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THE ANTHELMINTHIC EFFECTS OF CRUDE AQUEOUS AND ETHANOLIC EXTRACTS OF *OCIMUM GRATISSIMUM* AND *ZINGIBER OFFICINALE* ON EXPERIMENTAL *SCHISTOSOMA MANSONI* INFECTIONS IN MICE

P. M. Lar[1,3], A. S. Akintunde[2], C. I. Igwillo[2] and J. P. O. Oyerinde[3],
[1] Department of Microbiology, Faculty of Natural Sciences, University of Jos, PMB 2084 Jos, Nigeria
[2] School of Pharmaceutical Technology, School of Pharmacy, College of Medicine, University of Lagos, Nigeria
[3] Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria
Corresponding author: Dr. P. M. Lar

ABSTRACT

The anti-schistosomal effect of crude aqueous extract of *Ocimum gratissimum* and the ethanolic extract of *Zingiber officinale (Ginger)* on experimental S. *mansoni* infections in 120 mice were evaluated. Similarly, the effect of a mixture of these two extracts in equal proportions was also analyzed. Various oral concentrations of the extracts administered for 10 days (100 mg/ml, 75 mg/ml, 50 mg/ml and 25 mg/ml) were carried out and mice sacrificed 14 days after treatment for the mature mouse infections and 41 and 27 days after treatment for the 2 week old and 4 week old infections. A concentration of 100 mg/ml for 10 days of *O. gratissimum* caused 80% reduction and 50 mg/ml of the ethanolic extract of *Z. officinale* resulted in a 60% reduction of worm burden when mice were treated at 8 weeks of infection. The combination of the two extracts was ineffective (20% reduction of worms).The female worms were more susceptible to both extracts than the male worms. Only *O. gratissimum* (100 mg/ml) caused a 25% reduction of worm burden on the young adult at 4 weeks infections. Interestingly, the combination of the two extracts caused a 13% reduction in worm burden on the 2 week old infections. Effect of the extracts on liver weight and the average egg load per liver in *O. gratissimum* treated mice was significant, although the average liver egg load was increased but the difference was not significant compared with the control mice. This report confirms that both *O. gratissimum* and *Z. officinale* have anti-schistosomal properties, and *Ocimum* may be effective against immature Schistosome infections but also possess anti-inflammatory properties. These results would require further validation.

Keywords: *Ocimum gratissimum, Zingiber officinale, Schistosoma mansoni,* mice

INTRODUCTION

Efforts at reducing the morbidity caused by schistosomiasis have continued to be a cause for concern. As a result of re-infection, especially in children of school age, chemotherapy continues to be at the center of control programs (Savioli *et al.*, 2004). Praziquantel, the recommended chemotherapeutic compound for the treatment of urinary and intestinal schistosomiasis (WHO, 1997) is used in mass chemotherapy but is generally unavailable and the potential to develop resistance has been reported in Senegal (Stelma *et al.*, 1995) necessitating the search for more effective and cheap alternative chemotherapeutic compounds.

The use of plants for therapeutic purposes is widespread and in schistosomiasis, traditional herbal remedies provide cheap and reliable alternative. Several plants with lethal effects on Schistosoma species have been reported. The ethanolic extract of *Citrus reticulata* root or the oleo-resin extract from Myrrh of *Commiphora molmol* tree (Mirazid) have been recently reported to be effective against *S. mansoni* and are now used as anti-schistosomal drugs (Hammed and Hetta, 2005). Clinical cure has been reported in Egypt for both *S. mansoni* and *S. hematobium* using tablets of Myrrh. *Solanum nigrum* leaves was effective against the cercariea of *S. mansoni* in mice (Ahmed and Rifaat, 2005) and Artemether, a methyl ether derivative of dihydroartemisinin, derived from the Chinese plant *Artemisia annua* L. (Asteraceae) an effective anti-malarial agent, has also been reported to possess strong activity against the immature stages of *S. mansoni* in mice (Berquist *et al.*, 2005).

