academicJournals

Vol. 9(6), pp. 394-403, 11 February, 2015
DOI: 10.5897/AJMR2014.7214
Article Number: 3782DA950576
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antischistosomal impact of Albendazole and Nitazode on *Schistosoma mansoni* larval stages

Gehan L. El-Enain[1,2*], Sharaf, H.M[3] and Abd El-Atti M. S[3]

[1]Department of Parasitology, Theodor Bilharz Research Institute, Egypt.
[2]UC Abu Dhabi, University, United Arab of Emirates (UAE).
[3]Department of Zoology, Faculty of Science, Zagazig University, Egypt.

Received 26 October, 2014; Accepted 19 January, 2015

Albendazole and Nitazode were tested as molluscicidal agents against *Biomphalaria alexandrina* snails. The effect of Albendazole and Nitazode on snails infected with *Schistosoma mansoni* miracidia and hermaphrodite glands of *Biomphalaria alexandrina* snails were also carried out. In addition, the parasitological parameters, the dynamics of serum-specific immunoglobulins and splenic cytokines associated with changes in granuloma diameter were assessed. The results indicate that exposure of *B. alexandrina* to Albendazole and Nitazode, resulted in a considerable reduction in the infectivity of *S. mansoni* miracidia to the snails and a severe damage in the hermaphrodite gland cells of treated snails. In addition, immunization did not affect worm reduction, but a slight decrease in granuloma diameter, increase in immunoglobulins and cytokines was observed. Reduction in worm burden was associated with a reduction in ova count. Changes in oogram pattern were mainly due to Albendazole and Nitazode. In conclusion, treatment with Albendazole and Nitazode with immunization resulted in significant reduction of parasitological parameters and rise of specific immunoglobulins.

Key words: *Biomphalaria alexandrina*, Albendazole (ABZ), Nitazode (NTZ), cytokines, immunoglobulins.

INTRODUCTION

Control of snail intermediate hosts remains an essential component of integrated schistosomiasis control programs (El-Emam et al., 1990; Bakry, 2009a). Snail control strategies are considered a priority of the reduction of schistosomiasis transmission. Although the use of molluscicides has always been considered a major supportive procedure in integrated schistosomiasis control, yet, there are gaining increased attention for newly molluscicides, as they may be highly effective, rapidly biodegradable, less expensive than synthetic molluscicides and probably easily applicable with simple techniques (El-Khoby et al., 1998).

Schistosomiasis is a tropical disease that remains endemic in about 75 countries worldwide (Chitsulo et al, 2004; Gryseels et al., 2006). By using mass chemotherapy and molluscicides, efforts are being made to limit

*Corresponding author. E-mail: zan196022@yahoo.com.

disease transmission in some of these countries.

Schistosomal pathology is a direct consequence of theimmunological response to ovideposition in host tissue especially liver. Liver injury is typically associated with infiltration of inflammatory cells, leading to fibrosis (Friedman, 2003). Various investigators have focused on the protective immunization against schistoromiasis using several soluble egg antigens (SEA) fractions which were identified and tested in experimental models with the induction of variable levels of protection against infection (Tendler et al., 1996). Immunization of mice stimulates specific immunity which causes reduction in worm burden, intestinal egg load and liver pathology (Romeih et al., 2008; Garcia et al., 2008). Until recently, none of immunizing fractions was able to induce more than 67% protection, but the existence of at least partially protective immunity would make a logical complement to drug therapy (Bergquist et al., 2008; Maher et al., 2003).

The benzimidazole compound Albendazole (ABZ) has a fasciolicidal effect when administered orally. The effective quantity of the active compound in the oral application in sheep was found to be 20 mg Albendazole sulphoxide per kg bodyweight. Although ABZ is only effective against adult flukes, it is a broad-spectrum nematodicidal compound with well-known ovicidal activity (Alvarez et al., 2009).

Nitazode (NTZ) is a new thiazolide antiparasitic agent that shows excellent *in vitro* activity against a wide variety of protozoa and helminths (Fox and Saravolatz, 2005). It is given by the oral route with good bioavailability and is well tolerated, with primarily mild gastrointestinal side effects. Nitazode has been licensed for the treatment of *Giardia intestinalis*–induced diarrhea in children (Gilles and Hoffman, 2002). It is also recommended for treatment of diarrhea due to *Cryptosporidium* species in children and adults (Rossignol et al., 2001), and for use in treating immunocompromised hosts (Abboud et al., 2001). It represents an important addition to the antiparasitic arsenal.

