Ultrastructure alterations of adult male *Schistosoma mansoni* harbored in albino mice treated with Sidr honey and/or *Nigella sativa* oil

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Abstract In this investigation, the effects of Sidr honey, *Nigella sativa* oil (black-seed oil) and Sidr honey and black-seed oil together on the ultrastructure of tegument, gastrodermis and testes of adult male *Schistosoma mansoni* harbored in albino mice were studied. The tegument of adult male *S. mansoni* recovered from mice treated with various types of treatments revealed apparent damages which were severely clear in male worms harbored in mice treated with Sidr honey and black-seed oil together. On the other hand, the gastrodermis and testes of adult male *S. mansoni* obtained from mice treated with Sidr honey only or black-seed only revealed no apparent damage, while the gastrodermis and testes of worms recovered from mice treated with Sidr honey and black-seed oil together showed severe disorganization. Therefore, these treatments may represent a promising alternative treatment for the control of schistosomiasis mansoni, especially in endemic areas and where drug-resistant strains are found. These natural products may be recommended as useful, pleasant and popular accepted elements of food and drinks in such cases.

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1. Introduction

Schistosomiasis continues to occupy the second position in the world among parasites, after malaria, in the term of the extent of endemic areas and number of people infected. Two drugs have been widely used in the treatment of schistosomiasis with good efficacy and low toxicity: oxamniquine and praziquantel (Lescano et al., 2004). Meanwhile, repeated chemotherapy in endemic areas has resulted in the emergence of drug-resistant schistosome strains (Ismail et al., 1999; Kenworthy et al., 2003; Lawn et al., 2003).
The development of such resistance has drawn the attention of many authors to alternative drugs. Many medicinal plants were studied to investigate their antischistosomal potency and found to be effective, like: plant quinghao Artemisia annia (Abdel Aziz and El-Badawy, 2000), garlic Allium sativum (Zakhary, 1994; Riad et al., 2007, 2008), wild carrots Daucus carota (Shalaby et al., 1999), jungle weed Combretum sp. (McGaw et al., 2001), myrrh Commiphora molmol (Massoud, 1999), ginger Zingiber officinalis (Sanderson et al., 2002) and hound’s berry or night shade Solanum nigrum (Ahmed and Rifaat, 2005).

The crude oil of Nigella sativa (black-seed oil) is one of the promising alternative drugs of plant origin that have an antischistosomal effects (EL-Qadri and Emara, 1994; Mostafa, 2001; Mostafa and Soliman, 2002; Mahmoud et al., 2002; Tantawi and Mostafa, 2003; Ahamed and Mostafa, 2003; Mohamed et al., 2005).

The use of honey as a medicine eventually came to exist only in folk medicine but now has been redefined into modem medicine (Al-Wali, 2003). Some researchers began to document the medical properties of honey. The antimicrobial properties of honey may render it beneficial in the treatment of various oral ailments including periodontal disease and mouth ulcers (Molan, 2001; Engeseth et al., 2002; English et al., 2004). In the field of parasitological studies, Zeina et al. (1997) investigated the activities of honey dilutions against three species of Leishmania; their results were compared with the effects of the same concentrations of sugar; honey and sugar both have anti-leishmanial effects in vitro, but the results indicated that honey is superior to sugar. Mostafa (2003) studied the effects of Sidr honey alone, black-seed oil alone and Sidr honey and black-seed oil together on schistosomiasis mansoni in albino mice; his scanning electron microscopical observations revealed that the surface of male worms obtained from mice treated with Sidr honey alone showed extensive loss of spines. Moreover, the worm recovery and the eggs density were lowered in mice treated with Sidr honey.

Alterations in the surface ultrastructure of schistosome worms were used by several investigators for the evaluation of antischistosomal drugs (Mohamed and Fawzi, 1997; El-Sayed and Allam, 1997; Fawzi, 1999; Mostafa and Soliman, 2002; Jiraungkoorskul et al., 2005; Mostafa, 2005). Moreover, the alteration caused by antischistosomal drugs was more pronounced in the male tegument than in that of the female (Shaw and Erasmus, 1987; Shalaby et al., 1991). However, drug related testis alterations of schistosomes and other helminthes have received little attention (Bang and Hairston, 1946; Khayyal, 1964; Molokhia and Smith, 1968; Stammers, 1975; Otubanjo, 1981; Iriz et al., 1989; Basch and Clemens, 1989; You et al., 1992; Mohamed, 1999; Fawzi et al., 2001).

