Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.2.201414D107

Evaluation of the *in vivo* effect of ivermectin on *Schistosoma mansoni* in experimentally-infected mice

Amira Taman[1*], Samar El-Beshbishi[1], Nora El-Tantawy[1], Amira El-Hawary[2], Manar Azab[1]

[1]*Department of Medical Parasitology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt*

[2]*Department of Pathology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt*

ARTICLE INFO

Article history:
Received 2 Apr 2014
Received in revised form 20 Apr 2014
Accepted 10 May 2014
Available online 23 Sep 2014

Keywords:
Schistosoma mansoni
Ivermectin
Praziquantel
Scanning electron microscopy
Tegument
Glutamate

ABSTRACT

Objective: To evaluate the effect of ivermectin on mice experimentally infected with *Schistosoma mansoni*.
Methods: Ivermectin was given to mice 42 days *p.o.* in two treatment regimens: a single dose of 25 mg/kg or the same dose for two consecutive days.
Results: In both regimens, there were significant reductions in female worms, hepatic tissue egg count and early immature ova, in addition to significant reductions in the count and size of hepatic granuloma. The same dose for two consecutive days resulted in significant reductions in male, total worms, and intestinal tissue egg load. In both regimens, scanning electron microscopy revealed tegumental alternations as bleb formation, erosion and necrosis more evident with the double dose. In addition, high dose showed extensive erosions of male worms with destruction of the gynaecophoric canal. In females, marked destruction of the tegumental surface was extending to both ventral and oral suckers.
Conclusions: Ivermectin has promising anti-schistosomal effects. However, further research is needed to test the effect of ivermectin on schistosomiasis especially in combination with other antischistosomal agents, to avoid any possible resistance from monotherapy.

1. Introduction

Schistosomiasis, caused by the trematode flatworms of the genus *Schistosoma*, is one of the most neglected tropical diseases with major public health significance in terms of morbidity and mortality. Recent estimates indicate that more than 230 million people are affected in 76 countries[1], about 779 million are at risk of contracting the infection and 280 000 cases died in each year in sub-Saharan Africa because of schistosomiasis[2,3]. The burden of infection occurs in rural areas; however, with increased travelling, European and North American travellers are occasionally become infected. Five major species causing human schistosomiasis of which *Schistosoma mansoni* (*S. mansoni*), the aetiological agent of intestinal schistosomiasis, is endemic in 54 countries[4].

Currently, praziquantel is the only anti-schistosomal agent. Although it is safe and highly effective in a single dose regimen, the problem of worm resistance is a big threat, as low efficacy of praziquantel in the treatment of schistosomiasis has been reported in Senegal and Egypt[5,6].

No safe, effective alternative drug for treatment of schistosomiasis exists. Moreover, in spite of more than 40 years of research to develop a vaccine for schistosomes, no effective vaccine candidate exists, and praziquantel remains the mainstay for schistosomiasis control[7]. This makes the discovery and development of praziquantel alternatives is very important.

Great efforts by various research groups are going to develop novel anti-schistosomal agents either synthetic or from natural compounds. As a relatively time-saving and cost-effective approach, scientists started to test the available compounds in the market, which have some antiparasitic activities. Among them, the antimalarials

*Corresponding author: Amira Taman, PhD, Department of Medical Parasitology, Faculty of Medicine, Mansoura University, 2 El-Gomhouria Street, Mansoura 35516-Egypt.
Tel: +2-050-2244873
Fax: +2- 050-2263717
E-mail: amirataman@mans.edu.eg

artemether, mefloquine, and compound artemisinin-naphthoquine phosphate, have shown activity against schistosomiasis[8-10].

Recently, the compound BTP-Iso, a novel benzimidazole-derived compound, with well-known broad spectrum anthelmintics activity of this group of compounds, has also revealed anti-schistosomal effects[11].

Ivermectin is a semi-synthetic broad-spectrum antiparasitic drug with high efficacy and a wide margin of safety, affecting most of parasitic nematodes as filarial worms, *Ascaris*, *Enterobius*, and *Strongyloides*. Moreover, ivermectin affects different ectoparasites such as lice, mites, ticks, and fleas[12]. The effect of ivermectin on nematodes can be attributed to its strong agonist effect to muscular and neuronal glutamate-gated chloride channels[13-15]. Early researchers recorded the lack of efficacy of ivermectin on trematodes and cestodes[16-19]. However, a pronounced *in vitro* effect of ivermectin when incubated with the metacestode stage of the tapeworm *Echinococcus granulosus* (*E. granulosus*)[20]. Moreover, degenerative changes have been observed in the *E. granulosus* protoscoleces incubated with ivermectin in the form of tegumental blebbing, which revealed that the tegument is the primary site of damage in this parasite[21]. Similar tegumental changes have been recorded recently[22], when ivermectin was incubated with the trematode *Fasciola gigantica* (*F. gigantica*). These recent studies highlight the potential activity of ivermectin on trematodes and possibly on schistosomes.

