Applied Biological Research 15 (1): 47-52; 2013

EFFECT OF *Allium sativum* AQUEOUS EXTRACT ON EGG LOAD AND WORM BURDEN IN IMMUNOSUPPRESSED GUINEA PIGS INFECTED WITH *Schistosoma haematobium*

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(Received 8 February, 2013; accepted 3 April, 2013)

ABSTRACT

Current research was designed to evaluate the prophylactic and therapeutic potential of garlic (*Allium sativum*) aqueous extracts in *Schistosoma haematobium* infection, using immuno-suppressed guinea pigs as animal model. Twenty four infected male guinea pigs were divided into various groups that were treated with *A. sativum* extracts over different periods, praziquantel treated group and control. Eggs recovered in various groups were counted and recorded. A substantial reduction in egg load of *S. haematobium* was observed in *A. sativum* extract-treated and praziquantel treated groups in comparison to untreated control. Further, praziquantel and *A. sativum* extract treatments significantly reduced worm burden. *S. haematobium* infection was established in guinea pigs under controlled immunosuppression. The study suggests that *A. sativum* aqueous extract is a convenient prophylactic and promising therapeutic agent for the control of *S. haematobium* infection.

Key words: *Allium sativum,* guinea pig, praziquantel, prophylactic potential, *Schistosoma haematobium*

INTRODUCTION

Schistosomiasis is considered the most important human helminthiasis in terms of morbidity and mortality (Copeland *et al.,* 2002); and despite major advances in its control schistosomiasis continues to spread to new geographic areas. Currently, schistosomiasis affects more than 250 million people (Hu *et al.,* 2004). In many countries schistosomiasis affects a large proportion of under-17 children due to their habit of swimming in water that harbours snail, the intermediate host of these parasites. Schistosomes belong to the phylum: Platyhelminthes, class: Trematoda, subclass: Digenea, family: Schistosomatidae, and genus: Schistosoma. Five most common species of schistosomes infecting man and causing human schistosomiasis are*: Schistosoma haematobium* (which affects 54 countries in Africa and the Eastern Mediterranean, and causes urinary schistosomiasis) (Copeland *et al.,* 2002); *S. mansoni* (responsible for African intestinal schistosomiasis), *S. japonicum* and *S. mekongi* (these two species cause intestinal schistosomiasis in Asia and the Pacific region) (Ojewole, 2004); and *S. intercalatium.* Like other digenetic trematodes, the schistosomes are equipped with suckers with which the worms attach themselves to the walls of the blood vessels in which they live.

The life-cycle of the trematodes that cause schistosomiasis in man is atypical of the other parasites of class Trematoda in that the worms are unisexual. Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species. For instance, *S. haematobium* normally lives and mates in the veins of urinary bladder of man (the only

important definitive host), producing eggs with a terminal spine, which pass into the bladder wall and finally into the urine.

There are limited options available for the chemotherapeutic treatment of schistosomiasis with the drug of choice still being praziquantel (WHO, 1990). However, after some 30 years of praziquantel usage, a decreased susceptibility to the drug and the emergence of drug-resistant strains of schistosomes has been observed in several countries (Ismail *et al.,* 1999; Botros *et al.,* 2005). Thus emphasizing on the need to develop new safe antischistosomal agents and evaluate the potency of traditional medicinal plants for treating schistosomiasis.

Ancient Egyptians realized the benefits of garlic as a remedy for a variety of ailments. Garlic (*Allium sativum),* belonging to the family Liliaceae, has recently shown multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, hypoglycemic and antitumor activities (Thomson and Ali, 2003). *A. sativum* is cultivated allover the world including Nigeria and is used as meat tenderizer and spice in many delicacies. The traditional medical practitioners have considered *A. sativum* as an excellent medicinal plant with a lot of therapeutic properties (Krishnaraju *et al.,* 2006). It has received special attention for its beneficial effects to man and animals (Auer *et al*., 1990), but until recently there is little scientific support for the chemotherapeutic and prophylactic capacity of *A. sativum* against *S. haematobium* (Soffar and Mokhtar, 1991; Abdel-Rahman *et al.,* 1998).The anthelmintic effects of *A. sativum* in recent years has been a matter of interest for most researchers (Ayaz *et al.,* 2008; Riad *et al*., 2007, 2008). In view of above, the present research was carried to assess the survival of *S. haematobium* in guinea pigs through immunosuppression and also to determine whether *A. sativum* aqueous extract could be harnessed and used as a prophylactic and antischistosomal agent in *S. haematobium* infection.