On the other hand, the schistosome digestive tract has been more or less ignored in several investigatory efforts (Senft, 1969; Erasmus, 1977); however, there have been a few reports on drug action relative to this system. For instance, Yarinsky et al. (1970), Bogitsch (1975) and Clarkson and Erasmus (1984) have shown that either in vitro or in vivo administration of the drugs astiban, hycanthone, lucanthone and niridizole can precipitate morphological changes in the gastrodermis of schistosomes similar to changes resulting from starvation.

This work was aimed to study the ultrastructure alterations in the tegument, gastrodermis and testes of adult male Schisto-
soma mansoni harbored in albino mice treated with Sidr honey and/or black-seed oil.

2. Materials and methods

2.1. Infection of mice

S. mansoni cercariae and clean CD1 male albino mice were supplied by the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Male CD1 albino mice were infected with 100 S. mansoni cercariae via subcutaneous route. The ethical obligations to experimental animals were followed. The experiments were carried out at Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

2.2. Treatment of mice

Mice were treated orally with pure Sidr honey at a dose of 0.5 ml/mouse and/or N. sativa oil (black-seed oil) at a dose 250 µl/kg body weight, day after day from the first day of infection till the end of the 7th week post-infection. Pure Sidr honey was purchased from Al-Yahya Bees Farms, Abha, Asser, Saudi Arabia. Sidr honey obtained from bees collected nectar of Zizyphus spina-christi flowers. The plant Z. spina-christi is of the Rhamnaceae family, and it grows wild in the mountains of Asser district in southwestern of Saudi Arabia. Black-seed oil is available in drug stores in gelatin capsules under the name of Baraka produced by Pharco Pharmaceuticals, Alexandria, Egypt.

2.3. Experimental design

This study was carried out on 40 mice divided into four groups, 10 mice each: first group was infected with S. mansoni cercariae and non-treated; second group was infected with S. mansoni cercariae and treated with Sidr honey only; third group was infected with S. mansoni cercariae and treated with black-seed oil only and fourth group was infected with S. mansoni cercariae and treated with Sidr honey and black-seed oil together. All mice were sacrificed at the end of the 7th week of the experiment (starting from the day of infection).

2.4. Worm recovery

The recovery of S. mansoni worms from the hepatic portal system and mesenteric veins of sacrificed mice was done by the perfusion technique described by Smithers and Terry (1965).

2.5. Transmission electron microscopical techniques

Worms were fixed in 4% glutaraldehyde in sodium cacodylate buffer for 2 h and washed in the same buffer at a pH of 7.4. Post-fixation was performed with osmium tetroxide in sodium cacodylate buffer. Specimens were dehydrated in graded ethanol before embedding in Spur resin. Ultrathin sections of the male worms were stained with uranyl acetate and lead citrate and examined with JOEL-1200EX2 electron microscope.
3. Results

3.1. Tegument

Tegumental ultrastructure features of adult male *Schistosoma mansoni* recovered from infected, non-treated mice revealed no apparent damage, showing a superficial syncytial layer forming tubercles with evident surface pits leading to branched intercommunicating channels. The syncytial layer was externally covered by an outer tegumental membrane studded with electron dense tiny discoid bodies. The spines appeared as large densely osmophilic more or less triangular bodies. Underneath the syncytial layer there is an intact layer of longitudinally and transversely cut muscle fibers and tegumental cells (Fig. 1).

The tegument of adult male *S. mansoni* recovered from mice treated with various types of treatment revealed apparent damages which were severely appeared in worms harbored in mice treated with Sidr honey and black-seed oil together. The tegumental features of adult male *S. mansoni* recovered from infected mice treated with Sidr honey only showed disappearance of most tegumental spines but the muscle layer appeared normal. The cytoplasm of the tegumental cell was vacuolated and the secretory granules were decreased tremendously; in addition the cells showed moderately degeneration in the cytoplasm and nuclear materials (Figs. 2–4).

The tegumental surface of adult male *S. mansoni* recovered from infected mice treated with black-seed oil only, showed loss of spines in tegumental tubercles, swelling of the muscle layer and vacuolated loose material appeared beneath the outer tegumental layer. The secretory granules greatly reduced. Some sensory papillae are sunken in the swelling tegument (Fig. 5).

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**Figure 1** Transmission electron micrograph (TEM) of adult male *Schistosoma mansoni* tegument recovered from infected, non-treated mice showing numerous spines covering the tubercles (Sp), small papilla with minute pore (P), circular muscle (Cm) and longitudinal muscle (Lm), parenchyma cell (Pc) with large nucleus (N) and nucleolus (Nu). Note also a canal (C) coming from a tegumental cell. Bar = 1 μm.