This study was conducted to evaluate the possible antischistosomal effect of ivermectin against adult *S. mansoni* harboured in mice in terms of worm burden, oogram pattern, tissue egg load, granuloma measurement. In addition, we used scanning electron microscope (SEM) to study any alterations in the tegumental surface of schistosomes. To our knowledge this is the first experimental study to test the activity of ivermectin on *S. mansoni*.

2. Material and methods

2.1. Mice infection

Cercariae of *S. mansoni* Egyptian strain (CD) were obtained from infected *Biomphalaria alexandrina* snails after light exposure. Snails were purchased from Schistosome Biologic Supply Program (SBSP) at Theodor Bilharz Research Institute (Giza, Egypt).

Female BALB/c mice (age=6 weeks), obtained from the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, Mansoura, Egypt, weighing 25-30 g were used in the experiments. Each mouse was infected with 80±10 cercaria through subcutaneous injection. Mice were maintained under environmentally controlled conditions and fed on standard diet and normal drinking water.

All animal experiments in this study were carried at MERC, following the institutional and national animal welfare regulations.

2.2. Drug

Ivermectin (Sigma-Aldrich, USA) was dissolved in dimethyl sulfoxide with gentle heating in a water bath immediately before use.

2.3. Experimental design and mice treatment

The study was carried on 21 mice, randomly divided into three groups each with 7 mice: Group I was infected with *S. mansoni* cercaria and received the vehicle only (infected-untreated control), Group II was infected and treated with ivermectin in a single oral dose of 25 mg/kg and Group III was infected and treated with 25 mg/kg/day of ivermectin for two consecutive days.

All mice were deprived of food 3 h before drug or vehicle intake. The dosing protocols were administered by oral gavage using a ball-tipped feeding needle six weeks *p.o.* Mice were allowed to eat one hour after treatment. Two weeks post-treatment all animals were euthanized by *i.p.* injection of sodium thiopentone (100 mg/kg).

2.4. Parasitological studies (worm recovery, oogram pattern and tissues egg count)

The portal vein was perfused using citrated saline to recover adult worms. The collected worms were counted and sexed, to calculate the percentage of worm reduction[10]. For oogram studies, one hundred eggs per oogram were randomly chosen and classified as immature (stages I-IV), mature and dead, then the percentage of each stage was determined[11]. One gram of liver and intestine was incubated in 4% KOH at 37 °C for 18 h. The number of eggs was counted to calculate the number of eggs per gram of tissue[10].

2.5. Scanning electron microscopy (SEM)

Worms recovered from the three groups used in the study were washed with saline and incubated in 2.5% glutaraldehyde in phosphate-buffered saline (pH 7.4) for 24 h at 4 °C, then processed for examination by SEM, using JEOL, JSM-6510LV (USA).

2.6. Histopathological studies

Specimens of the liver were fixed in 10% formalin and processed into 4 μm thick sections. Later sections were

stained with haematoxylin and eosin (H&E), and examined under light microscope. Counting of the granuloma was conducted in five successive microscopic fields (×40) of serial tissue sections of more than 250 μm apart, and the average diameter of granulomata was calculated as well.

2.7. Statistical analysis

SPSS software version 16.0 was used for statistical comparison between infected treated groups and the infected untreated control group. A nonparametric Mann-Whitney *U* test was used to test for significant differences between groups. A *P* value>0.05 was considered statistically insignificant. The percentage of reduction between treated and untreated groups was assessed using the following formula: (mean value of untreated group-mean value of treated group)×100/mean value of untreated group. Descriptive statistics including the mean±SD were used.

3. Results

3.1. Parasitological studies

Ivermectin administered to mice 6 weeks *p.o.*, in a single oral dose of 25 mg/kg (Group II), resulting in significant reduction in female worms ($P<0.01$), but non-significant reduction ($P>0.05$) in male worms and total worms burdens compared with Group I (controls), with percentage reductions of 21.6%, 7.9%, and 7.6%, respectively (Table 1). However, ivermectin given for two consecutive days (Group III) showed statistically significant reductions in female worms, male worms and total worms ($P<0.01$), with reductions of 45.4%, 27.6%, and 28.8%, respectively, compared with controls (Table 1).

Table 1

Effects of ivermectin given 6 weeks *p.o.* on worm burden in mice infected with Egyptian strain of *S. mansoni*.

