppm and 48ppm-111ppm respectively. The results showed that there
were significant increase in the mortality rate when the snails are exposed to the different concentrations of the two tested plants compared to the control group. This finding (when *Lantana camara* tested against *Biomphalaria pfeifferi*) agrees with Mohmoud M.R and Refaie L.A (2001). They showed marked reduction in the survival rate of snails (*Biomphalaria alexandrina*) with different concentration of different *Lantana* parts (leaves, flowers branches and whole plants). In this case the differences may be due to the differences of the snails strains and method of plant extraction. In this study, the differences in the toxicity response of the snails to the same plant parts may be due to genetic and immunological variation with in the species.

The work described above may be considered as a pilot experiment designed to detect molluscidal activity of methanol extract of *Steculia setigera* bark and *Lantana camara* leaves in the laboratory, but it is not recommended for their application in laboratory or/and field, and hence more research is needed to identify the active ingredients of each plant parts and study the effects of these plant parts on both the physical and biotic component of the environment. Several workers have engaged themselves in the process of preparation of extracts from different plants and subsequently in testing them for schistosomicidal and molluscidal activities. In most cases preparation of extracts and chemicals used at present is expensive and cannot be used, unless they proved to be cost effective for field application. Other wise the idea behind the use of such natural products in vectors control will be defeated (at least in the developing countries). In the Sudan, the idea behind the use of natural products is to seek them from naturally abundant trees. In such cases a cheap source of safer controlling agents will be guaranteed, application of such products must also be as easy as possible to the rural community.
Crude water extracts of plant parts or by-products of trees would be ideal to fulfill these criteria.

Tartar bark and *Lantana camara* leaves can be used as antischistosomals, prophylactic and molluscicidal in the remote area until more investigation is done.
Conclusion and Recommendation

Sterculia setigera bark and Lantana camara leaves has a moderate anti schistosomal activity and promising prophylactic activity in vivo, so they posses curative effect against Schistosoma mansoni.

Further elucidation of the phytochemistry, plant species, time of cultivation, and origin of plant and method of extraction as well as extraction with different solvents are needed. Elucidation of the active constituents in plant parts are important before conducting further experimental studies.
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