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Effects of garlic on albino mice experimentally infected with *Schistosoma mansoni*: A parasitological and ultrastructural study

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Abstract. Due to increasing problems of the resistance associated with praziquantel, the drug of choice for treatment of schistosomiasis, alternative therapies are being sought. Scientific interest in the healing power of garlic has resulted in thousands of published papers on the ability of garlic to maintain good health. The current study was designed to evaluate the prophylactic and therapeutic values of garlic treatment against *Schistosoma mansoni.* Albino mice were infected with *S. mansoni* cercariae and were classified into: (a) treated with garlic before infection (prophylactic group), (b) treated with garlic after infection (therapeutic group), (c) treated with garlic before and after infection; (d) infected non-treated (control) group. Seven weeks post-infection, all mice were necropsied, and their livers and ilea were obtained for parasitological assessments. Schistosomes recovered from all groups were processed for ultrastructural investigations.

Garlic treatment significantly evoked a reduction in the egg and worm burden. Garlic also resulted in various ultrastructural alterations in the tegument of the surviving worms including tubercular disruption, oedema, blebbing, ulcers, and vacuolization of other tegumental structures. Our findings suggest that garlic is a convenient prophylactic and a promising therapeutic agent for *schistosomiasis mansoni* infection.

INTRODUCTION

Schistosomiasis is considered the most important of the human helminthiasis in terms of morbidity and mortality (Copeland *et al.*, 2003). 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). There are limited options available for the chemotherapeutic treatment of schistosomiasis with the drug of choice still being praziquantel (WHO, 1999). However, after some 30 years of praziquantel usage, a decreased susceptibility to the drug and the emergence of drug-resistant strains of schistosomes have been observed in several countries (Ismail *et al.*, 1999; King *et al.*, 2000; Botros *et al.*, 2005).

Because of the great need to develop new antischistosomal agents, trials were designed to test the potency of traditional medicinal plants for treating schistosomiasis. Ancient Egyptians realized the benefits of garlic as a remedy for a variety of ailments. In recent times, garlic has been shown to have multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, hypoglycemic and antitumor activities (Thomson & Ali, 2003). Lately, the anthelmintic effect of garlic has been a matter of interest of researchers (Soffar & Mokhtar, 1991; Abdel-Rahman *et al.*, 1998; Sutton & Haik, 1999; Streliaeva *et al.*, 2000; Ayaz *et al.*, 2008). However, research was mostly confined to fight intestinal parasites. Thus, this work was carried out to assess the effect and potency

of garlic in treating *Schistosoma mansoni*, a blood vascular system helminth.

MATERIAL AND METHODS

Forty naïve adult male Swiss albino mice (CD1 strain), weighing approximately 20 grams, were purchased from the Schistosome Biological Supply Programme (SBSP) unit at Theodore Bilharz Research Institute (TBRI), Giza, Egypt. The animals were transported to the animal care facility of the Zoology Department, Faculty of Science, Ain Shams University, one week prior to the initiation of the experiments for acclimatisation to the laboratory conditions. Mice were housed in polypropylene cages at 25 ± 2°C with 12 h/12 h light/dark cycle, and were given access to water and standard rodent food pellets *ad libitum*.

Garlic was intragastrically administered, as crude juice, day after day, in a dose of 50 mg/kg body weight/mouse. The crude extract was prepared as follows: Garlic bulbs were separated, peeled, and washed with distilled water. After drying in a shed, about 500 g of garlic bulbs were crushed in a blender until a uniform consistency was achieved. The resulting paste was diluted with distilled water to obtain a 1 g/ mL aqueous solution. Raw garlic juice was placed in 1.5 mL tubes and stored in a -20°C freezer. Working solution (50 mg/kg body weight) was made from the stock solution by dilution with distilled water. The dose selected for the present work is equivalent to the daily amount of garlic recommended for an average human to maintain good health (~ 4 gm).

The cercariae used in this study are of the Egyptian strain of *Schistosoma mansoni*, shed from laboratory-bred infected *Biomphalaria alexandrina* snails at SBSP, TBRI, Giza, Egypt. Mice were subcutaneously infected with ~200 cercariae each, according to Peters & Warren (1969).