The present work aims to evaluate the effect of Albendazole and Nitazode on infection of *Biomphalaria alexandrina* snails with *Schistosoma mansoni* and the histological effects on the snail's hermaphrodite gland. In addition, parasitological parameters and the dynamics of serum-specific immnoglobulins and splenic cytokines associated with changes in hepatic pathogenesis and granuloma diameter were assessed in an attempt to study the effect of treatment with Albendazole and Nitazode on immunized infected mice model.

MATERIALS AND METHODS

Snails

Laboratory bred *B. alexandrina* snails (5-6 mm in shell diameter) were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI).

Schistosoma mansoni ova

They were obtained from Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute (TBRI).

Animals

Mice (six to eight weeks old), (18 - 20 g) were bred and maintained at Schistosome Biology Supply Center (SBSC) of Theodor Bilharz Research Institute (TBRI) and kept under standard housing conditions. The animal experiments were been carried out according to the internationally valid guidelines in an institution responsible for animal ethics (TBRI) (Nessim et al., 2000).

Drugs

Albendazole was obtained as 5% suspension from the Egyptian Company. For chemicals and pharmaceuticals (ADWIA, 10 th of Ramadan City), each ml contains 50 mg Albendazole. The sulphoxide was not used for Albendazole. A dose of 140 mg/kg three times/week was administered until the day of sacrifice.

Nitazode was obtained as suspension produced by Sigma in pharmaceutical industries for Al Andalous Medical Company ARE. Each ml suspension contains 20 mg Nitazode. Nitazode (nitazoxamide) or Clos-Atak (closantel) in addition to Albendazole as drug control, were prepared and added to worms in culture plates. The experimental design included 16 groups; each drug was studied in five groups for the five doses in addition to control untreated worms. A dose of 140 mg / kg three times/week was administered until the day of sacrifice.

Preparation of *Shistosoma mansoni* soluble egg antigen (SEA)

SEA was prepared (Carter and Colley, 1978) and purified from host antigen by affinity chromatography using cyanogens bromide activated Sepharose-4B beads (Nordon and Strand, 1984). SEA was sterilized by filtration and protein content was estimated using Bio-Rad kit (Bradford, 1976).

Biological study

Molluscicidal screening

A series of concentrations was prepared from each experimental plant powder on the basis of weight/volume as water suspensions. Another series of concentrations from cold water extract, boiled water extract or organic solvent extracts was prepared (WHO, 1965). For each experimental concentration, three replicates were prepared; each of 10 snails/L. Another three replicates were prepared in dechlorinated water as a control. Exposure and recovery periods were 24 h each. Mortality rates were recorded and corrected according to Abbot's formula (1925), then the SPSS computer program under windows, Litchfield and Wilcoxon (1949) and Finny (1971) methods were used to calculate LC_{50} pen and LC_{90} ppm values.

Effect of LC_{25} from Albendazole and Nitazode on infectivity of *S. mansoni* miracidia to *B. alexandrina* snails

The first group of snails (5 - 6 mm) were simultaneously exposed for 24 h to LC_{25} from Albendazole and miracidia (10 freshly hatched miracidia/snail). The second group was exposed for 24 h to LC_{25} from Nitazode, miracidia (10 freshly hatched miracidia/snail).

Thereafter, they were removed and continuously maintained in the tested compound concentration (25 ± 1 _C). Three replicates, each of 10 snails/L, were prepared for each compound. The compound concentration was renewed every 3 days. The third group used as control group was exposed to miracidia concurrently with the experimental snails and treated similarly until cercarial emerged. The snails were daily fed, oven dried lettuce leaves and dead snails were daily removed. After, 15 days of post miracidial exposure, the survived snails were individually examined for cercarial shedding (3 h/3 days). The cercarial production/infected snails were recorded (Oliver, 1962).

Effect of LC_{25} from Albendazole and Nitazode on histological aspects of *Biomphalaria alexandrina*

The first group of *B. alexandrina* snails (8-10 mm) were continuously exposed to LC_{25} of Albendazole for one week. The second group was continuously exposed to LC_{25} of Nitazode for one week. The third group was not treated (control). Hermaphrodite gland of treated and control snails was removed from their shells, fixed in Bruin's fluid for five hours, then transferred to 70% alcohol. Further procedures included dehydration in 100% alcohol, clearing in xylol and paraffin embedding were followed. Five (5) µm sections were stained with hematoxylin and eosin. Stained slides were also examined under polarized light microscope (Mohamed and Saad, 1990).