Animal groups	Male burden	Female burden	Total worms burden
Group I	18.1±2.9	13.9±2.0	37.7±6.8
Group II	16.7±1.8 (7.9)	10.9±1.2 (21.6**)	34.9±1.3 (7.6)
Group III	13.1±1.1 (27.6**)	7.6±2.6 (45.4**)	26.8±2.9 (28.8**)

Values are expressed as means±SD. Group I: infected-untreated mice; Group II and III: infected mice and treated with ivermectin at single oral dose of 25 mg/kg or the same dose for two consecutive days, respectively. Numbers between parentheses indicate the percentage of reduction compared with infected-untreated controls. **: Significant difference from control group at $P<0.01$.

Analysis of the oogram pattern of the treated groups revealed significant reduction ($P<0.01$) in immature eggs (especially early immature stages) in Groups II and III (19.4 % and 38.8%, respectively), and significant ($P<0.01$) increase in dead eggs in both groups, respectively (56.2% and 76.8%, respectively), compared with the infected-untreated controls. However, non-significant reduction in mature eggs in both treated groups ($P>0.05$), compared with the control group, was recorded (Figure 1).

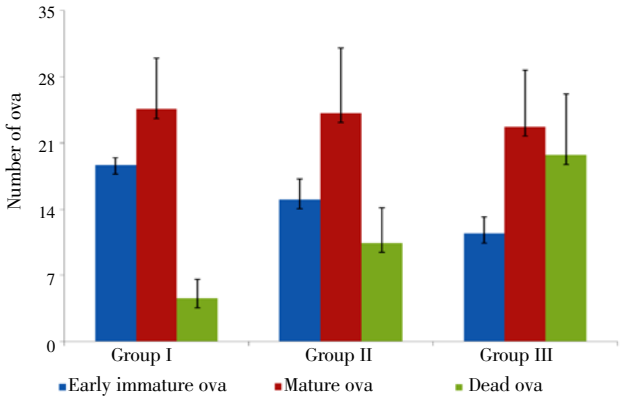

Figure 1. Oogram pattern in *S. mansoni*-infected mice under different treatment regimens of ivermectin.

Group I: Untreated control; Group II: Received 25 mg/kg ivermectin; Group III: Received 25 mg/kg ivermectin for two consecutive days.

Mean number of eggs per gram of intestinal and hepatic tissues was significantly reduced ($P<0.01$) in Group III compared with Group I, with percentage reductions of 21.0% and 27.4%, respectively. While Group II revealed non-significant reduction in the mean number of intestinal eggs, significant reduction ($P<0.01$) in hepatic eggs of 8.7% and 14.5%, respectively (Table 2).

Table 2

Effects of ivermectin given 6 weeks *p.o.* on tissue egg loads in mice infected with Egyptian strain of *S. mansoni*

Animal groups	Intestinal ova count (×10^3)	Hepatic ova count (×10^3)
Group I	36.0±4.3	46.4±3.0
Group II	32.9±3.1 (8.7)	39.7±2.4 (14.5**)
Group III	28.4±2.4 (21.0**)	33.7±5.9 (27.4**)

Values are expressed as means±SD. Group I: infected-untreated mice; Group II and III: infected mice and treated with ivermectin at single oral dose of 25 mg/kg or the same dose for two consecutive days, respectively. Numbers between parentheses indicate the percentage of reduction compared with infected-untreated controls. **: Significant difference from control group at $P<0.01$.

3.2. Results of SEM

To detect the possible effect of ivermectin on schistosomes' tegument, recovered worms were examined by SEM. The shape and appearance of untreated control worms of *S. mansoni* (Figure 2A), were similar to those previously described in the literature[23]. The morphological alterations of the tegument occurred in a dose-dependent manner and were more pronounced in female than in male adults. Male worms recovered from mice treated with a single oral dose of ivermectin showed tegumental damage as roughness of the surface, peeling and erosion with exposure of the basement membrane, reduced number and collapse of tubercles (Figure 2B and C) as well as shortened spines on tubercles (Figure 2C). Swelling of the tegumental ridges and vesicles formation were seen on the ventral surface of the worm next to the gynaecophoric canal (Figure 2D). No morphological alterations could be observed in the oral and ventral suckers of male worms except for minor erosions, emergence of few tiny vesicles, and some deformities of the ventral sucker (not shown). Ivermectin given in the high dose (25 mg/kg for two

consecutive days), resulting in extensive destruction of the male tegument in the form of peeling and erosions on the dorsal surface; exposing the basement membrane (Figure 3A), in addition to massive destruction of the ventral surface, damage and deformity of gynaecophoric canal (Figure 3B). Several vesicles were also seen in the gynaecophoric canal (Figure 3C). However, female worms had pronounced destructive changes all over the body with massive erosion and peeling of the oral sucker, ventral sucker, area between both suckers (Figure 3D) in addition to deformity of the ventral sucker and area below ventral sucker (Figure 3E).