Mice were divided into four groups and treated as follows: (a) Infected control-mice infected and given distilled water, day after day, from the 1st day of infection till the end of the 7th week post-infection; (b) Prophylactic group-mice treated with garlic for a week before infection; (c) Therapeutic group-mice treated with garlic from the 1st week of infection till the end of the 7th week post-infection; (d) Continuously-treated group-mice treated with garlic one week prior to infection till the end of the 7th week post-infection.

Seven weeks after cercarial exposure, all mice were necropsied, and worms were recovered from the portal system and mesenteric veins by perfusion technique according to Smithers & Terry (1965). The worms were counted and sexed, and adult male worms were prepared for scanning and transmission electron microscopic inspection. The egg burden of the liver and ileum was assessed according to Cheever (1968).

Numerical data were expressed as mean ± standard error. The significance of the interrelation of the treated groups to the control were tested using One-Way Analysis of Variance (GraphPad Prism, version 3.00 for Windows, GraphPad software, San Diego, CA, USA). Values were considered significant when $P \leq 0.05$, and highly significant when $P \leq 0.001$.

RESULTS

Statistical analysis of the obtained data, presented in Table 1, showed that the application of garlic evoked a highly significant reduction ($P \leq 0.001$) in the mean worm count as compared to the infected non-treated mice. This reduction was 67.2%, 56.3%, and 77.5% in the prophylactic, therapeutic, and the group treated before and after infection, respectively. Moreover, the mean egg load in hepatic and ileal tissues of all garlic-treated groups dropped extremely beyond the control levels. The reduction in the number of egg/g liver and ileum reached 82.8% and 81.3% ($P \leq 0.001$) in the prophylactic group, 81.7% and 78.1% ($P \leq 0.001$) in the therapeutic group, and 91% and 89% ($P \leq 0.001$) in the group treated pre- and post-infection.

The ultrastructural features of the tegument and subtegumental structures of

Table 1. Worm and egg burden of *Schistosoma mansoni* harboured in mice under different garlic-treatment regimens

Group	Total worm burden	Total worm reduction (%)	Egg burden/ gliver	Hepatic egg reduction (%)	Egg burden/ gileum	Ileal egg reduction (%)
Infected Group	92.2 ± 6.9	—	37,701 ± 3014	—	47,731 ± 3379	—
Prophylactic Group	30.2 ± 4.2*	67.2	6,472 ± 1439*	82.8	8,934 ± 2294*	81.3
Therapeutic Group	40.3 ± 7.7*	56.3	6,891 ± 1459*	81.7	10,469 ± 974*	78.1
Continuous Group	20.7 ± 3.1*	77.5	3,430 ± 593*	91	5,239 ± 1472*	89

Asterisks indicate the significant difference of treated groups versus control group within the same column, * $P \leq 0.001$.

S. mansoni adult males, recovered from untreated mice, are presented in Figs. 1a, 2a, 3a, 4a & 5a. These control specimens revealed no apparent damage and confirmed with the description of the worms by Hockley (1973) and Machado-Silva *et al.* (1997) in a similar study.

Treating mice with garlic resulted in a variety of changes in the structure of the tegument of the worms that survived the effect of garlic and developed into adults. The range and extent of these changes were not significantly different among the three treated groups.

Scanning electron microscopy of garlic-treated groups revealed that the numerical and volume density of the tubercles and spines were reduced (Figs 1b & 1c). In some specimens, the tubercles were disrupted and retracted and the spines became short and few (Fig. 1b). Other tegumental alterations included blebbing, and tubercular fusion as seen in Figs. 1b & 1c. Nevertheless, the tegument of severely injured worms suffered from focal lesions and peeling, and possessed ruptured and pinched-off tubercles, exposing the underneath muscles (Fig. 1c).

With regard to transmission electron microscopy, the main injury observed in the tegumental integrity of male worms was the widespread vacuolization. The outer membrane expanded to form deep and tortuous ramifications, leading to the increase in the number of the surface pits (Fig. 3b). Further and ensuing the degeneration of the syncytial matrix and the extensive swelling of the inner membrane folds, countless large vacuoles appeared (Figs. 3b & 4b).