Parasitological parameters

Experimental design

One hundred and forty (140) mice were immunized with SEA (10 ug x 3). Six weeks later, they were infected by tail immersion with 100 cercariae of *S. mansoni* and were divided into 3 groups. The first group was treated with Albendazole (140 mg/kg). The second group was treated with Nitazode (140 mg/kg). The third group immunized, infected untreated mice were used as immunized infected control. The fourth group infected was not immunized; untreated animals were used as infected control. Clean uninfected, untreated animals were used as normal control. All animals were sacrificed 12 weeks post infection.

Worm burden

Infected animals were perfused to recover hepatic and portomesenteric worms for subsequent counting (Duvall and DeWitt, 1967).

Tissue egg load

The number of eggs per gram tissue (liver and intestine) was studied according to the procedure described by Cheever (1968).

Oogram pattern

The percentages of immature, mature and dead eggs in the small intestines were computed from a total of 100 eggs per intestinal segment and classified according to categories previously defined by Pellegrino et al. (1962).

Immunological study

Determination of anti-SEA immunoglobulin subclasses IgG1, IgG2 and IgG4 were measured using indirect enzyme linked immunosorbent assay (ELISA), based on the method of Engvall and Perlman (1971). ELISA microtiter plates were coated with 100 ul/well of 30 ug/ml of SEA. Sera were diluted 1:20 and anti-mouse IgG subclasses (Binding site, Birmingham, UK) were used at a dilution of 1:500. Absorbance at 492 nm was measured.

Cytokine assay

Serum IFN-γ, IL-4 and IL-10 levels were measured by a sandwich ELISA technique. Briefly, plates were coated with capture antibodies and 100 ul of serum samples or recombinant cytokines were added. Following the addition of the biotinylated detection antibody and streptavidin-alkaline phosphatase conjugate, the reaction was developed with paranitrophenyl phosphate (Sigma) and absorbance was measured at 405 NM.

Granuloma measurement

The hepatic granuloma diameter was measured according to Von Lichtenberg (1962). The percent reduction in granuloma diameter relative to infected control was calculated as follows:

$$\text{Reduction of granuloma diameter (\%)} = \frac{\text{Mean diameter of controls - mean diameter of test}}{\text{Groups mean diameter of control group}} \times 100$$

Statistical analysis

The data were presented as mean (standard error of the mean (X (SE). The means of the different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if p values were less than 0.05.

RESULT

Effect of Albendazole and Nitazode on *B. alexandrina* snails

Effect of LC_{25} from Albendazole and Nitazode on infectivity of S. mansoni miracidia to B. alexandrina snails

The molluscicidal activity of Albendazole and Nitazode against adult *B. alexandrina* snails after 24 h of exposure under laboratory conditions is presented in Table 1. The obtained data indicated that LC_{50} values were 4.5, 11.2 and 20.4 ppm, respectively. It is clear from Table 2 that the survival rates at 1st shedding of snail groups exposed to LC_{25} of Albendazole and Nitazode during exposure were less than that of control ones. The rates for snails exposed to LC_{25} of Albendazole and Nitazode during miracidial exposure were 60 and 80%, respectively, compared to 92% of control ones ($P < 0.05$). The infection rate of snails by *S. mansoni* miracidia (Table 2) was lower than that of the control snails. The rates were 40 and 50% for snails exposed to LC_{25} of Albendazole and Nitazode, respectively, compared to 82% of the control group. There is no significant difference between the prepatent period of the snails exposed to LC_{25} of

Table 1. Molluscicidal activity of Albendazole and Nitazode against *Biomphalaria alexandrina* snails.

Treatment	LC_{25} (ppm)	LC_{50} (ppm)	LC_{90} (ppm)	Slope function
Albendazole	4.66 (3.8 - 5.52)	6.8 (5.7 - 8.16)	11.2	1.5
Nitazode	8.9 (6.35 - 12.46)	13.5 (9.6 -18.9)	21.6	1.7

Table 2. Effect of LC_{25} from Albendazole and Nitazode on the infectivity of *S. mansoni* miracidia to *Biomphalaria alexandrina* snails
.

Compound	Survival at 1st shedding		Infection of snails		Prepatent period (Days)		Cercarial production/ infected snail		Shedding duration (Days)	
	No.	%	No.	%	Range	Mean ± S.D	Range	Mean ± S.D	Range	Mean ± S.D
Albendazole	30	60	12	40	32 - 46	40.5 ± 1.6	72 - 433	511 ± 24.2***	3 - 9	6.7 ± 1.2***
Nitazode	40	80	20	50	36 - 48	41.4 ± 2.3	82 - 822	800 ± 12.1**	4 - 12	10.2 ± 2.6**
Control	46	92	38	82	24 - 49	42 ± 3.5	154 - 1344	1121 ± 322	8 - 17	16.4 ± 3.44