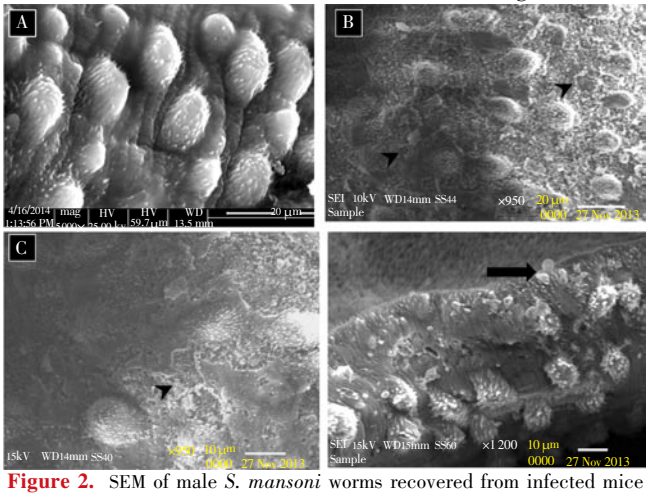

Figure 2. SEM of male *S. mansoni* worms recovered from infected mice treated with 25 mg/kg of ivermectin.

A is male control. B, C, and D are treated male worms with roughness of the surface, peeling, erosions (arrowheads) and collapsed tubercles with short spines (C). Swelling of the tegumental ridges and vesicles formation (arrows) on the ventral surface next to the gynaecophoric canal (D).

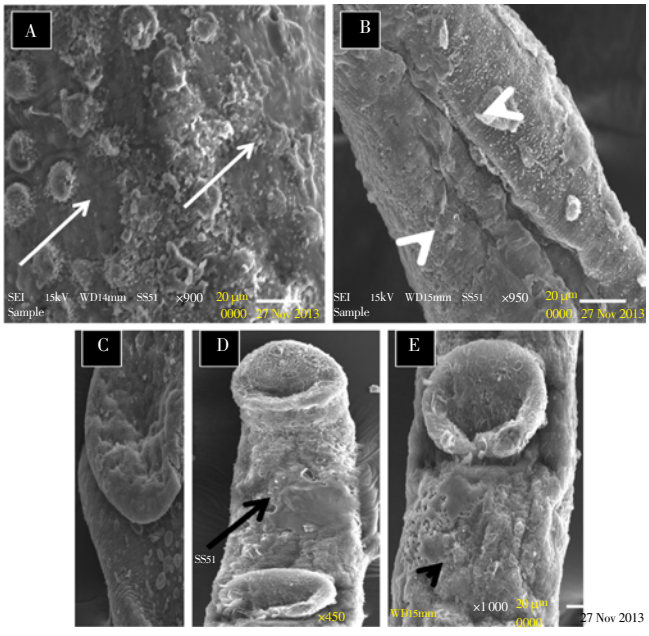

Figure 3. SEM of male and female worms recovered from infected mice treated with 25 mg/kg of ivermectin for two consecutive days.

A: Male worm showing extensive peeling, erosion, and exposure of the basement membrane (arrows). B: Damage of the male ventral surface extending to the gynaecophoric canal (arrowheads). C: Several vesicles are detected in the gynaecophoric canal (open arrow). D, E: Female worm with massive erosion and peeling on the oral sucker, ventral sucker, area between both suckers (black arrows) and below ventral sucker (arrowhead).

3.3. Histopathological studies

Histopathological examination of liver sections of untreated infected mice revealed marked inflammation of liver parenchyma, many eosinophilic abscesses and multiple cellular granuloma around mature ova (Figure 4A), with mean diameter of (858.6±52.1) µm and number of 15.6 ±1.3 (Table 3). Mice treated with single dose of ivermectin showed moderate parenchymal inflammation, some eosinophilic abscesses with significant reductions (P<0.01) in granuloma count (7.1±1.1) and diameter (480.7±60.6) µm (Figure 4B). While mice treated with two ivermectin doses, revealed less inflammatory reactions and small-sized granulomata in the liver parenchyma (Figure 4C) with significant high reductions in the granuloma number and diameter (P=0.001) of (5.0±1.3) and (425.0±17.1) µm, respectively, compared with the control group (Table 3).