MATERIALS AND METHODS

Experimental animals: A total of 24 healthy, male guinea pigs weighing between 125-200 g were obtained from Animal Genetics and Breeding Laboratory, University of Nigeria, Nsukka. These guinea pigs were treated in accordance with the internationally accepted principles for laboratory animal use and care (Nahed *et al.*, 2009). Further, **s**nail species *Bulinus truncates,* which serve as the intermediate hosts of *Schistosoma haematobium,* were obtained from Ishielu (Nigeria) where they are abundant (Okafor, 1990).

Collection of urine and exposure of *S. haematobium* eggs in diluted urine to shed miracidia: *S. haematobium* infection is endemic in Ohaukwu, Nigeria (Okafor, 1990). Urine samples of 75 pupil attending public primary schools in this area were collected in 75 clean plastic containers between 8.00 am - 12.00 noon and transferred to Parasitology Research Unit, University of Nigeria. Infected urine containing viable *S. haematobium* eggs were exposed to artificial illumination to shed miracidia (Niels *et al*., 1984).

Infection of snails with miracidia and exposure of snails to shed cercariae: Snail intermediate hosts were transferred into plastic containers containing miraciadia and were allowed to stay for 3 h under bright light. They were later transferred into fresh water contained in a plastic bowel and fed with lettuce for 28 days. Infected snails were kept in total darkness for a period of 48 h prior to carrying out cercariae harvesting procedures, after which they were made to shed cercariae by subjecting them to high stress through strong artificial illumination for 3 h and later transferred into fresh water in another container (Niels *et al*, 1984).

Preparation of *A. sativum* aqueous extracts: *A. sativum* bulbs were separated, peeled, and washed with distilled water and 1 kg *A. sativum* bulbs crushed in a blender with 1000 ml water until a uniform consistency was achieved (Nahed *et al.,* 2009). From the resulting paste 1 g ml^{-1} aqueous solution was obtained for LD_{50} determination.

Experimental design: Twenty four male guinea pigs were immunosuppressed by administering orally 5 mg kg^{-1} of prednisolone before infection. Cercariae just harvested were intra-peritoneally injected into the guinea pigs with limited amount of fresh water (Niels *et al.,* 1984). Animals were divided into 4 groups, with each group containing 6 male guinea pigs and treated as follow: i) Infected, *A. sativum*-treated group (CG) wherein animals were infected with 200-300 *S. haematobium* cercariae and allowed to stay for 10 weeks prepatent period before commencing another 4 weeks treatment with 5 ml kg^{-1} of *A. sativum* aqueous extracts daily; ii) Infected, *A. sativum*-prophylactic group (PG) wherein animals were treated with 5 ml kg^{-1} of *A. sativum* aqueous extracts daily for 7 days before infecting them with same number of *S. haematobium* cercariae; iii) Infected, praziquantel treated group (DC) wherein animals were infected with 200-300 cercariae and treated for 4 weeks with praziquantel after 10 weeks pre-patent period and iv) Infected, untreated group (IU) wherein animals were infected with *S. haematobium* only and v) untreated control.

Screening of guinea pig's urine for *S. haematobium* eggs: After 10 weeks prepatent period, urine samples of guinea pigs were collected daily from various groups for additional 4 weeks. This was achieved by keeping them in a suspended iron cages with a plastic container under for urine collection. The urine collected from various groups was screened for *S. haematobium* eggs as per Niels *et al.* (1984). The number of eggs recovered daily in the various groups were counted using a light microscope with 10-40x magnification and recorded. Mean of three accepted egg counts were used for the various groups daily. The percentage reduction in egg load was calculated using the formula:

$$\frac{\text{Egg load of infected untreated} - \text{Egg load of infected and treated}}{\text{Egg load of infected untreated}} \times 100$$

Recovery of adult worms from guinea pigs: After 4 weeks urine collection period, animals were sacrificed by intra-peritoneal injection of Nembutal solution. Worms were recovered from the venules of their urinary bladders by perfusion technique (Niels *et al.,* 1984). The worms were counted and separated according to their sexes using 10-40x microscope magnification.