**Figure 2** TEM of the tegumental surface of adult male *Schistosoma mansoni* recovered from infected mice treated with Sidr honey showing blebs (B) on the outer surface of the tegument, numerous membrane bound vesicles (Mv) under outer tegumental surface (T) and rod shaped vesicles (Rv). Note swelling of circular (Cm) and longitudinal (Lm) muscle layers. Some muscle bands riddled with many vacuoles (arrows). Bar = 1 μm.

**Figure 3** TEM of the ventral side of tegumental surface of adult male *Schistosoma mansoni* recovered from infected mice treated with Sidr honey. The tegumental cells (Tc) showed tremendous vacuolation (V) in cytoplasm and nuclear material (N). The muscle layers (Cm and Lm) appear normal. Bar = 1 μm.
The tegumental surface of adult male *S. mansoni* recovered from infected mice treated with Sidr honey and black-seed oil showed loss of spines and swelling of the muscle layer. Distortion of the tegumental cells was observed, vacuolation was spread everywhere in subtegumental tissue and between muscle bundles. Secretory granules were disappeared completely (Figs. 6–9).
3.2. Gastrodermis

The gastrodermis of adult male *Schistosoma mansoni* recovered from infected, non-treated mice appeared as syncytial with normal nuclei and provided with numerous cytoplasmic extensions (Fig. 10). The gastrodermis of worms recovered from mice treated with Sidr honey only or black-seed only revealed no apparent damage, however the gastrodermis of worms recovered from mice treated with both Sidr honey and black-seed oil showing several disorganization. The first signs of anomaly appeared as blebbing of small components from the apical surface of the gastrodermis into the lumen and a dramatic decrease in the number of surface cytoplasmic extensions compared to control. Moreover, flattening of the gastrodermis, degeneration of endoplasmic reticulum and darkening of the cytoplasm were noticed (Fig. 11).

3.3. Testis

Ultrastructural examination of the testis of *S. mansoni* males recovered from infected mice treated with Sidr honey and black-seed oil, showing distortion of the tegumental cells (Tc), with highly vacuolated cytoplasm. Vacuolation spreads everywhere in subtegumental tissue and between muscle bundles (V). Bar = 1 µm.

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mice treated with Sidr honey and black-seed oil revealed several changes. The non-germinal cells exhibited different damage and various degree of degeneration. Their cytoplasm became dark and their nucleus appeared vacuolated while mitochondria appeared as dark bodies with indistinct cristae. Large intercellular spaces were noticed containing debris of degenerating cells. Membranous bodies and aggregation of lipid granules were also observed. Disruption of follicular wall and degeneration of circular muscles were observed (Fig. 13).

The spermatogonia appeared swelling developing large vacuoles. Chromatin patches disappeared and the nucleolus became condensed. Lipid granules, swelling mitochondria and whorled bodies were observed in the cytoplasm. Degenerating spermatogonia sometimes contained membrane whorls which lay directly below the cell surface and around other cytoplasmic organelles (Fig. 14).

Degenerative changes occurred in spermatocytes, a prolonged swelling and vacuolation was observed in cytoplasm and nucleus. The swollen nuclei lost their characteristic chromatin patches. Mitochondria exhibited varying morphological changes: some lost their cristae and appeared distended. In addition, some spermatocytes were completely disintegrated (Figs. 15 and 16). Rosette of early spermatids appeared pale with numerous mitochondria accumulated at nuclear anterior end. Lipid granules were observed in the cytoplasm. Nuclei became very pale with very few chromatin patches while some nuclei degenerated completely. Degeneration and regression of late spermatids were also noted in testicular follicles of treated worms and the cytoplasm develops vacuolation (Figs. 17 and 18). As to spermatozoa, some of them showed abnormal morphology and their nuclei lost characteristic electron-lucent areas (Fig. 19).

Figure 11 TEM of gastrodermis of adult male *Schistosoma mansoni* recovered from mice treated with Sidr honey and black-seed oil showing disorganized gastrodermis containing lipid droplets (L) and blebbing (B) of small components from the apical surface. Note extensively decreased cytoplasmic extensions (Ce). Bar = 1 µm.

Figure 12 TEM of a testis of adult male *Schistosoma mansoni* recovered from infected, non-treated mice showing germinal cells (G), non-germinal cells (Ng), nucleus (N), nucleolus (Nu), basal lamina (arrows) and circular muscle (Cm). Bar = 2 µm.

