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Comparative worm load recovered from laboratory animals treated with local plant extracts and Praziquantel

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ABSTRACT: The efficacy of cold water, acetone and methanol extracts of *Maytenus senegalensis, Terminalia glaucescens* and *Colocassia antiquorum* were tested on 3-4 weeks old mice and rats, infected with *Schistosoma mansoni* cercariae. Oral treatments of six batches of mice and three batches of rats with the plant extracts administered at 40g/kg body weight gave a cure rate ranging from 91.56% to 87.76% in rats and 71.56% to 76.30% in mice. Praziquantel administered at 60mg/kg body weight gave a cure rate of 87.76% in rats and 83.41% in mice respectively. These results were significantly different ($P<0.05$). 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 for *M. senegalensis* and 6.2 and 5.0 for the acetone and methanolic extracts of *T. glaucescens* in rats and mice respectively. Prazaiquantel gave also similar values of 4.4 and 4.6 respectively in rats and mice. There was no significant difference in the therapeutic scores between Praziquantel and selected plant extracts ($P<0.05$). This study confirmed the efficacy of these plants as potent antischistosomal agents.

Key Words: Schistosomiasis, antischistosomal agents, rats, mice, Praziquantel.

Introduction

Schistosomiasis (Bilharziasis), a parasitic disease of man and other vertebrate animals, is caused by blood-flukes of the genus *Schistosoma.* The disease is wide spread in various parts of the world.
It is a public health problem with considerable magnitude. The infection is second only to the malaria as a cause of human morbidity and mortality (WHO, 1990). It was 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 infection (WHO, 1990). However, the extend of morbidity and mortality due to schistosomiasis has probably been underestimated. It is widely accepted that most individuals show no symptoms or signs upon physical examination and only a small proportion develop serious chronic disease (Chen and Mott, 1989). Another reason is the lack of epidemiological data on the state of the infection in rural areas.

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At least 19 species of schistosomes 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 (Mouchet, 1918), based on Chesterman's reports from Zaire (now Democratic Republic of Congo (DRC)) in 1923 (Chesterman, 1923). *S. mekongi*, found in the Laos, Thailand (WHO, 1980).

Other mammalian species such as *S. bovis, S. matheei, S. curasoni* and possibly *S. capense* can produce infection in man (Payet *et al.,* 1966).

However, taxonomic problems remain obscure because of morphological similarities between the four main known species listed above and the other animal 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 intermediate snail hosts among others (Ukoli, 1984).

The pathology of schistosomiasis varies according to the species and strains. Most of the 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 are produced.
- Stage of late infection: Late chronic infection which may be characterized by corpulmonale, fistula, obstructive uropathy, renal failure, portal hypertension and abdominal distension (Edington and Gilles, 1981, Butterworth *et al.*, 1994). In rare cases elephantiasis may be induced in some individuals (Kela and Bowen, 1995).

The prevalence of the infection in Africa varies greatly from one country to the other and from one region to another. Some of the hyper endemic regions in Africa are the Nile Region where Egypt leads in *S. haematobium* infection with almost 100% of the population being infected in some places (Ukoli, 1984). Other hyperendemic regions are the Zambezi River region, with 67.8% of *S. haematobium* in Mozambique in the Northern provinces. However, there are very few publications on the Mozambican infection (Traquinho *et al*., 1998). In the Senegal River region, De Clerg *et al.* (1994) found that Dogon people in Mali have 51.3% and 12% infections with *S. haematobium* and *S. mansoni* respectively. *S. mansoni* is also the main species with 78% in the Delta of the same Rivers in the Republic of Senegal (Picquet *et al.*, 1996). *S. intercalatum* is prevalent along the main rivers of the rain forest region of Africa such as Zaire/Congo River, with 5.7-19.7% in school children in Libreville (Gabon) (Dazo and Gilles, 1972) or the Democratic Republic of Congo, where the infection rate ranges among the highest of the continent with 30-50% in school children around Kinshasa (De Clerg, 1987). In Cameroon, *S. intercalatum* infection prevails along the Wouri River, where it is confined in the Littoral Province around Edea, Puma and Douala where 23.6-32% of school children are infected.

S. japonicum is prevalent in the Middle East. Indonesia is the most infected country with 65%. In the Philippines, the infection rate is about 26.44% depending on the localities. In China, 15 million people are infected, giving an infection rate of between 14-26% (Chen and Mott, 1988). From the foregoing, schistosomiasis still remain a public health issue despite the combined effort in the integrated control programme including chemotherapy, snail control, health education, improvement of nutritional status (WHO, 1990).

Materials and Methods

Information on the Plants

Information on the medicinal values of the plants used *Maytenus senegalensis*, *Terminalia glaucescens* and *Colocassia antyquorum* was obtained from traditional healers in Bauchi Local Government Area of Bauchi State Nigeria. A 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. Plants and plant parts were 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

Useful parts of the plants were collected and dried under shade. Dried parts were then pulverized separately in a wooden mortar and pestle, sieved through an ordinary flour's sieve and the powder from each separately stored in labeled polyethene bags for use. The extraction of plants materials was done using the solvent polarity technique with three solvents according to the recommendation of : Moore and Winston (1996). These were Acetone, methanol and water in increasing polarity as described by Ibrahim *et al.* (1984), Kela *at al.* (1989).