Owing to the rigorous swelling of the tegument and due to the diminution of the size of the spines, some spines were completely surrounded by the tegumental matrix (Figs. 3b & 4b). In those specimens, where swelling of the tegument surface was extensive, such protuberance was accompanied by an increase in the width and loosening of the matrix (Fig. 4b). Alongside the swelling, many of the male worms had thin-walled evaginations of the outer surface of the tegument filled with lucent material (blebs) (Fig. 2b). Concerning the tubercles, loss of most of the spines, increase of lipid globules, emergence of myelin-like bodies, and degenerative vacuoles were also noticed (Fig. 2b).

Most parenchymal cells suffered from necrosis, indicated by their shrinkage, increased electron-density, and absence of their nuclei. Furthermore, the mesenchymal matrix endured widespread vacuolative degeneration (Fig. 3b). On the other hand, the injury assaulting the tegument-forming cells was the cytoplasmic lysis. Additionally, and other than the disappearance of the nucleoli and insignificant dilatation of the nuclear membrane, the nuclei of the tegument cells did not show any sign of injury (Fig. 5b).

DISCUSSION

The search for bioactive plants which can be used as non-conventional anthelmintics has received considerable attention in recent times because of the increasing, worldwide development of resistance to chemical anthelmintics in worm populations.

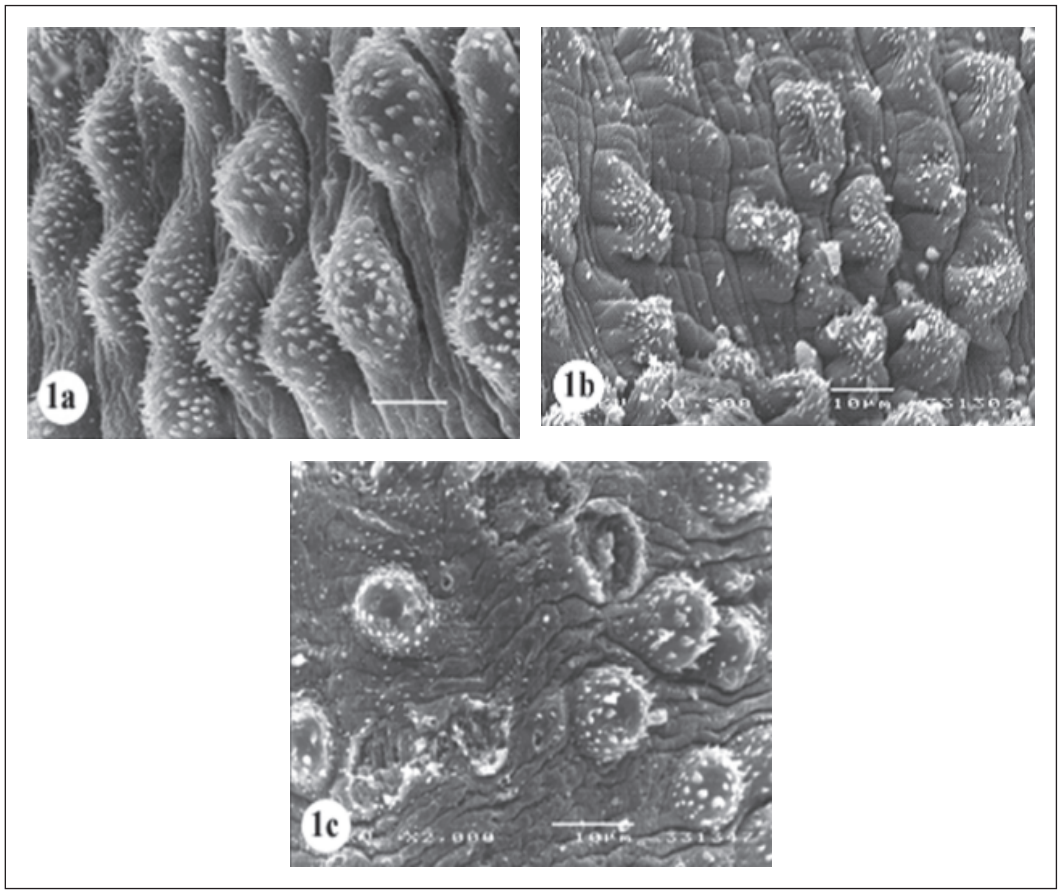

Figure 1. Scanning electron micrographs of the dorsal surface of adult male *Schistosoma mansoni*. (a) Control, showing normal tubercles. (b) Garlic-treated, showing numerous blebs (arrowheads) on the retracted tubercles. (c) Garlic-treated, showing ruptured tubercles. Bar= 10 µm.