Ginger is a rhizome that is cultivated worldwide. Its activity against *S. mansoni* miracidia, cercariae and adult worms has been reported (Adewumni *et al.,* 1990; Sanderson, 1998; Sanderson *et al.,* 2002), but its effect on immature worms has not been reported. *Ocimum gratissimum* L. (Labiatae family), also known as alfavaca have been report ed to poccess antimicrobial and antibacterial properties (Nakamura *et al.,* 1999; Ngassoum *et al.,* 2003).
The aim of this work is to evaluate the anti-schistosomal effect of the ethanolic extract of *Zingiber officinale* and aqueous extracts of *Ocimum gratissimum* In addition, the effect of combinations of these extracts on mature and immature and *S. mansoni* in mice will be evaluated.

MATERIALS AND METHODS

Plant extracts: Ethanol extract of *Z. officinale* was obtained by standard methods with 70% ethanol using a soxhlet apparatus. *O. gratissimum extract* was extracted using hot water (African Pharmacopoeia, 1986). For both ethanolic and water extracts, three different concentrations were prepared namely, 100 mg/ml, 75 mg/ml and 25 mg/ml. A mixture of both extracts in equal proportions, served as the third treatment.
Experimental animals: Male and female albino mice with age range between 5-6 weeks old were obtained from the Animal Centre of the College of Medicine, University of Lagos for the study. The animals were fed with standard mice cubes (Pfizer) while water was provided *ad libitum.*
Parasite: Cercariae *of S. mansoni were* obtained from infected snails of the species, *Biomphalaria pfeifferi,* collected in Jos, Nigeria. Infected snails were placed in a beaker containing 10 – 15 ml distilled water and exposed to light for 60 minutes to stimulate the emergence of the cercariae. The density of the released cercariea was determined by counting the number of cercariea in aliquots of 0.5 ml of water. The average counts of 5 aliquots was used to determine the concentration of the cercariea to infect individual mice, which were then infected by the paddling method described by Moore *et al.* (1949).
Two groups of mice (40 each) were infected with a total of 200 cercariae, and this constituted the acute and sub-acute infection group. Another set of mice (40) were infected with 60 cercariae and served as for the chronic infection group.
Experimental procedure: For the group of mice that were infected with 200 cercariae, 40 mice were divided into 2 groups of 20 mice each (making group I and II). Each of groups I and II were further divided into 4 subgroups of 5 mice each. Treatment in group I started at 2 weeks post cercarial exposure, and treatment for animals in group II started at 4 weeks post exposure. Group III is the chronic infection group was subdivided into 4 sub-groups of 10 mice each, and treatment was initiated from 8 weeks post cercarial exposure.
Plant extracts for *Ocimum gratissimum* (A) and *Z.officinale* (B) and the mixture of both (C) were administered to three of the sub-groups for the acute and sub-acute infections (I and II) while the 4th sub group served as the control and was given distilled water. Treatment comprised daily oral dose of 1 ml of each extract concentration (that is 100 mg/ml; 75 mg/ml; 50 mg/ml; 25 mg/ml) for 10 consecutive days. The mice in the various groups were sacrificed 41, 27 and 73 days respectively post cercarial exposure, for the acute, sub-acute and chronic groups. The entire gastrointestinal tract was removed and teased out into physiological saline for the recovery of worms from the mesenteric veins. The liver was also removed and worms lodged within the intra-hepatic portal veins were recovered and counted for each mouse.
In order to determine the total liver egg counts, livers of infected mice were digested in 40% potassium hydroxide at 37^{0}C for approximately 18 hours for the recovery of eggs within the tissues.

RESULTS

Effect of aqueous, ethanolic and combination of ginger and *Ocimum gratissimum* extracts on 8, 4 and 2 week old *S. mansoni* infections in mice was evaluated. The administration of 100 mg/ml of *Ocimum* for 10 days to 8 week old *S. mansoni* infections in mice caused an 80% reduction in the average female worm count and similarly 50 mg/ml of aqueous ginger extract resulted in 60% reduction in mean female worm counts in mice (Table 1). The action of the individual plant products was obvious on the female worms than the male worms. The combination of the two extracts however was ineffective (20% female worm reduction). Average liver egg loads was not

taken for these infections. Neither ginger nor *Ocimum* extract was effective on the acute (2 week-old) infections but the combination of the two extracts resulted in a 13% reduction in worm burden. For the 4 week-old *S. mansoni* infection in mice, 100 mg/ml of the ethanolic extract of *Ocimum* resulted in the reduction of 25% total worm count in the treated compared with the untreated mice.