***P<0.05, **P<0.01, ***P<0.001.**

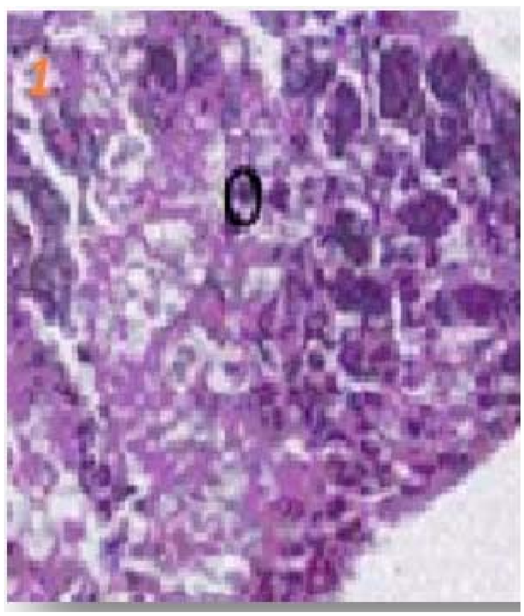

Figure 1. T.S. in normal *Biomphalaria alexandrina* snail showing the hermaphrodite gland. Sp= Sperms, Gc = Gametocytes, O = Oocyte (Hematoxylin & eosin, X=400).

Albendazole and Nitazode and the control group. The duration of cercarial shedding for snails treated with LC_{25} of Albendazole and Nitazode decreased to 6.7 ± 1.2 and 10.2 ± 2.6 days, respectively, compared to 16.4 ± 3.44 days for the control snails.

For cercarial production/snail, it was reduced by snails exposure to the tested plants. Thus, this parameter for snail groups exposed to LC_{25} of Albendazole and Nitazode during miracidial exposure was significantly different from that of control snails, being 511 ± 24.2 and 800 ± 12.1 cercariae/snail, respectively, compared to 1121 ± 322 cercariae/control snail ($P < 0.001$).

Histological investigation

The hermaphrodite gland of normal snails is composed of number of acini connected together by a connective tissue, containing various developmental stages of oogenic and spermatogenic cells. The hermaphrodite region in Figure 1 shows mature ova (o), gametocytes (GC), oocyte (O), sperm (sp) and spermatocytes (S). The

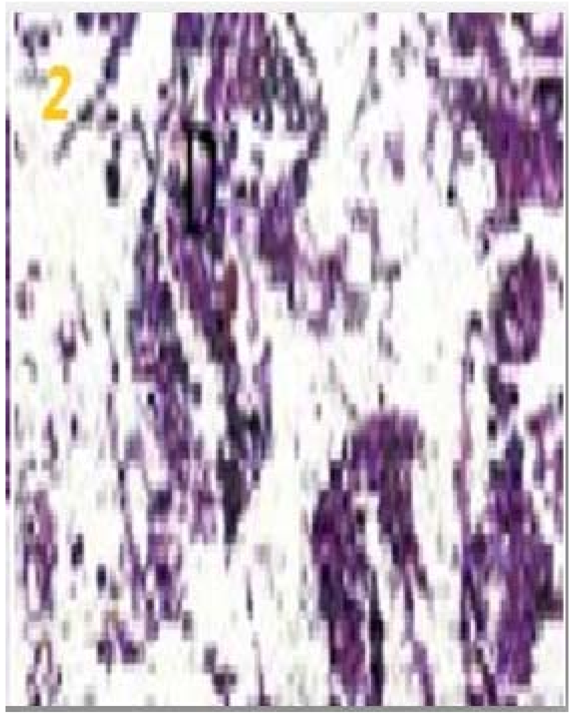

Figure 2. T.S. in *Biomphalaria alexandrina* treated with *LC25* of Albendazole showing the damage hermaphrodite follicle. V= Vacuoles, D= Degeneration (Hematoxylin & eosin, X=400).

results (Figures 2 and 3) of snails treated with Albendazole and Nitazode showed great histological changes in hermaphrodite gland.

Exposure of snails to LC_{25} of Albendazole caused great damage in the hermaphrodite gland. A severe destruction of the germinal epithelia of the acini and complete inhibition of all stages of gametogenesis and spermatogenesis was observed (Figure 2). Inhibition of spermatogenesis is realized, but little sperm could be detected. Coyotes and spermatocytes were degenerated.

Treated *B. alexandrina* with LC_{25} of Nitazode (Figure 3) showed some hermaphrodite acini appeared empty from gametocytes with the destruction of germinal epithelial layer, while the hermaphrodite region showed sperms with the absence of immature stages of spermatogenic cells.