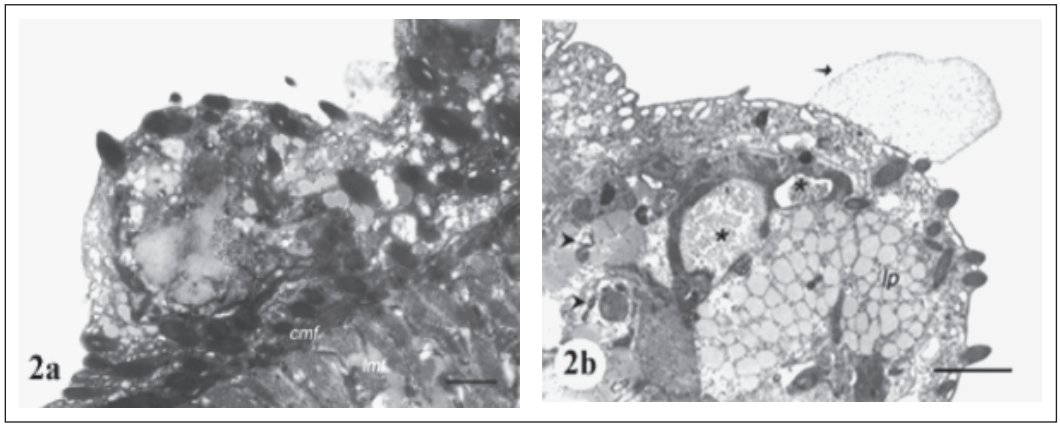

Figure 2. Transmission electron micrographs of the dorsal tubercles. (a) Control. Bar=4 ìm. (b) Garlic-treated, showing tubercle with few spines, blebs (arrow), lysis (asterisks), lipid droplets (lp) and myelin figures (arrowheads). Bar=3 µm.