Collection of Snails

Snails (*Biomphalaria* and *Bulinus* species) that are known intermediate hosts of schistosomiasis were collected from the Yelwa stream flowing through the campus of the Abubakar Tafawa Balewa University (ATBU), Bauchi following the method described by the Danish Bilharziasis laboratory (Madsen, 1985).

Snails collected were sorted out and screened for infection according to the methods of Frandsen (1981) and Madsen (1985) and finally identified as either *Bulinus physopsis globosis*, *B. truncatus* or *Biomphalaria pferfferi* using appropriate keys described by Kristensen (1989), Brown and 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 Christensen *et al.* (1979) 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 in a single dose of 60mg/kg of body weight (WHO, 1980) formulated as a suspension of the tablet made of 30% water and 70% glycerin. The drug was administered 5-6 weeks post infection with schistsosome cercariae as described by El Marsy *et al.*, 1988 and Van Lieshout *et al.*, 1994.

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

Dissection

Two weeks after the administration of Praziquantel and plant extracts, the mice and rats were sacrificed. Their abdominal parts were carefully opened and organs were identified, using anatomic keys (Manton and Brown, 1977 and Rowett, 1977). Viscera were collected and the degree of pathological changes were recorded.

Results

The results of worms recovered from infected, treated test organisms and controls were summarized in Table 1. From the table it could be seen that there was a marked reduction of worms from animals treated with both Praziquantel and plant extracts.

Table 2 summarized the worm recovery rate in test mice. It could be further observed that worm burden was greatly reduced in mice treated with Praziquantel and a moderate reduction in mice treated with plant extracts.

Table 1. Worm burden recovered from infected rats.

Extract Worms	A_1	A_2	A_3	PZQ	$Control_1$	$Control_2$	Total
Paired	6	8	10	14	222	0	260
Single	14	19	19	15	15	0	342
Total	20	27	29	29	237	0	342

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
$Control_2$ - Non infected.

Table 2: Worms burden recovered from infected mice

Extract Worms	B_1	B_2	B_3	C_1	C_2	C_3	PZQ	$Control_1$	$Control_2$	Total
Paired	30	18	20	18	20	18	4	184	0	312
Single	23	32	37	42	39	42	31	27	0	273
Total	53	50	57	60	59	60	35	211	0	585

Key
B_1: *T. glaucescens* acetone extract
B_2: *T. glaucescens* methanol extract
B_3: *T. glaucescens* water extract
C_1: *C. antiquorum* acetone extract
C_2: *C. antiquorum* methanol extract
C_3 *C. antiquorum* water extract.
PZQ – Praziquantel
$Control_1$ Infected but non treated
$Control_2$ Non infected.

Discussion

Worms were recovered from all the infected animals. This was an indication of successful establishment of schistosomes in these animals. The reduction in the number of worms from infected treated rats and mice with both praziquantel and plants extracts demonstrate the antischistosomal effect of the extracts and praziquantel. The efficacy of the extracts, however differ. This is supported by the considerable reduction in worm burden, varying from 87.76% to 91.56% in infected rats treated with extracts of *M. senegalensis*. The Praziquantel treated group had a cure rate of 87.76% compared to that of extracts of *M. senegalensis*. In mice, praziquantel also had a moderate efficacy with a cure rate of 83.41%. The efficacy of praziquantel recorded in this investigation is within the normal range of 70-100% as recommended by El Marsy *et al.* (1988).

The fact that animals treated with praziquantel had the lowest worm burden confirms that this drug remains superior in reducing the pathogenecity of schistosomes. The differences in potency of this drug could be attributed to differences in the physiology of the animals. It is also probable that the differences could be attributed to the real quantity of praziquantel taken up by animals. Since the administration of the drugs was oral, the individual rats especially the larger ones might have taken a much lower dose. In mice the plant extracts yielded cure rates varying from 71.56% to 76.30%.

In rats, extracts from *M. senegalensis* had a better reduction of worm burden as evident by the low number of granulomas seen in the viscera of infected rats reduction in worm burden could be explained by the gradual decrease of the amount of the active ingredient extracted by the different solvents used. Acetone, being the first solvent used in the series of the extraction had the best results with water being the last solvent used having the least efficacy. Acetone as a solvent could have removed most of the active antischistosomal components. Similarly, few of the unextracted potent antischistosome components left by acetone could have been removed by methanol leaving trace of the active antischistosomal ingredients that was removed by water, thus accounting for the decrease in the efficacy of the different extracts.

It was observed that the reduction in worm burden was proportional to the number of paired adult worms recovered. This is due to the fact that the number of paried worms determines the egg output and consequently the entire pathogenecity of schistosomiasis. The low number of paired worms in infected treated mice revealed a clear indication of the efficacy of plant extracts and praziquantel. The finding shows good potential of the plant extracts in the control of schistosomiasis, since morbidity is lowered. Since acetone extract of *M. senegalensis* got the least number of paired worms recovered from infected treated rats, it is the most potent antischistosomicide, thus confirming its efficacy and potentials in future treatment of schistosomiasis. The uncoupling nature of this extract further suggests that it has the ability to disrupt copulation. All the extracts from *C. antiquorum* had the poorest results in reducing the worm load as well as having the highest number of paired worms. The apparent lack of potency exhibited by water extract from *M. senegalensis* may be that, the serial extraction could have removed most of the active antischistosomal principles, when acetone was used as the first solvent. This confirms the efficacy of the solvent polarity approach when the extract has to be purified as noted by Moore and Winston (1996).

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