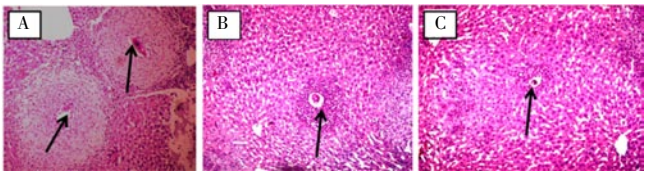

Figure 4. Effect of oral administration of ivermectin on hepatic granuloma diameter in mice infected with *S. mansoni* (Egyptian strain).

A: Untreated control; B: Received 25 mg/kg ivermectin; C: Received 25 mg/kg ivermectin for two consecutive days(H&E ×100). Arrow points to *Schistosoma* ova.

Table 3

Effects of ivermectin given 6 weeks *p.o.* on granuloma characteristics in mice infected with Egyptian strain of *S. mansoni*.

Animal groups	Granuloma count/40×	Reduction (%)	Granuloma diameter (µm)	Reduction (%)
Group I	15.6±1.3		858.6±52.1	
Group II	7.1±1.1	54.1**	480.7±60.6	44.0**
Group III	5.0±1.3	67.9**	425.0±17.1	50.5**

Data are presented as means ±SD. Group I: infected-untreated mice; Group II and III: infected mice and treated with Ivermectin at single oral dose of 25 mg/kg or the same dose for two consecutive days, respectively. **: Significantly different from control (P<0.01).

4. Discussion

Schistosomiasis is one of the most important parasitic diseases that continue to threaten millions of people in many tropical and subtropical countries[24]. The dependence on a single drug to treat people infected worldwide is a big threat, particularly when considering the emergence of praziquantel-resistant strains of schistosomes[6]. Accordingly, outstanding researches for the discovery and development of novel antischistosomal compounds are eminent to allow alternation of drug regimens and prevent or delay further development of resistant strains.

Despite being developed for veterinary uses, ivermectin is used to control several nematodes and arthropods,

which cause human diseases such as lymphatic filariasis, onchocerciasis, strongyloidosis, and scabies, respectively[12].

There are contradictory results about the effect of ivermectin on trematodes. Early studies reported that avermectins and milbemycins had no activity against *Fasciola hepatica* (*F. hepatica*) and *S. mansoni*[18,19]. However, another research reported that IVOMEC ®-F, which is composed mainly of ivermectin, reduced the fecal egg count by 98%, given to sheep infected by *F. hepatica*[25]. Recently, researchers preferred to use ivermectin in combination with other drugs to enhance/potentiate its effect on Fasciola[26,27].

This study is the first one to highlight the potential *in vivo* antischistosomal effect of ivermectin. Oral administration of a single dose of ivermectin at 25 mg/kg, resulted in significant reduction of 21.6% in female worms. While, administration of the same dose for two consecutive days, revealed reduction of 45.4%, 27.6%, and 28.8%, respectively, in female, male worms and total worm burdens, respectively compared to infected controls receiving the vehicle only. Worm reductions could be related to the tegumental damage found in some worms and verified by SEM. Reduction in female worms is of great significance, as compounds targeting female schistosomes will affect egg production, the main cause of disease morbidity[28], and hence lead to improve the clinical status or even treat infected individuals, and reduce disease transmission. Moreover, reduction of male worms is of importance since males are in frequent contact with the host microenvironment[29].

Previous research showed that a single dose of praziquantel at 300 mg/kg (approximately one third of the curative dose) resulted in 16.8% schistosomes' burden reduction[30]. This result illustrates that high dose of praziquantel (6 times that of ivermectin) remains less effective than lVM dose (25×2 mg/kg) used in our study.

Both regimens of ivermectin showed significant reduction in immature eggs (early stages) leading to reduction in tissue egg load. Reduction of ova and significant increase in the number of dead eggs indicates the strong ovicidal activity of ivermectin. These results are in agreement with previous studies reporting the *in vitro* and *in vivo* effects of ivermectin against *F. gigantica* and *F. hepatica*, respectively[22,26,27]. The decreased egg production could be attributed to the reduction in the number of the adult females or tegumental and suckers destruction that may interfere or even impair feeding in adult worms, which could affect egg-laying[31,32].