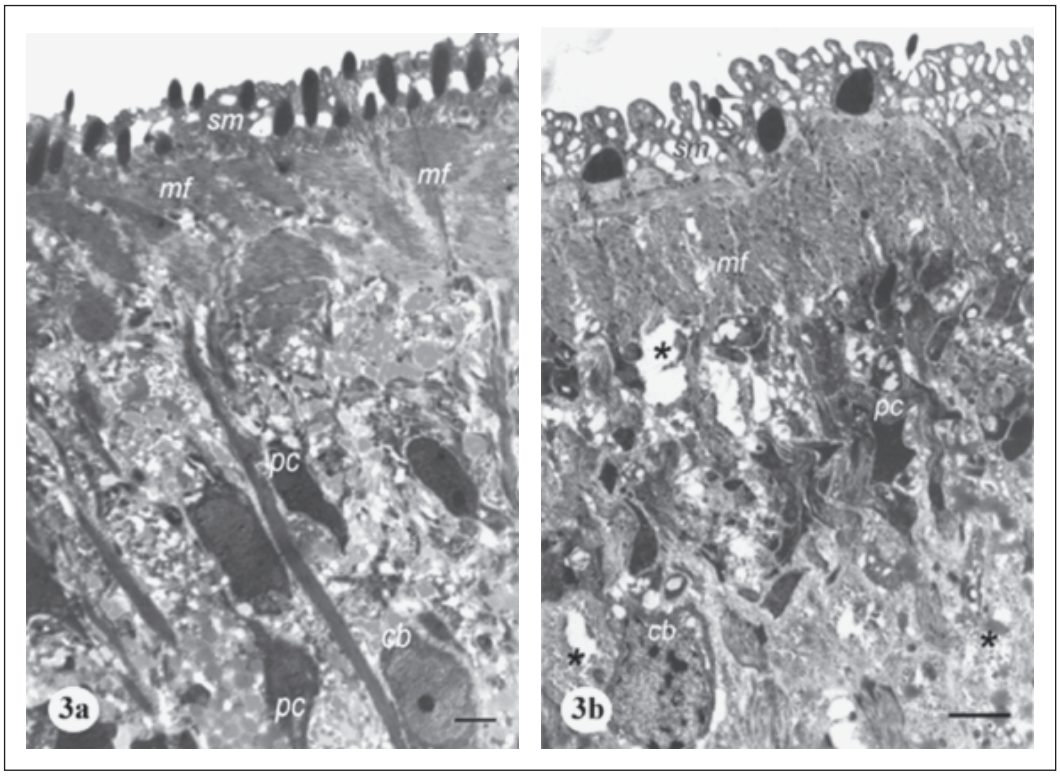

Figure 3. Transmission electron micrographs of the dorsal tegument and sub-tegumental structures. (a) Control. (b) Garlic-treated, showing disruption of tegument. sm, syncytial matrix; mf, muscle fibers; pc, parenchymal cells; cb, cell bodies of tegument-forming cells; asterisks, mesenchymal vacoulation. Bar=2 µm.

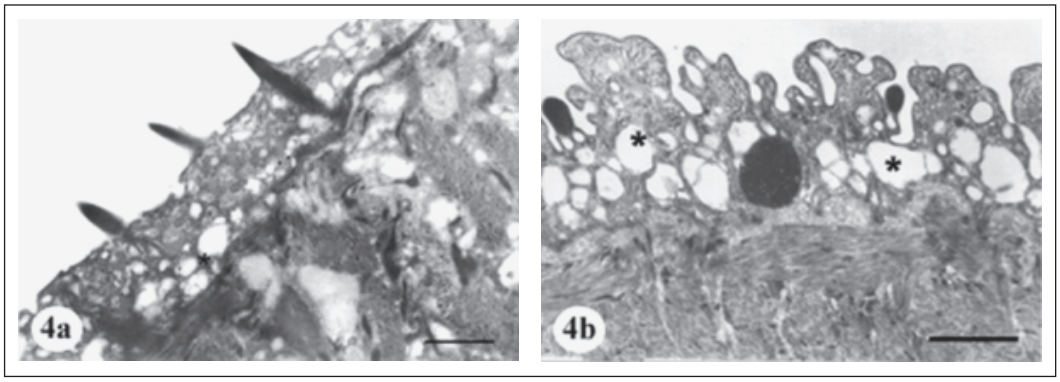

Figure 4. Magnification of the syncytial matrix. (a) Control. (b) Garlic-treated, showing swelling and vacuolation of the matrix (asterisks). Bar=2 µm.

However, scientific evidence to validate the use of plants remains limited (Hoste *et al.*, 2008). Thus, this study was oriented to evaluate the protective and curative capacity of garlic against *S. mansoni* through different means. The assessment of the influence of garlic administration on infected mice has been attained by comparing the results of treated infected mice to that of the corresponding non-infected (control) mice.

The results obtained here showed that treating infected mice with garlic evoked a significant reduction in the worm load. Garlic is reported to have an anthelmintic potency

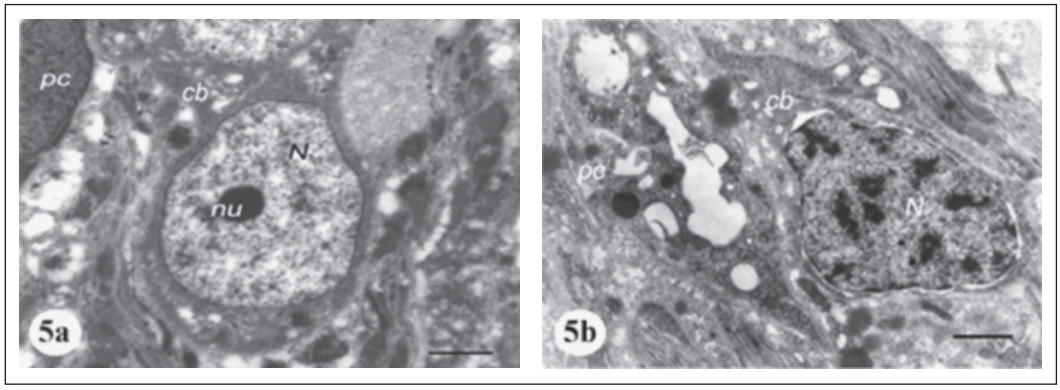

Figure 5. Magnification of the tegumental cells (a) Control. (b) Garlic-treated, showing necrotic parenchymal cells (pc) and cell bodies of tegument-forming cells (cb). Bar=1 µm.

