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The efficacy of *Maytenus senegalensis* (L) extracts on experimentally infected rats with *Schistosoma mansoni*

G. A. Domo*[1] and S. L. Kela[2]

1-Department of Biological Sciences, Adamawa State University, P.M.B. 025, Mubi, Nigeria
2-Department of Biological Sciences, Abubakar Tafawa Balewa University, Bauchi, Bauchi State , Nigeria

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ABSTRACT: *Maytenus senegalensis* extracts, were tested on 3-4 weeks old rats, infected with *Schistosoma mansoni* cercariae. Oral treatments of three batches of rats with plant extracts of cold water, acetone and methanol were administered at 40g/kg body weight gave a cure rate ranging from 91.56% to 87.76% in rats; while praziquantel administered at 60mg/kg body weight gave a cure rate of 87.76%. These results show statistically significant difference ($P<0.05$). The therapeutic nature of the extracts and praziquantel reduced the pathological conditions of infected treated animals as evident by mottling of the liver with mean liver mottling score of 4.4 and 4.6 granolumas recorded and the level of damages done on organs and viscera in rats for *M. senegalensis*. Prazaiquantel gave similar value of 4.4 and 4.6 in rats. There was no significant difference between therapeutic scores of plant extracts and praziquantel ($P<0.05$) as shown by the pathological changes observed in the test animals.

Key Words: Schistosomiasis, *Schistosoma mansoni* Praziquantel, antischistosomal agents, snails, rats, *Bulinus*, organs, *Maytenus senegalensis.*

Introduction

Schistosomiasis or Bilharziasis, a parasitic disease of man and other vertebrate animals, is caused by blood-flukes of the genus *Schistosoma.* The disease is widely spread in various parts of the world. It is a public health problem with considerable magnitude. The infection is second only to malaria as a cause of human morbidity and mortality (WHO, 1990). It is estimated that over 250 million people in 76 countries of the world are infected with the disease with over 600 million others exposed at the risk of contacting the infection (WHO, 1990). The extent of morbidity and mortality due to schistosomiasis however has probably been underestimated. It is widely accepted that most infected individuals show no symptoms or signs of the disease upon physical examination and only a small proportion develop serious chronic disease. Another reason is the lack of epidemiological data on the state of the infection in rural areas.

*To whom correspondence should be addressed.
E-mail: domogalbert@yahoo.co.uk

At least 19 species of schistosome are recognized though only few are pathogenic to man and domestic animals (Johnson *et al.*, 1993). The most important species which infect man include: *Schistosoma haematobium* found in Africa and the Middle East; *S. mansoni* which occurs in Africa, the Arabian Peninsula, West Indies and Southern America; *S. japonicum* which is found in the Far East and *S. intercalatum* which occurs in Central and West Africa. The first case of *S. intercalatum* infection in man was probably reported in 1914, based on Chesterman's reports from Zaire (now Democratic Republic of Congo (DRC)) in 1923. *S. mekongi*, occur in the Laos, Thailand (WHO, 1990).

Other mammalian species such as *S. bovis, S. matheei, S. curasoni* and possibly *S. capense* can produce infection in man and termed as zoonotic species.

The geo-epidemiology of infection shows disparity around the globe. Although it infects people of all ages, it is more prevalent in children, farmers and fishermen.

The life-cycle of schistosomiasis is complex, involving many hosts. Man and other warm-blooded animals being the definitive hosts with the freshwater snails (*Biomphalaria* spp; *Bulinus* spp. and *Oncomelinia* spp) as intermediate hosts, while water bodies provide the link between them.

Epidemiology of schistosomiasis is characterized by many factors such as level of sanitation, association with water-body and the snail intermediate hosts among others.

The pathology of schistosomiasis varies according to the species and strains. Most infected people do not show any signs or symptoms of the disease. However, the pathology can be subdivided into the following phases:

- Invasion stage: During the penetration of cercariae and migration of schistosomula, the clinical signs observed are the skin reactions, fever, cough and Katayama syndrome.
- Stage of maturation, characterized by febrile illness.
- Stage of established infection, during which, large number of eggs is produced. In early chronic cases there are haematuria and other intestinal changes with inflammatory reactions, resulting from formation of granuloma.
- Stage of late infection: Late chronic infection which may be characterized by corpulmonale, fistula, obstructive uropathy, renal failure, portal hypertension and abdominal distension (Butterworth *et al.*, 1994). In rare cases elephantiasis may be induced in some individuals (Kela and Bowen, 1995).

However, since the introduction of tartar emetic by Christopherson in 1918, the development of antischistosomal drugs had really decreased compared to other areas of drug development. The few drugs available in the market are either scarce in endemic areas (Anthony *et al.*, 1994) or the organisms have developed resistance to them (Stelma and Talla, 1993; Picquet *et al.*, 1996). In addition most of them have serious side effects.