Effect of Albendazole and Nitazode on *mice*

Parasitological parameters

The total number of worms and the percent reduction of worm burden showed no significant difference between infected control and the immunized infected control. On the other hand, the groups treated with Albendazole and Nitazode showed a highly significant decrease ($P<0.001$) compared to immunized infected control. The mean ova count in the intestine and liver showed a significant reduction ($P<0.01$) in immunized infected control compared to infected control, while all treated groups showed a highly significant reduction ($P<0.001$) compared to immunized infected control (Table 3). As regards oogram pattern, there was no significant change between the infected control and immunized infected control. On the other hand, highly significant decrease was shown only in the groups treated with Albendazole or Nitazode ($P< 0.001$) as compared to immunized infected control (Table 4).

Granuloma diameter

Granuloma diameter showed slight decrease in immunized infected control as compared to infected control ($P<0.05$), while in all treated groups, it showed a highly significant decrease ($P<0.001$) (Table 5).

Immunological parameters

Serum-specific immunoglobulin isotypes

In infected control group, there was no significant change

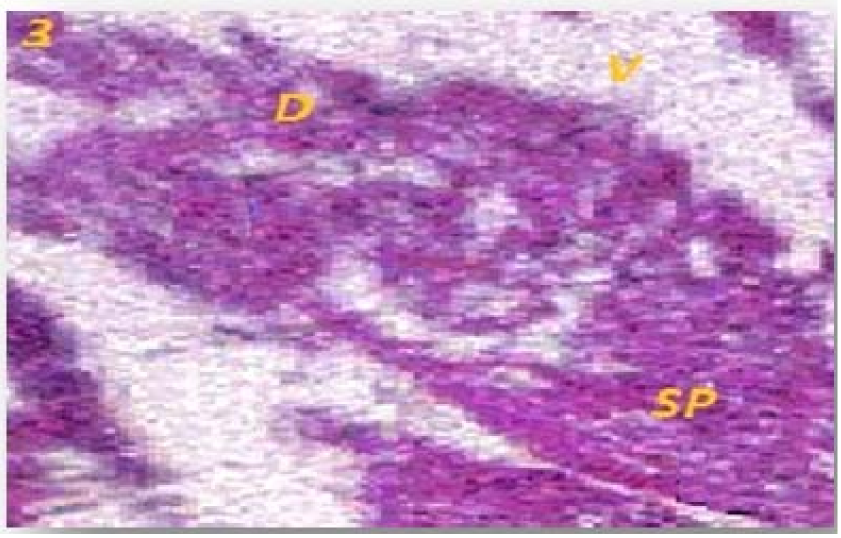

Figure 3. T.S. in *Biomphalaria alexandrina* treated with LC25 of Nitazode showing damage hermaphrodite follicle. Sp = Sperms, V = vacuoles, D= degeneration (Hematoxylin & eosin, X=400).

Table 3. Worm burden and tissue load in mice immunized with SEA (10 µg X 3) 6 weeks before infection and treated with Albendazole and Nitazode then sacrificed 12 weeks post infection.

Animal group	Mean no. of worms ± SEM	% reduction	Mean no. of ova count ± SEM / g tissue			
			Intestine	% Reduction	Liver	% reduction
Infected control	42.2 ± 0.62		15102 ± 177		4201 ± 374	
Immunized infected control	38.2 ± 0.32	9.5	**5130 ± 114	66	**1533 ± 210	63.5
Treated groups						
Albendazole	*23.4 ± 0.33	27.5	**3211 ± 143	78.7	**964 ± 277	77
Nitazode	**24.2 ± 0.51	42.7	**2421 ± 145	84	***454 ± 90	89.2

*** P<0.001, ** P < 0.001 , * P < 0.01, * P <0.05 relative to infected control.

in IgG isotypes compared to normal control. However, in immunized infected control there is a significant increase in IgG1 (P< 0.01) and IgG4 (P<0.05) as compared to the infected control. The level of IgG1 showed no significant change in the treated groups as compared to immunized infected control. On the other hand, there was a highly significant increase in IgG2 level in all treated groups (P<0.001) (Table 6).

Serum cytokines level

The profile of Th-1 related cytokine IFN-γ showed significant increase in infected control (P < 0.001) as compared to the normal control. On the other hand it showed a slightly significant decrease in immunized infected control compared to infected control (P<0.05). In treated groups, the groups treated with Albendazole showed a significant increase (P < 0.05) as compared to immunized infected control. On the other hand, significant decrease in IFN- γ level was observed in groups treated with Nitazode alone (P<0.01 – P< 0.001, respectively) compared to immunized infected control.