The SEM analysis revealed several morphological changes in adults worms recovered from mice treated with both regimens of ivermectin, which were more extensive with the double dose, particularly in female worms. Several agents exhibit their antischistosomal activity by targeting the tegument, as praziquantel[27], artemether[33], atorvastatin alone or in combination with medroxyprogesterone acetate[34], mefloquine[9,35], miltefosine[36], and recently the compound BTP-Iso, a novel benzimidazole-derived compound[11]. Likewise, tegumental alterations induced by ivermectin on adult schistosomes were similar to those observed for parasites incubated with the aforementioned compounds. In addition, the lethal effect of ivermectin on the cestode; *E. granulosus* and the trematode[21]; *F. gigantica* is mainly attributed to tegumental destruction[22]. This may suggest that the tegument is the possible target for ivermectin in platyhelminths.

The tegument has a vital role in schistosomes' nutrition, host immune evasion, and sensation[37]. Therefore, it is an ideal drug target since its destruction will expose the worm antigens to the host immune system for eventual destruction and elimination. Ivermectin in high dose showed tegumental disruption on the ventral surface of male worms with deformity of some parts of the gynaecophoric canal and formation of vesicles inside. Intimate contact between male and female schistosomes, which is achieved by female residing in the male gynaecophoric canal, is important for stimuli transfer from male to female. These signals are not only important for physical and sexual maturation of females but also to maintain their mature status[38]. Ivermectin in double dose affects integrity of the gynaecophoric canal and separation of female is a possible result. Separated mature females will suffer from regression of the reproductive organs for affecting egg production and development[39]. Additionally, females were more affected than males with extensive destruction of the oral and ventral suckers and area between them. The extensive effect of ivermectin on females than males might be attributed to the fact that male tegument is much thicker with highly folded surface and tubercles[38]. Damage of the suckers in females could result in loss of the ability to attach to the blood vessels and interfere with ingestion of nutrients from blood[40], which could be another possible mechanism of killing more females than males and reduced egg deposition in tissues.

One of the most important findings in our study has highly significant reduction in bilharzial granuloma count and size after both dosing protocols. This could be attributed to the reduction in the tissue egg load due to ivermectin effect. However, an immunomodulatory effect after treatment might be another possible cause as cytokines are believed to modulate the granuloma size and to play a fundamental role in the pathology of schistosome infection[41].

Herein as in previous studies[21,22], the tegument

is probably the primary target for ivermectin action. Macrocytic lactones such as ivermectin are potent agonist of nematode and arthropod glutamate-gated chloride channels (GluCl) and potentiate the effect of glutamate[42]. Glutamate signalling has been recorded previously on the surface of schistosomes[43,44], which supports the presence of ivermectin target in the tegument.

Our data encourage researchers to look for other uses of ivermectin other than being used to treat nematodes and arthropods causing human diseases. Interestingly, a recent work increases the possibility of using ivermectin in combination with other antimalarials compounds to control malaria caused by *Plasmodium falciparum* species[45].

Based on the outcome of this study, we could consider ivermectin as a potential antischistosomal agent due to its schistosomicidal activity on adult worms especially females and its ovicidal effect in addition to its potentiality in improving hepatic lesions. Further laboratory work is needed to elucidate the effect of ivermectin on different schistosomes' species, particularly if used in combination with other antischistosomal drugs such as praziquantel and artemether.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

The authors would like to express their gratitude to Prof. Mohamed A. Sobh, Director of the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, Egypt, for providing all facilities required for this work. We greatly acknowledge Dr. Sahar Hamed, Genetics Unit, Mansoura University Children Hospital, Faculty of Medicine, Mansoura University, and Dr. Yomna Khater, MERC, for assistance in animal experiments. We wish to thank Dr. Basem Nageeb, Electron Microscope Unite, Mansoura University for the technical assistance in SEM.

References

[1] Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet* 2006; **368**: 1106-1118.

[2] Utzinger J, Brattig NW, Kristensen TK. Schistosomiasis research in Africa: how the CONTRAST alliance made it happen. *Acta Trop* 2013; **128**: 182-195.

[3] Utzinger J, N'goran EK, Caffrey CR, Keiser J. From innovation to application: social-ecological context, diagnostics, drugs and integrated control of schistosomiasis. *Acta Trop* 2011; **120**(Suppl 1): S121-S137.

[4] Fallon PG, Doenhoff MJ. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. *Am J Trop Med Hyg* 1994; **51**: 83-88.

[5] Ismail M, Botros S, Metwally A, William S, Farghally A, Tao LF, et al. Resistance to praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. *Am J Trop Med Hyg* 1999; **60**: 932-935.

[6] Coeli R, Baba EH, Araujo N, Coelho PM, Oliveira G. Praziquantel treatment decreases *Schistosoma mansoni* genetic diversity in experimental infections. *PLoS Negl Trop Dis* 2013; **7**: e2596.

[7] McWilliam HE, Driguez P, Piedrafita D, McManus DP, Meeusen EN. Novel immunomic technologies for schistosome vaccine development. *Parasite Immunol* 2012; **34**: 276-284.