Table1. The anti-schistosomal effect of *Ocimum gratissimum*, *Zingiber officinale* and the Mixture of both on chronic (8 week-old) *S. mansoni* infections in mice

Extract	Dose (Mg/mlx10 days	Average recovery	Worm count per mouse	Percentage female worm reduction per control	
		Female worm	Total worm count		
Ocimum	100	2	6	80	6
Ginger	50	4	8	60	6
Occimum/Ginger	Mixture	8	18	20	6
Ccontrol	Untreated	10	29	0	6

There was no significant reduction in the average total egg load per liver of treated mice compared with the untreated control mice (Table 2). Ginger extract was however, ineffective on the 4 week-old infections as evidenced by higher liver load egg load. When extract treatment was given at 2 weeks of cercarial infection, there was a marked reduction in liver weight for the treated mice (with both the extracts and their combinations). Nether the ethanolic extract of 100 mg/ml of neither *Ocimum* nor ginger was effective against the 2 week-old infections, but the mixture of the two extracts resulted in a 13% reduction in female worm burden. There was no significant reduction in egg load of liver weight.

Table 2.The anti-schistosomal effect of *Ocimum* and *Ginger* extracts on 4 week-old (sub acute) *S. mansoni* infections in mice

Extract type	Dose of Plant extract Mg/ml x 10days	Average worm count recovery per mouse		% female worm reduction relative to control	Average weight of liver (gm)	Average total egg load per liver
		Female worms	Total Worm count			
Ocimum	100	58	156	25.64	1.4786	1,100
Ginger	100	76	180	2.56	1.5788	2,750
Control		78	188	0.00	1.4541	1,800

DISCUSSION

The anti-schistosomal effect of *Zingeber officinale* against adult *S. mansoni* has been reported in Nigeria (Adewumni *et al.*, 1990; Sanderson *et al.*, 2002), but its action against immature worms has not been reported. Similarly, the antibacterial effect of *Ocimum gratissimum* has been reported but the anti-schistosomal action has not been reported (Nakamura *et al.*, 1999; Ngassoum *et al.*, 2003). We evaluated the effect of the aqueous and ethanolic extracts of these plants against experimental *S. mansoni* infections in mice.

Following a 10 days course of therapy 100 mg/ml of *O. gratissimum* caused 80% reduction and 50mg/ml of the ethanol extract of *Z. officinale* resulted in a 60% reduction of worm burden in adult worm infection (8 weeks old) confirming the efficacy of ginger against *S. mansoni* infections. But the combination of the two extracts was ineffective (20% reduction of worms) on the adult worm infections. The female worms were more susceptible to both extracts than the male worms. The anti-schistosomal properties of these two extracts is an indication that both products may be useful for the reduction of symptoms and morbidity of schistosomiasis in endemic areas as they both caused a marked reduction in liver weight of the treated mice compared to that of the untreated controls. This is suggesting a reduction in the inflammatory

lesions caused by the eggs deposited in the livers. Histological sections were however not taken to confirm the development of the lesions.

Plant extracts have been shown to be valuable alternatives to the control and treatment of schistosomiasis. The alcoholic extract *Pavetta owariensis* was effective against adult male worms in chronic *S. mansoni* infections in mice. In Sudan, Koko *et al.* (2005) evaluated the efficacy of a single dose of *Balanites aegyptiaca* fruit mesocarp against the Sudanese strain of *S. mansoni* in mice. It showed promise by effecting a reduction in egg load. Also, the powder form of *Ferula asafetida,* a hard, resinous, oily herbaceous gum of the plant family Umbelliferae in Egypt was effective against *S. mansoni* experimentally infected mice. It will thus serve to further evaluate the efficacy of combinations of ginger and *Ocimum* extracts for the treatment and relief of symptoms of schistosome infections.

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