The effects of *A. sativum* aqueous extracts on worm burden were compared using a one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The treatment of infected animal with *A. sativum* aqueous extracts and praziquantel drug inflicted drastic reduction in egg load of *S. haematobium* in comparison to control (IU). The reduction was 89.6, 81.7, and 92.4% in CG, PG and DC groups, respectively (Fig. 1). The *S. haematobium* eggs recovered in various groups in 1st week after 10 weeks prepatent period when treatment commenced in some groups (CG, PG and DC) included 84.2 ± 8.7 for IU, 28.2 ± 29.8 for CG, 7.5 ± 2.9 for PG and 29.3 ± 26.9 for DC (Table 1). When IU group was compared with CG, PG and DC groups using multiple comparisons the difference were significant ($p<0.05$); however, the differences between CG, PG and DC were non-significant.

During 2nd week of treatment, the eggs recovered were 82.5±6.98 from IU, 5.2±5.0 from CG, 10.0±1.4 and 1.0±0.89 from DC group and these values significantly ($p<0.001$) differed from IU group

Table 1: Mean values of eggs recovered from various groups during 4 weeks treatment after 10 weeks prepatent period

Weeks	Infected, untreated group (IU)	Chemotherapeutic group (CG)	Prophylactic group (PG)	Praziquantel treated group (DC)
1	84.17 ± 8.64	28.17 ± 29.80	7.50 ± 2.88	29.33 ± 26.94
2	82.50 ± 6.98	5.17 ± 5.00	10.00 ± 1.41	1.00 ± 0.89
3	62.33 ± 16.72	1.33 ± 1.211	6.33 ± 1.63	0.50 ± 0.55
4	57.33 ± 7.92	1.67 ± 1.37	0.83 ± 0.75	0.83 ± 0.75

Table 2: Mean worm burden of *S. haematobium* in guinea pigs in different groups; IU, CG, PG and DC

Variables	Infected, untreated group (IU)	Chemotherapeutic group (CG)	Prophylactic group (PG)	Praziquantel treated group (DC)
Mean worm burden	59.17± 38.83	6.33 ± 4.32*	14.17± 6.49*	4.67± 4.54*

but were at par with one other (Table 1). The eggs recovered from the urine samples during 3rd week of treatment were 62.33 ±16.72 for IU, 1.33 ±1.21 for CG, 6.33 ±1.63 for PG and 0.50 ± 0.55 for DC which significantly differed from IU but were at par with one another. Similarly, on 4th week the eggs recovered from urine sample were 57.33 ± 7.97 for IU, 1.67 ± 1.37 for CG, 0.83 ± 0.75 for PG and 0.83 ± 0.75 for DC which were statistically at par but varied from infected and untreated control group (IU) ($p<0.001$). The worm burden of the experimental animals in the various groups examined was 6.33 ± 4.32 for CG, 14.17 ± 6.49 for PG, 4.67 ±4.54 for DC and 59.17 ± 38.83 for infected and untreated group (IU) (Table 2).

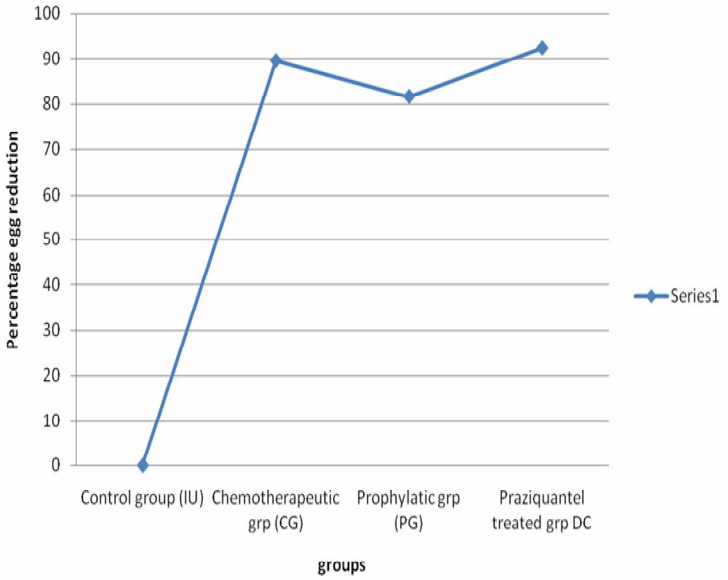

Fig. 1: Percentage reduction of *Schistosoma haematobium* eggs in guinea pigs in different groups; IU, CG, PG and DC