Niridazole (Ambilar) that was effective against *S. haematobium* is removed from the market because of its carcinogenic effects and cytogenic action on spermatogenesis (WHO, 1990) coupled with other side effects such as cramps, dizziness, head ache, nausea, vomiting, immunosuppression, rash, insomnia, convulsion, haemophilic aneamia in glucose 6-phosphate Dehydrogenase (G6PD) deficiency and psychosis among other (Bogistsh and Cheng, 1990).

Oxamniquine, though effective against *S.mansoni* (WHO, 1990), causes rashes, drowsiness, headache, diarrhoea, insomnia as well as hepatic enzyme changes (Bogistsh and Cheng, 1990).

Praziquantel introduced in the 1970's as a drug of choice for *S. haematobium; S. mansoni* and *S. japonicum* infections is however costly and not readily available in rural areas where they are most needed (Anthony *et al*., 1994). The present investigation is aimed at testing local plant extracts with potent antischistosomal agents.

Materials and Methods

Information on the Plants

Information on the medicinal values of the plants used was obtained from traditional healers in Bauchi Local Government Area of Bauchi State, Nigeria. Field trip together with the informants was arranged and during the trip samples of the plants was collected and the parts used in the traditional treatment obtained. The local Hausa names of the plants were documented. In the laboratory the plant was identified based on the characteristics of their leaves, flowers, fruits, bark, using appropriate keys described by Standfield and Hopkins (1966) and Hutchinson and Dalziel (1968).

Processing of Plant Materials

The parts of the plants used were collected and dried under shade for two weeks. They were then pulverized in a wooden mortar and pestle, sieved through ordinary flour's sieve and the powder stored in labeled polyethene bags for use. The extraction of plants materials was done using the solvent polarity technique with three solvents namely acetone, methanol and water in increasing polarity as described by Moore and Winston; (1996).

Collection of Snails

Snails (*Biomphalaria* and *Bulinus* species) that are known intermediate hosts of schistosomiasis mansoni were collected from the Yelwa stream flowing through the campus of the Abubakar Tafawa Balewa University (ATBU), Bauchi. Snails collected were sorted out and screened for infection according to the method described by the Danish Bilharziasis laboratory (Madsen, 1985), and finally identified as either *Bulinus Physopsis globosis, B. truncatus* or *Biomphalaria pferfferi* using appropriate keys described by Christensen (1993) and Brown (1994).

Collection and Rearing of Mice/Rats

Albino mice and rats purchased from the Animal House, University of Jos and the National Institute of Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria were infected by using paddling method as per Danish Bilharziasis laboratory (Madsen, 1985) recommendations.

Formulation and Administration of Praziquantel and Plant Extracts

Praziquantel tablets Batch NO. DISTT 3009 (Shinpoong Pharmaceutical Company, Korea) were purchased from Tinna Pharmaceutical Chemists Ltd., CI Kobi Street, Bauchi. The drug was orally given as a suspension of the tablet made of 30% water and 70% glycerin in a single dose of 60mg/kg body weight. Administration of the drug was done 5-6 weeks post infection with schistosome cercariae as described by Van Lieshout *et al.* (1991).

Plant extracts were similarly administered orally as a single dose of 40g/kg body weight, dissolved in aqueous suspension of 30% water and 70% glycerin as in the case of Praziquantel.

Phytochemical Analysis

Phytochemical screening of the plant extracts was carried out, to test the presence of saponins, tannins, phenol flavanoids, alkaloids and volatile oils as described by Sofowora (1984).

Results

Table 1 shows result of phytochemical analysis of the plants extracts. This result shows that *Maytenus senegalensis* apparently lacks tannin and volatile oils, and the water extract does not contain flavonoids.

Table 2 shows the liver mottling in rats infected with schistosomes. The acetonic and methanolic extracts of *M. senegalensis* had similar results to that of the praziquantel. The granulomas recorded are shown in Table 3. From the table, we discovered that the number of granulomas recorded in the rats treated with acetonic extracts is significantly lower than other extracts and to that of praziquantel. Table 4 shows the levels of damages caused by the infection in other vicera. We observed that the spleen was apparently normal in all the treated batches of animals except that it was darker in all the animals treated with the water extract. Also two cases of spleenomegaly were observed in the batch treated with Praziquantel.The kidney was not affected by infection while the major pathology observed in lungs was patechial hemorrhage with affected lungs having spots of dark coagulated blood on the surface as shown on the tail. Other pathological changes such as ascites, ulcers and perforations of the bowel were also observed on many organs.

Experimental infection.

The result of the experimental infection of mice with cercariae was impressive as shown by the number of worms recovered from animals with an average of 47 worms per rat. As result of this infection, some of the animals infected with *Schistosoma mansoni* cercariae passed out watery stools seven weeks post infection. Furthermore some infected animals lost their hair eight weeks post infection. Most of the affected animals that lost their hair were looking dull, week, emanciated and anaemic

Physical observations after treatment.