as proved by the work of Soffar & Mokhtar (1991) and Streliaeva *et al*. (2000), who studied the effect of garlic on *Hymenolepis nana*. Moreover, our results confirm the schistosomicidal effect of garlic, previously reported by Riad *et al*. (2007) and El Shenaway *et al.* (2008). However, Sutton & Haik (1999) stated that the antiparasitic mode of action of garlic is not by pharmacological elimination of the parasite, but it is rather by enhancing the immunity of the host to attack the parasite. This assumption was verified by the biochemical results of the work of El Shenaway *et al.* (2008) on the antioxidative properties of aqueous garlic extract in *Schistosoma*-infected mice.

Concurrently, remarkable decrements in the tissue egg count were observed in all garlic-treated groups. Similar findings where obtained by Riad *et al.* (2007), who reported a significant reduction in the egg load after treating infected mice with aqueous garlic extract in acute and early chronic stages. El Shenaway *et al.* (2008) stated that aqueous garlic extract impaired the development and maturity of *Schistosoma* eggs, as the treatment resulted in the appearance of high numbers of dead eggs in the oogram assessment. This is possibly due to a positive linear relationship between the egg output and the worm burden, where the reduction of the number of worms is correlated with the reduction in the ova count. This is also suggested by Gryseels & Polderman (1991). However, several other factors may explain such reduction in schistosomal egg count. These factors are a probable diminished fecundity of the worm pairs and an increased rate of egg excretion due to the egg death.

In general, the antischistosomal effect of garlic could be correlated with its immunomodulatory effect, as garlic is recognized to inhibit the formation of inflammatory compounds (Hodge *et al.*, 2002). The tumour necrosis factor-alpha (TNF-α) is one of the proinflammatory cytokines, which is known to kill schistosomula in high concentrations, and to limit hepatocellular damage in response to schistosome eggs by stimulating granuloma formation. However, it has been implicated in the maintenance of schistosomes viability in the portal system (Davies *et al.*, 2004), and the induction of egg laying by female worms (Haseeb *et al*., 2001). Thus, the viability and fecundity of schistosomes is most likely affected by garlic, since garlic decreases TNF-α as stated by Hodge *et al*. (2002).

From the current parasitological results, it seems that treating mice with garlic before infection brings about better results than giving garlic post-infection. Garlic efficacy was highest in the group treated with garlic before and after bilharzial infection. However, the statistical difference between the three treated groups was not significant.

On the other hand, the importance of studying the tegument of schistosomes arises because it acts as an interface between the parasite and its environment in the host. This interface which is used to

evade the immune responses of the host also has sensory, secretory, and absorptive functions (Xiao *et al.*, 2002). Thus, a comprehensive knowledge of tegumental components would be helpful in the development of new drugs.

Many workers have documented the ultrastructural alterations encompassing *S. mansoni* as a result of using antibilharzial drugs, such as: Amoscanate (Voge & Bueding, 1980); Oxamniquine (Kohn *et al.*, 1982; Popiel & Erasmus, 1984; Amin & Mikhail, 1989; Fallon *et al.*, 1996), and Praziquantel (Mehlhorn *et al.*, 1981; Shaw & Erasmus, 1983, 1987; Shaw, 1990; Modha *et al.*, 1990; Staudt *et al.*, 1992; Fallon *et al.*, 1996; Liang *et al.*, 2002; Mohamed *et al.*, 2006).

In fact, the tegumental alterations in most of the previous studies were more pronounced in the male tegument than that of the female. This might be explained by the fact that the female is not usually in direct contact with the microenvironment of the host (Mostafa & Soliman, 2002). Therefore, the present study examined the surface topography of only male worms.

In the current investigation, garlic administration has been found to cause various changes to the tegument of the male schistosomes. The tubercles of the worms recovered after treatment were reduced in number, disrupted, and retracted. Tubercle distortions may result in the inability of the worm to adhere to the walls of the host blood vessels, and this will almost certainly cause the schistosome to be dislodged and moved with the blood stream from the mesenteric veins to the portal vein and intravenous hepatic capillaries, and become lodged in the liver (Mehlhorn *et al.*, 1981). In the hepatic blood vessels, the worms will be trapped and encapsulated with fibrous matrix (da-Silva & Noel, 1990).