Figure 13 TEM of testis of adult male *Schistosoma mansoni* recovered from mice treated with Sidr honey and black-seed oil showing degeneration of non-germinal cells (Ng), aggregation of lipid granules (L). Note also disruption of follicular wall (arrows). Bar = 2 µm.
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Figure 14  TEM of testis of adult male *Schistosoma mansoni* recovered from mice treated with Sidr honey and black-seed oil showing disorganized testicular tissue. Note swollen spermatogonia (Sg) develop pronounced vacuolation (V), whorled bodies (Wb) swollen mitochondria (M), nucleus (N) and nucleolus (Nu). Bar = 500 nm.

Figure 15  TEM of testis of adult male *Schistosoma mansoni* recovered from mice treated with Sidr honey and black-seed oil showing degenerated spermatocyte (Sc) with abnormally vacuolated cytoplasm (V), nucleus (N), lipid granules (L), aggregation of dark material in cytoplasm (arrowheads). Note degenerated follicular wall and circular muscles (arrow) and some regressed spermatozoa (Sp). Bar = 1 μm.

Figure 16  TEM of testis of adult male *Schistosoma mansoni* recovered from mice treated with Sidr honey and black-seed oil showing another degenerated spermatocyte (Sc) with vacuolated cytoplasm (V). Note seriously degenerated nucleus (N). Bar = 1 μm.

Figure 17  TEM of testis of adult male *Schistosoma mansoni* recovered from mice treated with Sidr honey and black-seed oil showing rosette of early spermatids (St1), with pale cytoplasm containing numerous mitochondria, pale swollen nucleus (N) with nucleolus (Nu) and few chromatin patches. Note degenerated late spermatid (St2). Bar = 2 μm.
4. Discussion

Tegumental and subtegumental alterations induced by different types of treatment used in the present investigation were characterized by swelling, disappearance of spines, cytoplasm vacuolation, reduction or disappearance of secretory granules, degeneration in the cytoplasm and nuclear materials and sunken of some sensory papilla in the swelling tegument. However, the combined treatment was more severe in action on the tegument compared to single one. More or less similar changes were induced in response to different chemical drugs. Vogel and Bueding (1980) presented a detailed study on S. mansoni tegumental surface alterations induced by subcurative doses of the schistosomicide amoscanate. They observed swelling, wrinkling, constriction, collapse of sensory bulbs and erosion of large areas of the surface. Mehlhorn et al. (1981) reported that the primary effect of praziquantel that eventually lead to the death of S. mansoni was the disruption of the tegument of those parasites that are chemotherapeutically susceptible to praziquantel. Tegumental changes induced by oxamnique treatment of adult S. mansoni worms included marked oedema, wrinkling, distortion, complete disorganization of suckers, destruction of tubercles and collapse of sensory bulbs, as observed by Amin and Mikhail (1989). The tegument of S. mansoni was immediately destroyed after exposure in vitro to triclabendazole leading to the formation of defects in the surface of the worms (El-Sayed and Allam, 1997). Also, Ro15-5458 and R-354 were recorded to cause tegumental damage of S. mansoni in the form of vacuolation (Fawzi, 1999; Taha, 2007).

Kusel et al. (1989) reported some of the functions of glycoproteins in parasite surface: act as receptors for growth substances, as a physical or immunological barrier to cells and antibodies of the host immune system and maintain the structure of the surface membrane. Therefore, the tegumental changes induced by Sidr honey and/or black-seed oil in S. mansoni worms could have exerted a profound effect upon the metabolic activities of the parasite. Moreover, the alterations produced in the tegumental surface make the worms vulnerable to the host immune system and attacked by the host’s inflammatory cells. Mehlhorn et al. (1981) reported that after treatment of S. mansoni with praziquantel, leucocytes of the host attacked the damaged surface and penetrated to the interior tissues of the parasite. In addition, it is well known that male worms use tubercles and spines in holding to the wall of the blood capillaries. Since the treatment with Sidr honey and/or black-seed oil causes partial or complete destruction to these structures, the worms can be drifted with the blood stream.

The present finding coincide with the scanning electron microscopical observations that obtained by Mostafa (2005) who found that the surface topography of male worms obtained from mice treated with Sidr honey alone showed extensive loss of spines. The tegument of worms developed in mice treated with black-seed oil showed moderate structural changes, since the tubercles on the dorsal surface of the male showed partial loss of spines. However, the worms developed in mice treated with Sidr honey and black-seed oil together showed the greatest changes; they lost their normal surface architecture, and erosion of the tegument and spines loss were noted. In addition, the obtained results are in harmony with that observed by Mostafa and Soliman (2002) in their study on the surface topography of adult worms of S. mansoni harbored in albino mice treated with black-seed oil; they reported that the tubercles on the dorsal surface of the mature males developed in mice treated with black-seed oil from 0 day of infection showed extensive loss of spines. Spines may be partially or completely disappeared in some worms. Moreover, the size of the tubercles was greatly reduced. The inter-tubercle
tegmental regions showed extensive swelling (oedema) while erosion of the surface was observed.