Clinically, the worst affected animals were treated with Praziquantel and the plant extracts of *M. senegalensis.* Acetonic and methanolic extracts gave the best results as hair regeneration was achieved within 2-4 days after oral administration. The water extract gave poor results. Animals treated with Praziquantel recovered their hair 4 days after the beginning of the treatment and were fully regenerated seven days post treatment. Animals passing watery faeces stopped on day 2, when Praziquantel and the plant extracts were administered. The water extract of *M .senegalensis* gave positive results by preventing diarrhea, but failed to regenerate hair. There was also improvement in the general body condition of the animals during treatment, both with Praziquantel as well as the plant extracts. Treated animals became active and feeding well

Table 1. Phytochemical screening of plant extracts in *M. senegalensis*.

Plant extracts	Components					
	Saponins	Tannins	Phenol	Flavonoids	Alkaloids	Volatile oil
Acetone	+	–	+	+	+	–
Methanol	+	–	+	+	+	–
Water	+	–	+	–	+	–

Key - absent
\+ present

Table 2. Liver mottling induced by schistosomes in rats

Extract Animals	A1	A2	A3	PZQ	Control 1	Control 2	Total
1	5	3	7	4	23	0	42
2	4	5	8	6	20	0	43
3	2	4	9	5	17	0	37
4	6	6	5	4	25	0	46
5	5	5	6	3	19	0	38
Total	22	23	35	22	104	0	206

Key:

A_1: *M. senegalensis* acetone extract
A_2: *M. senegalensis* methanol extract
A_3: *M. senegalensis* water extract
PZQ – Praziquantel
$Control_1$ Infected but non treated
$Contol_2$ Non infected

Table 3.Granulomas recorded in Some Organs of schistosome infected Rats

Organs Extracts	Stomach	Colon	Caecum	Intestine	Total
A_1	0	5	3	5	13
A_2	0	3	5	24	32
A_3	0	5	0	34	39
PZQ	1	8	3	23	35
Control 1	4	16	17	94	131
Control 2	0	0	0	0	0
Total	5	37	28	180	250

Key:

A_1 : *M. senegalensis* acetone extract
A_2: *M. senegalensis* methanol extract
A_3: *M. senegalensis* water extract
PZQ – Praziquantel
$Control_1$ - Infected but non treated
$Contol_2$ - Non infected.

Table 4. Level of damages scored in the viscera of schistosome Infected rats

Viscera Extracts	Spleen			Lungs		Kidneys		Total
	N	D	SM	N	PH	N	AF	
A_1	5	0	0	4	1	55	0	15
A_2	5	0	0	5	0	5	0	15
A_3	0	5	0	2	3	5	0	15
PZQ	3	0	2	4	1	5	0	15
Control 1	5	0	0	5	0	5	0	15
Control 2	5	0	0	2	3	5	0	15
Total	23	5	2	22	8	30	0	90

Key:

A_1: *M. senegalensis* acetone extract
A_2: *M. senegalensis* methanol extract
A_3: *M. senegalensis* water extract
PZQ – Praziquantel
$Control_1$ Infected but non treated
$Contol_2$ Non infected.
AF - affected
N - Normal
D - Darkened
SM. Spleenomegaly
PH. Patechial haemorrhage

Discussion

The eggs of schistosomes are the main cause of pathology in schistosomiasis infection. The eggs penetrate the blood vessels of the host tissue by secreting proleolytic enzymes, (Bogistsh and Chen, 1990). The host reactions to the eggs may vary from granulomas to intensive fibrosis. As a result of experimental infection of rats and mice with schistosomes, granulomas were observed in all the infected animals, however, with a variable intensity. The stomach was the least affected organ, with only 0.86% of the total granulomas recorded in rats. The intestine had the highest concentration of granulomas in contrast to the stomach which had the least concentration. In the control batches of infected but non treated animals, the total score of the granulomas was very high. However, the few granulomas recorded could be attributed to the large quantity of eggs as direct consequence of the heavy infection.

There were marked differences in the number of granulomas recorded among the treated rats. The non-treated infected control animals had granuloma scores that was highly significant ($P<0.05$). This could be due to the large number of eggs laid by the numerous worms recovered. There was significant difference ($P<0.05$) in the number of granulomas in infected rats treated with extract from *Maytenus senegalensis*. Animals treated with the acetone and methanol extracts recorded the lowest number of granulomas along the alimentary canal. This could be explained if we admit that the extracts had antischistosomal potency that might have either reduced drastically the number worms or at least succeeded in reducing the number of egg output. All the extracts from *M. senegalensis* had flavanoids as the chemical compounds except the water extract. Water extract had serious side effects on the lungs as observed in table 4. There is no difference in toxicity between the water extract treated batch and the control infected but not treated batch.