[8] Utzinger J, Xiao SH, Tanner M, Keiser J. Artemisinins for schistosomiasis and beyond. *Curr Opin Investig Drugs* 2007; **8**: 105-116.

[9] Manneck T, Haggenmüller Y, Keiser J. Morphological effects and tegumental alterations induced by mefloquine on schistosomula and adult flukes of *Schistosoma mansoni*. *Parasitology* 2010; **137**: 85-98.

[10] El-Beshbishi SN, Taman A, El-Malky M, Azab MS, El-Hawary AK, El-Tantawy DA. First insight into the effect of single oral dose therapy with artemisinin-naphthoquine phosphate combination in a mouse model of *Schistosoma mansoni* infection. *Int J Parasitol* 2013; **43**: 521-530.

[11] El-Bialy SA, Taman A, El-Beshbishi SN, Mansour B, El-Malky M, Bayoumi W, et al. Effect of a novel benzimidazole derivative in experimental *Schistosoma mansoni* infection. *Parasitol Res* 2013; **112**: 4221-4229.

[12] Gunning K, Pippitt K, Kiraly B, Sayler M. Pediculosis and scabies: a treatment update. *Am Fam Physician* 2012; **86**: 535-541.

[13] Martin RJ. Modes of action of anthelmintic drugs. *Vet J* 1997; **154**: 11-34.

[14] Wolstenholme AJ. Glutamate-gated Cl-channels in *Caenorhabditis elegans* and parasitic nematodes. *Biochem Soc Trans* 1997; **25**: 830-834.

[15] Dent JA, Smith MM, Vassilatis DK, Avery L. The genetics of ivermectin resistance in *Caenorhabditis elegans*. *Proc Nat Acad Sci* 2000; **97**: 2674-2679.

[16] Campbell WC, Fisher MH, Stapley EO, Albers-Schonberg G, Jacob TA. ivermectin: a potent new antiparasitic agent. *Science* 1983; **221**: 823-828.

[17] Whitworth JAG, Morgan D, Gilbert CE, Mabey DM, Maude GH, Taylor DW. Effects of repeated doses of ivermectin on ocular onchocerciasis: community-based trial in Sierra Leone. *Lancet* 1991; **338**: 1100-1103.

[18] Whitworth JAG, Morgan D, Maude GH, McNicholas AM, Taylor DW. A field study of the effect of ivermectin on intestinal helminths in man. *Trans R Soc Trop Med Hyg* 1991; **85**: 232-234.

[19] Shoop WL, Ostlind DA, Rohrer SP, Mickle G, Haines HW, Michael BF, et al. Avermectins and milbemycins against *Fasciola hepatica*: *in vivo* drug efficacy and *in vitro* receptor binding. *Int J Parasitol* 1995; **25**: 923-927.

[20] Casado N, Rodriguez-Caabeiro F, Jiménez A, Criado A, de Armas C. *In vitro* effects of levamisole and ivermectin against *Echinococcus granulosus* protoscoleces. *Int J Parasitol* 1989; **19**: 945-947.

[21] Elissondo MC, Ceballos L, Alvarez L, Bruni SS, Lanusse C, Denegri G. Flubendazole and ivermectin *in vitro* combination therapy produces a marked effect on *Echinococcus granulosus* protoscoleces and metacestodes. *Parasitol Res* 2009; **105**: 835-842.

[22] Diab TM, Mansour HH, Mahmoud SS. *Fasciola gigantica:* parasitological and scanning electron microscopy study of the *in vitro* effects of ivermectin and/or artemether. *Exp Parasitol* 2010; **124**: 279-284.

[23] Miller FH Jr, Tulloch GS, Kuntz RE. Scanning electron microscopy of integumental surface of *Schistosoma mansoni*. *J Parasitol* 1972; **58**: 693-698.

[24] King CH. Parasites and poverty: the case of schistosomiasis. *Acta Trop* 2010; **113**: 95-104.

[25] Ĉorba J, Várady M, Prasličkа J, Tomašovičová O. Efficacy of IVOMEC (R)-F against naturally acquired gastrointestinal worms, lung worms (*Dictyocalus filaria*) and liver flukes (*Fasciola hepatica*) in sheep. *Helminthologia* 1996; **33**: 133-135.

[26] Hanna REB, Cromie L, Taylor SM, Couper A. The effect of a parenteral ivermectin/closantel injection on the growth and reproductive development of early immature *Fasciola hepatica* in cattle. *Vet Parasitol* 2006; **142**: 78-90.