The current research established *S. haematobium* infection in immunosuppressed guinea pigs and does not support views of Vianna (1958) who reported that human is the only reservoir hosts of *S. haematobium*. In present study the treatment of infected guinea pigs with *A. sativum* aqueous extracts significantly reduced the worm load. *A. sativum* reportedly has anthelmintic potency in *Hymenolepis nana* (Soffar and Mokhtar, 1991; Streliaeva *et al*., 2000). Our study confirms the schistosomicidal effect of *A. sativum* previously reported by Riad *et al*. (2007), El Shenaway *et al.* (2008) and Nahed *et al.* (2009). However, Sutton and Haik (1999) have reported that the antiparasitic mode of action of *A. sativum* is not by pharmacological elimination of parasite, but by enhancing the host immunity to attack the parasite. El Shenaway *et al.* (2008) observed the antioxidative properties of aqueous *A. sativum* extracts in *Schistosoma* infected mice.

Analysis of variance indicates that statistical comparison of worm burden of any of CG and PG with IU was significant ($p< 0.01$) which is in compliance with the work of Nahed *et al.* (2009). Also comparing DC with IU showed that the difference between them was highly significant ($p<0.01$). Different observations were made when CG and PG groups, treated with *A. sativum* aqueous extracts over different periods, were compared together or in comparison to DC group. Analysis of variance showed that the difference between any of the groups were non-significant ($p> 0.05$).

Remarkable decrements in urine egg count were observed in all *A. sativum* treated groups. The finding is supported by Riad *et al.* (2007) who noticed significant reduction in egg load on treating *S. mansoni*-infected mice with aqueous *A. sativum* extract in acute and early chronic stages. The aqueous *A. sativum* extracts impaired the development and maturity of *Schistosoma* eggs, as evidenced by the appearance of high number of dead eggs in oogram assessment (El Shenaway *et al.*, 2008). Our observations are in agreement with Nahed *et al*. (2009) who noticed a significant reduction in tissue egg count of *S. mansoni* infected mice. This is possibly due to a positive linear relationship between egg output and worm burden, where reduction in the number of worms is correlated with the reduction in ova count (Gryseels and Polderman, 1991). In general, the antischistosomal effects of *A. sativum* can be correlated with its immunomodulatory effect as *A. sativum* inhibits the formation of inflammatory

compounds (Hodge *et al.*, 2002). The tumour necrosis factor-alpha (TNF-α) is one of the pro-inflammatory cytokines known to kill schistosomula in high concentrations and limit hepatocellular damage in response to schistosome eggs by stimulating granulomas formation. However, it has led to reduction of schistosome viability in the portal system and reduction of the amount of eggs laid by female worms (Davies *et al.*, 2004). From present investigation it appears that treatment of guinea pigs with *A. sativum* aqueous extracts before infection gives better results than giving *A. sativum* post-infection. Treatment of *A. sativum* weeks after prepatent confers protection against parasite. However, the *A. sativum* treated groups (CG) were at par with PG group, while the difference between any of the *A. sativum* treated groups and the praziquantel treated group were non-significant.

Conclusion: This present study suggests that under proper controlled immunosuppression with prednisolone, guinea pigs are susceptible to *S. haematobium* infection. *A. sativum* treatment significantly evoked reduction in egg load and worm burden thereby revealing *A. sativum* aqueous extract to be a convenient prophylactic and a promising therapeutic agent for the control of *Schistosoma haematobium* infection.

Ethics statement: The study and collection of urine sample were approved by the Ethics Committee, Federal Ministry of Health, Nigeria and Ebonyi State Ministry of Health. All animal experimental procedures were performed in accordance with the International Guidelines for Laboratory Animal Use and Care (Nahed, *et al.* 2009).

Competing interests: The authors declare that they have no competing interests.

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