Unfortunately, no information is available concerning the effect of garlic on tegumental ultrastructure of schistosomes. Anyhow, Amin & Mikhail (1989), Mostafa & Soliman (2002), Shaohong *et al.* (2006) and Taha & Soliman (2007) used different antischistosomal drugs and reported the tuberclar disruption and the loss of tuberclar spines in their work.

In this study, the most prominent damage observed in the tegument of garlic-treated worms was in the form of oedema and blebbing. The formation of membranous whorls and blebs is a common feature on the tegument of worms treated with other schistosomicides (Voge & Bueding, 1980; Mehlhorn *et al.*, 1981; Kohn *et al.*, 1982; Amin & Mikhail, 1989; Shaw & Erasmus, 1983, 1987; Fallon *et al.*, 1996; Mostafa & Soliman, 2002; Shaohong *et al.*, 2006; Taha & Soliman, 2007). Nonetheless, comparable symptoms were also observed in immune-damaged worms (Hockley, 1973) and in worms maintained under a variety of unfavourable *in vitro* conditions (Wilson & Barnes, 1974). The production of blebs and membranous whorls is regarded to be an emergency repair mechanism utilized by the worms to replace damaged surface membranes (Shaw & Erasmus, 1983).

In addition to the topographic lesions discussed above, worms recovered from garlic-treated mice also suffered from ulcers. This conforms to the results reported by Voge & Bueding (1980), Mostafa & Soliman (2002), and Shaohong *et al.* (2006), in their studies testing various antibilharzial compounds on *S. mansoni*. Such peeling is claimed to result from extensive vacuolation in the tegumental syncytium (Becker *et al.*, 1980). Another explanation given by Modha *et al.* (1990), is that, tegumental damage results from the explosion of the blebs or protuberances due to the treatment.

The main damage seen in the TEM is the extensive swelling of the basal infoldings and vacuolization of various tegumental structures after garlic administration. Vacuolization of the tegument was observed as an effect of several chemotherapeutic agents as seen in other studies (Mehlhorn *et al.*, 1981; Kohn *et al.*, 1982; Shaw & Erasmus, 1983, 1987; Popiel & Erasmus, 1984; Shaw, 1990; Xiao *et al.*, 2002; Taha, 2007). Shaw & Erasmus (1983) assumed that the vacuolation and the loss of tegumental matrix are because of the formation of blebs.

Apart from causing vacuolation and disruption of the tegumental matrix, garlic caused lytic degeneration to the tegumental cells and necrosis in the parenchymal cells. Similar results were obtained under the influence of antibilharzial agents studied by Shaw & Erasmus (1983 & 1987), Xiao *et al.* (2002) and Taha (2007).

From the above discussion it seems that schistosomal tegument react in a classic mode under the influence of different schistosomicides, irrespective of their modes of actions. No matter what the mechanisms of these antischistosomals are, the alterations on the tegument will lead to the disappearance of the immunological disguise of the worm. Thus, it could be easily attacked by the host's immune system (Xiao *et al.*, 2001). In addition, tegumental changes will impair the absorptive functions of the tegument, as schistosomes are known to be avid glucose consumers, and glucose particles are mostly absorbed via the tegument. Therefore, such tegumental alterations induced by garlic could probably be exerting a profound effect on the worm's metabolism and consequently resulting in their death. Similar results were seen in the current study as evidenced by the reduced worm burden in all garlic-treated groups. Another *in vitro* study on the cestode, *H. nana* using garlic extract has also shown similar lethal effects on the worm (Soffar & Mokhtar, 1991).

From our study it could be deduced that the effect of garlic is more pronounced on the developing worms than on the mature ones. This conclusion seems sensible, since the schistosome tegumental changes in the present study were nearly the same in all garlic-treated groups, though using different treatment regimens.

Based on our results it can be concluded that garlic has good efficacy in reducing the severity of murine schistosomiasis. The data also point to garlic, at its chosen dose (50 mg/kg body weight), as a convenient prophylactic and a promising therapeutic agent for this disease. Therefore it is recommended that further investigations be carried out on the applications of garlic as a complement to praziquantel in the treatment of schistosomiasis.

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