It may suggest that the acetone and methanol have removed that substance since these two extracts did not damage the lungs. Flavanoids according Kumar and Singh (1979) are, not strong therapeutic drugs, but rather toxic to cells of the host organisms. According to the same source, some of the flavanoids are antifungal and antipathogenic agents. The most important substances of pharmacological values are the alkaloids, which were found to be present in all the extracts from *M. senegalensis*. The differences in the potency of the acetone, methanolic and water extracts could be due to solvent polarity extraction technique used which might have removed the active ingredients in the first solvent used. The acetone and methanol as solvents, produced extracts that had better results than the water extracts. The serial extraction might have reduced immensely the therapeutic value of the subsequent extracts by removing some of the potent antischistosomal compounds. There is, however, the need for further analysis of the components of the different extracts to ascertain this claim.

Conclusion and Recommendations

The present study confirmed that acetonic and methanolic extracts of *Maytenus senegalensis* are potent antischistosomal components, with efficacy on schistosomes comparable with that of praziquantel. There was apparently lack of activity of water extract. It is recommendated that *M. senegalensis* be investigated further in the treatment of schistosomiasis using animal models together with characterization and toxicity tests.

References

Anthony, E.A.; Alison, J.C. and David, W.D. (1994). Immunity and morbidity in Human schistosomiasis mansoni. *Tropical and Geographical Medicine,* **16:** 197-203.

Bogistsh, B. J. and Cheng, T.C. (1990): *Human Parasitology***.** Sounders College Publishing. Holt Reinhard and Winston, Inc. New-York, 435pp.

Brown, D.S. (1994). *Fresh water snails of Africa and their Medical Importance.* Revised 2nd ed. Taylor and Francis, London, 487pp.

Brown, D.S. and Christensen, T.K. (1993). *A field guide to African Freshwater Snails. 1. West African species,* Danish Bilharziasis Laboratory, 55pp.

Butterworth, A. E. Allison, J.C., David, D., Anthony, J.C., Fulford, G.K., Curtis, H. K., Raph, K. Davy, K., Gabriel, M., Ouma, J.M., Morneu, R., Frederick, W. T., Andre, C., Sturrock R. F. (1994). Immunity and Morbidity in Human Schistosomiasis mansoni. *Tropical and Geographical Medicine,* **46** (4) 197-206.

Hutchinson and T.H. Dalziel, T. M. (1968). *Flora of West Tropical Africa*. Vol. III, Part I. Crown of agents for oversea governments and administrations. Nullbank, London, pp. 450-489.

Johnson, D.A., Dias, N.E.; Simpson, A.J.G. and Rollingson, D. (1993). Opening of a can of worms: Molecular analysis of schistosome population. *Parasitology Today,* **9**: 286-296.

Kela, S. L. and Bowen, D. (1995). Control of snails and snail borne diseases. *Pesticides Outlook*. 1: 22-27.

Kumar, H. D. and Singh, H. N. (1979). *Plant Metabolism.* The MacMillan Press Ltd. Great Britain, London, pp 256-275.

Mandsen, H. (1985). *Ecology and Control of African Freshwater Pulmonate Snails. Part I. Life-cycle and Methodology*. Danish Bilharziasis Laboratory, pp. 36.

Moore, W.R. and Winston, A. (1996). *Laboratory Manual for Organic Chemistry.* A microscale Approach. The Mac Graw-Hill Companies. Inc. pp. 59-68.

Picquet, M.; Ernould, J.C.; Vercruysse J.; Southgate, V.R.; A Mbaye; B. Sambou; M. Niang and D. Rollinson (1996). Epidemiology of human schistosomiasis in Senegal River Bassin – *Transactions of Royal Society for Tropical Medicine and Hygiene,* **90**: 340-346.

Sofowora, A. (1984). *Medical Plants and Traditional Medicine in Africa.* Spectrum Book Ltd. Ibadan, 256pp.

Standfield, D. P. and Hopkins, P. (1966). *A Field Key to the Savanna Trees of Nigeria.* Ibadan University Press, pp. 63.

Stelma, J. and Talla, M. (1993). Epidemiology of *schistosoma mansoni* infection in recently exposed community in Northern Senegal. *American Journal of Tropical Medicine and Hygiene,* **49**: 701-706.

Van Lieshout, L.; N. de Jonge; N.A. El Marsy; M.M. Mansour S. Bassily; F.W. Krijger and A. M. Deedder (1994). Monitoring the efficacy of different doses of Paraziquantel by quantification of circulating antigens in serum and urine of schistosome patients. *Parasitology,* **108**: 519-526.

World Health Organization (1990). *Tropical Diseases* WHO Technical Report Series, **978**: TDR-CTDHH 90-91.