Ultrasound changes in the gastrodermis following treatment with Sidr honey and black-seed oil may interfere with digestion and, consequently, have a detrimental effect on nutrient assimilation. The schistosome digestive tract has been more or less ignored in several ultrastructure investigations, although it long has been recognized, because of its role in the physiology and development of the organism (Senft, 1969; Erasmus, 1977). However, there have been a few reports on drug action relative to this organ appeared to be comparable with the present finding. For instance, Leitch and Probert (1990) observed a severe damage to the gastrodermis with the development of autophagic vacuoles containing whorls of myelin and sequestered portions of damaged tissue in S. haematobium recovered from hamsters treated with astiban. Moreover, focal and extensive lysis, decrease in granular endoplasmic reticulum, vacuolation and degeneration of mitochondria were observed in gut epithelium of S. haematobium harbored in mice following amoscanate (Leitch and Probert, 1984), and artermether administration (Xiao et al., 2002, 2006). The increase in number of gastrodermal vacuoles has been documented by Bogitsh (1975) and Clarkson and Erasmus (1984) and is considered to be consistent with changes occurring in the gastrodermis of S. mansoni when subjected to stress conditions, such as starvation or drug treatment, either in vitro or in vivo.

Various alterations in testicular structure of S. mansoni have been recorded in this study as a result of treatment with Sidr honey and black-seed oil. Such alterations included the disintegration of non-germinal cells, cellular swelling, distortion and disorganization of germ cells, vacuolation within the testis and regression of spermatocytes. Non-germinal cells were partially disintegrated in this investigation. Complete disintegration has been reported in the testis of S. mansoni after treatment with astiban (Otubanjo, 1981), Ro15-5458 (Mohamed, 1999) and Ro-354 (Soliman, 2008). Moreover, vacuolation of the non-germinal cells was reported in testis of S. haematobium treated with Ro15-5458 (Fawzi et al., 2001). Non-germinal cells of S. mansoni showed increased phagocytic activity in astiban-induced testicular damage (Otubanjo, 1981).

In this study, the germinal cells show swelling and contained very large vacuoles. Nucleus squeezed by these vacuoles. Chromatin patches disappeared and the nucleus became condensed. Cytoplasm developed lipid granules while mitochondrial abnormalities increased and appeared as dark bodies with indistinct cristae. Leitch and Probert (1984) similarly reported abnormal increase of mitochondria as evident after treatment of S. haematobium with amoscanate. Mohamed (1999) reported shrinkage of seminal cells leads to separation and intercellular spaces after Ro15-5458 administration in case of S. mansoni. Similarly, Fawzi et al. (2001) found that shrinkage and cell separation in S. haematobium. Jirungkoorskul et al. (2005) reported swelling of the testicular tissue followed by degeneration leaving several hollow spaces in Eurytrema pancreaticum after using PZQ and triclabendazole.

Vacuolation within the testis and regression of spermatocytes observed in this study are comparable to those recorded by Irie et al. (1989) in their work on S. mansoni treated with PZQ and dextro-PZQ, Mohamed (1999) and Soliman (2008) in their investigation on S. mansoni treated with Ro15-5458 and Ro-354. The anticancer drug procarbazine was found to be profoundly damaging to the primary and secondary spermatocytes and spermatids which were replaced by amorphous granular material (Basch and Clemens, 1989). Degeneration and atrophy of the testis of S. japonicum was also demonstrated by You et al. (1992) after artemether administration. Otubanjo (1981) mentioned that testicular disorganization was prominent initially in spermatozoa and spermatids but became more generalized with drug accumulation. Basch and Clemens (1989) reported disruption of meiotic process, spermatocytes and spermatids were destroyed and replaces by amorphous granular material in S. mansoni by procarbazine treatment.

In conclusion, it is obvious that Sidr honey, black-seed oil and Sidr honey and black-seed oil together had schistosomocidal effects upon the tegument of S. mansoni in albino mice, but treatment with Sidr honey and black-seed oil together was the most effective. On the other hand, Sidr honey alone or black-seed oil alone appeared non-effective against the gastrodermis and testes. The last two organs are affected only by treatment with Sidr honey and black-seed oil together. Therefore, these treatments may represent a promising alternative strategy for control of schistosomiasis mansoni, especially in endemic areas and where drug-resistant strains are found. These natural products may be recommended as useful, pleasant and popular accepted elements of food and drinks in such cases.

References