[27] Borgsteede FH, Taylor SM, Gaasenbeek CP, Couper A, Cromie L. The efficacy of an ivermectin/closantel injection against experimentally induced infections and field infections with gastrointestinal nematodes and liver fluke in cattle. *Vet Parasitol* 2008; **155**: 235-241.

[28] Fahel JS, Macedo GC, Pinheiro CS, Caliari MV, Oliveira SC. IPSE/alpha-1 of *Schistosoma mansoni* egg induces enlargement of granuloma but does not alter Th2 balance after infection. *Parasite Immunol* 2010; **32**: 345-353.

[29] Riad NH, Taha HA, Mahmoud YI. Effects of garlic on albino mice experimentally infected with *Schistosoma mansoni*: a parasitological and ultrastructural study. *Trop Biomed* 2009; **26**: 40-50.

[30] Botros S, Soliman A, El-Gawhary N, Selim M, Guirguis N. Effect of combined low dose praziquantel and oxamniquine on different stages of schistosome maturity. *Trans R Soc Trop Med Hyg* 1989; **83**: 86-89.

[31] Barth LR, Fernandes APM, Rodrigues V. Oviposition by *Schistosoma mansoni* during *in vitro* cultivation. *Rev Inst Med Trop Sao Paulo* 1996; **38**: 423-426.

[32] Barnes TM, Hekimi S. The *Caenorhabditis elegans* avermectin resistance and anesthetic response gene unc-9 encodes a member of a protein family implicated in electrical coupling of excitable cells. *J Neurochem* 1997; **69**: 2251-2260.

[33] Shuhua X, Binggui S, Chollet J, Utzinger Jr, Tanner M. Tegumental changes in adult *Schistosoma mansoni* harbored in mice treated with artemether. *J Parasitol* 2000; **86**: 1125-1132.

[34] Soliman MF, Ibrahim MM. Antischistosomal action of atorvastatin alone and concurrently with medroxyprogesterone acetate on *Schistosoma haematobium* harboured in hamster: surface ultrastructure and parasitological study. *Acta Trop* 2005; **93**: 1-9.

[35] Xiao SH, Mei JY, Jiao PY. Effect of mefloquine administered orally at single, multiple, or combined with artemether, artesunate, or praziquantel in treatment of mice infected with *Schistosoma* japonicum. *Parasitol Res* 2011; **108**: 399-406.

[36] Eissa MM, El-Azzouni MZ, Amer EI, Baddour NM. Miltefosine, a promising novel agent for schistosomiasis mansoni. *Int J Parasitol* 2011; **41**: 235-242.

[37] Faghiri Z, Skelly PJ. The role of tegumental aquaporin from the human parasitic worm, *Schistosoma mansoni*, in osmoregulation and drug uptake. *The FASEB J* 2009; **23**: 2780-2789.

[38] Beckmann S, Quack T, Burmeister C, Buro C, Long T, Dissous C, et al. *Schistosoma mansoni*: Signal transduction processes during the development of the reproductive organs. *Parasitology* 2010; **137**: 497-520.

[39] Beckmann S, Quack T, Dissous C, Cailliau K, Lang G, Grevelding CG. Discovery of platyhelminth-specific α/β-integrin families and evidence for their role in reproduction in *Schistosoma mansoni*. *PLoS One* 2012; **7**: e52519.

[40] Walker AJ. Insights into the functional biology of schistosomes. *Parasit Vectors* 2011; **4**: 203.

[41] Aly IR, Hendawy MA, Ali E, Hassan E, Nosseir MM. Immunological and parasitological parameters after treatment with dexamethasone in murine *Schistosoma mansoni*. *Mem Inst Oswaldo Cruz* 2012; **105**: 729-735.

[42] Lynagh T, Lynch JW. Ivermectin binding sites in human and invertebrate Cys-loop receptors. *Trends Pharmacol Sci* 2012; **33**: 432-441.

[43] Mendonça-Silva DL, Pessôa RF, Noël F. Evidence for the presence of glutamatergic receptors in adult *Schistosoma mansoni*. *Biochem Pharmacol* 2002; **64**: 1337-1344.

[44] Taman A, Ribeiro P. Characterization of a truncated metabotropic glutamate receptor in a primitive metazoan, the parasitic flatworm *Schistosoma mansoni*. *PLoS One* 2011; **6**: e27119.

[45] Panchal M, Rawat K, Kumar G, Kibria KM, Singh S, Kalamuddin M, et al. *Plasmodium falciparum* signal recognition particle components and anti-parasitic effect of ivermectin in blocking nucleo-cytoplasmic shuttling of SRP. *Cell Death Dis* 2014; **5**: e994.