The Th-2-related cytokines IL-4 showed a highly significant increase in the infected control as compared to the normal control (P< 0.001). At the same time, it showed significant decrease in the immunized infected control (P<0.01) as compared to infected control. Also, it showed no significant change in groups treated with Albendazole and groups treated with Nitazode showed a slight decrease (P<0.05) as compared to immunized infected control. On the other hand, the Treg-related cytokine IL-10 level showed a highly significant increase in infected control (P<0.001) compared to normal control and slight increase in immunized infected control (P<0.05) as compared to infected control. In the treated group, it showed a slightly significant increase in the groups treated with Albendazole or Nitazode compared to

Table 4. Oogram pattern in mice immunized with SEA (10 µg x 3). 6 weeks before infection and treated with Albendazole and Nitazode then sacrificed 12 weeks post infection.

Animal group	Oogram pattern (% ova)		
	Immature	Mature	Dead
Infected control	62.1 ± 5	37.1 ± 2.6	2.7 ± 0.3
Immunized infected control	58 ± 2.4	36.1 ± 3.5	5.9 ± 0.2*
Treated groups			
Albendazole	42.3 ± 0.2**	22.6 ± 0.8**	35.1 ± 1.1***
Nitazode	41.1 ± 1.4**	28.5 ± 3.1**	30.4 ±1.1***

***P<0.001, **P<0.01, * P<0.05 relative to infected control.

Table 5. Hepatic granuloma diameter and % reduction in mice immunized with SEA (10 µg x 3). 6 weeks before infection and treated with Albendazole and Nitazode then sacrificed 12 weeks post infection

Animal group	Hepatic granuloma diameter(a) Mean µm ± SEM	% Reduction (b)
Infected control	234.2 ± 4.2	
Immunized infected control	*212 ± 5.6	9.48
Treated groups		
Albendazole	** 124 ± 14.4	41.5
Nitazode	** 136 ± 8.1	35.84

**P<0.01, *P<0.05 relative to infected control.

Table 6. Serum anti-SEA IgG subclasses levels in mice infected with SEA (10 µg x3). 6 weeks before infection and treated with Albendazole and Nitazode then sacrificed 12 wks post infection

Animal group	X\` O.D ± SEM Ig G1	X\` O.D ± SEM Ig G2	X\` O.D ± SEM Ig G4
Normal control	0.323 ± 0.4	0.51 ± 0.4	0.39 ± 0.2
Infected control	0.44 ± 0.18	0.6 ± 0.3	0.51 ± 0.17
Immunized infected control	0.99 ± 0.6**	0.65 ± 0.4**	0.98 ± 0.14**
Treated groups			
Albendazole	0.88 ± 0.7**	1.12 ± 0.4***	1.02 ± 0.3**
Nitazode	0.77 ± 0.3**	1.2 ± 0.2***	1.10 ± 0.3**

***P < 0.001, **P < 0.01, *P <0.05 relative to infected control.

immunized infected control (Table 7).

DISCUSSION

The infectivity of *S. mansoni* miracidia to *B. alexandrina* was greatly reduced by LC_{25} of Albendazole and Nitazode. This was supported by the interruptions in biochemical parameters, as well as the activities of enzymes of treated snails that render their physiological processes unsuitable for the parasite development and reduce cercarial production. Comparable results were obtained in the study (Bakry, 2006) using the plants, *Zygophyllum simplex, Furcraea gigantean* and *Lampranthus spectabilis.* However, there was no significant difference between the prepatent period of the snails exposed to LC_{25} of Albendazole and Nitazode and the control. Despite that, a highly significant reduction in the duration of cercarial shedding and total cercarial production per infected snails were detected. These phenomena were stated by many authors using different plant species as molluscicidal agents. Thus, Badawy (2007) and Gawish (2008) found that the plants *Viburnum tinus, Syzygium jambos, Eupanacra splendens* and *Aerangis stylosa* have a remarkable decrease in the duration of cercarial shedding and cercarial production/snail from *B. alexandrina* infected with *S. mansoni* miracidia. The authors attributed this, probably,

Table 7. Serum cytokine level in mice immunized with SEA (10 µg x 3) six weeks before infection and treated with different types of drugs the sacrificed 12 wks post infection

Animal group	IFN - γ Pg/ml ± SEM	IL - 4 Pg/ml ± SEM	IL - 10 Pg/ml ± SEM
Normal control	184 ± 44.3	24± 0.57	83 ± 4.3
Infected control	566± 31	74± 3	433± 15.6
Immunized infected control	**396± 15	**31 ± 1.3	**567 ± 18.7
Treated groups			
Albendazole	***128± 33	**23 ± 3.2	**644 ± 27.7
Nitazode	**197 ± 54	*34 ± 6.3	**622 ± 34.1

***P < 0.001, **P < 0.01, *P<0.05 relative to infected control.

to the disturbances in the activities of a snail's enzyme system, and the total protein concentration in their hemolymph that negatively affects the developmental stages of the parasite within their tissues.

The present results showed great damage in the hermaphrodite gland and complete inhibition of all stages of gametogenesis and spermatogenesis**.** These same findings were reported by Bakry (2009b) who found severe damages in the hermaphrodite gland of *B. alexandrina* post two weeks of exposure to LC_{25} of plant mollu-scicides. These observations are in accordance with the previous ones on Hematoporphyrin and Argon-ion laser against *B. alexandrina* snail's oviposition (El-Sayed and El-Sherbini, 2006). Moreover, Ibrahim et al. (2004) demonstrated a great histological damage of *B. alexandrina* ovotestis post exposure to the plants that stopped snail's oviposition after four weeks of exposure. Gawish (2009) recorded a rupturing of the gland cells and an evacuation of most of its tubules from gametogenic stages post exposure to 35, 60 and 85 ppm of carbamide perhydrate under direct sunlight for 4 h followed by 20 h in the laboratory without exposure to direct light.

The present study reveals that the immunization schedule used did not cause any significant change in worm burden but slightly significant reduction in the tissue egg load which agreed with Botros et al. (1996). The treatment of Albendazole or Nitazode in immunized infected animals caused almost similar high percentage of eradication of worms and the tissue egg load which also agree with the work of Suleiman et al. (2004). The death of the worms due to the treatment with antischistosomal drugs was attributed to metabolic disorders, mechanical destruction and muscular contraction of the treated worms (Abdel-Ghaffar et al., 2005). At the same time, percent reduction in the egg count in both immunized infected and treated groups was found to be higher in the intestinal tissue than in hepatic tissue. This variation was attributed to excretion of some ova from the intestine prior to digestion and to hepatic shift of worms after treatment (Abdel-Ghaffar, 2004). On the other hand, the treatment with Albendazole or Nitazode caused a decrease in immature egg stages and the number of mature eggs with a high increase in the number of dead eggs which agree with the findings of Botros et al. 1996. The parasitological improvement is due to Albendazole and Nitazode which causes direct or indirect toxic effect in combination with the effect of immunization with SEA which lead to reduction in tissue egg load. This may be attributed to a marked decrease in the worm number or fecundity due to hindering the process of oviposition (Guirguis, 2003).

The manifestations of schistosomiasis are mainly attributed to granulomatous inflammation around the parasite eggs (Abath et al., 2006). The formation of granulomas depends predominantly on CD4+ T cell specific for egg antigen and represents a delayed-type hypersensitivity (Garcia et al., 2008). At the same time, hepatic stellate cells (HSCs) comprise 10-15% of all hepatic cells and they are recruited to areas of hepatic injury and become activated (Cassiman et al., 2002). They adopted a myofibroblast–like phenotype, secreting extracellular matrix components (Mann et al., 2009).

In this study, although all treated groups revealed significant diminution of granuloma diameter, at the same time, the groups treated with Albendazole or Nitazode revealed lower pattern than the other treated groups and this may be due to the effect of previous immunization of the infected animals before treatment. In this study, immunization before infection increased the levels of production of IgG1 and IgG4. All treated groups had increased levels of IgG2, but slight increase in the level of IgG4 was observed in the groups treated with Albendazole. This increase in the production of immunoglobulins has an important role in the improvement of the pathology and the reduction in the ova count and worm burden (Soren et al., 2009; Njenga et al., 2014a, b).

Cytokines which act on lymphocytes are of special interest because of their role in regulating the cells of the immune response (Kim et al., 1997). During schistosomal infection, both Th1 and Th2 responses directed against egg antigen and produce IFN-γ, IL-4, IL-5 and IL-13 (Stadecker et al., 2004 and Keyel, 2014). In this study, the diminished production of Th1-cytokine IFN-γ and Th2-

cytokine IL-4 in the immunized group may be implicated in the down modulation of the granulomatous response due to immunization (Chensue et al., 1992). Groups treated with Albendazole and Nitazode showed significant decrease in IFN- γ and IL-4. Recent studies suggest that Treg cells play a pivotal role in suppressing Th1 cell development as well as limiting the magnitude of Th2 response directed against egg antigens by a process dependent upon IL-10 (Stadecker et al., 2004). The increasing level of IL-10 is probably implicated in the down regulation of granuloma formation as it reduces the intrahepatic inflammatory response and hence it has an antifibrotic effect (Nelson et al., 2003).

These results indicate the importance of the effect of Albendazole and Nitazode as it has a potent antifibrogenic role. In conclusion, treatment with Albendazole and Nitazode with immunization resulted in significant reduction of parasitological parameters and rise of specific immunoglobulins. The addition of antifibrotic drugs Albendazole and Nitazode, potentiated an antipathology effect which minimized and ameliorated liver fibrosis by inhibition of HSC activation and accentuation of the effect of suppressing Treg cells.

Conflict of Interest

The author(s) did not declare any conflict of interest.

REFERENCES

Abath FG, Morais CN, Montenegro CE, Wynn TA, Montenegro SM (2006). Immunopathogenic mechanisms in schistosomasis: what can be learnt from human studies? Trends Parasitol. 22: 85-91.

Abboud P, Lemee V, Gargala A, Brasseur P, Ballet JJ, Borsa-Lebas, Caron F, Favennec L (2001). Successful treatment of metronidazole- and albendazole-resistant giardiasis with Nitazode in a patient with acquired immunodeficiency syndrome. Clin. Infectious Dis. 32: 1792-1794.

Abdel-Ghaffar O (2004). Assessment of the efficacy of Ro16-2308 against the Egyptian strain of *Schistosoma mansoni* in mico: Parasitological, hematological and biochemical criteria. Egypt. J. Zool. 42: 173-203.

Abdel-Ghaffar O, Rawi SM, Ishaq AI (2005). Evaluation of the curative efficacy of RO 15-8843 against mansonian schistosomiasis in albino mice. J. Egypt. Ger. Soc. Zool. 47:15-22.

Alvarez LG, Moreno L, Ceballos L, Shaw I, Fairweather C, Lanusse J (2009). Comparative assessment of albendazole and triclabendazole ovicidal activity on *Fasciola hepatica* eggs. Vet. Parasitol. 164:211-216.

Badawy AM (2007). Studies on Dracaena draco (Agavaceae) and Viburnum tinus (Caprifoliaceae) as Plant Molluscicides Against the Snail Vectors of Schistosomiasis and the Larval Stages of this Parasite, Ph.D. Thesis, Zoology Dept. Girls College for Arts, Science and Education, Ain Shams University,Cairo, Egypt.

Bakry FA (2006). Effect of methanol extracts of *Furcraea gigantean* and *Lampranthus spectabilis* plants on *Biomphalaria alexandrina* infection by *Schistosoma mansoni* and on energy metabolism indicator, Egypt. J. Schist. Infect. Endemic Dis. 28:1-14.

Bakry FA (2009a). Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some biological effects. Pest. Biochem. Physiol. 95:159-165.

Bakry FA (2009b). Impact of some plant extracts on histological structure and protein patterns of *Biomphalaria alexandrina* snails. Global J. Mol. Sci. 4:34-41.

Bergquist R, Utzinger J, Mc Manus DP (2008). Trick or treat: the role of vaccines in integrated schistosomiasis control. PLoS. Negl. Trop. Dis. 2:244-252.

Botros S, Doughty B, Shaker Z, Akl M, Sharmy R, Diab T, Hassanein H (1996). Efficacy of antipathology vaccine in murine schistosomiasis administered with and without chemotherapy. Int. J. Immunopharmacol. 12:707-718.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-54.

Carter CE, Colley DG (1978). An electrophoretic analysis of *Schistosoma mansoni* soluble egg antigen. J. Parasitol. 64: 385-390.

Cassiman D, Lib brecht L, Desnet V, Denef C, Roskams T (2002). Hepatic Stelalte cell/myofibroblast subpopulations in fibrotic human and rat livers. J. Hepatol. 36:200-209.

Cheever AW (1968). Conditions affecting accuracy of KOH digestion techniques for counting *S. mansoni* eggs in tissues. Bull. WHO 39:329-331.

Chensue SW, Terebuch PD, Warmington KS, Hershey SD, Evanoff HL, Kunkel L (1992).Role of IL-4 and IFN- γ in *Schistosoma mansoni* egg-induced hypersensitivity granuloma formation. Orchestration, relative contribution, and relationship to macrophage function. J. Immunol. 148:900-906.

Chitsulo L, Loverde P, Engels D (2004). Schistosomiasis, Nat. Rev. Microbiol. 2:12-13.

Duvall RH, De Witt WB (1967). An improved perfusion technique for recovering adult Schistosomes from laboratory animals. Am. J. Trop. Med. Hyg. 16:483-486.

El-Emam MA, El-Amin SM, Ahmed WS (1990). Molluscicidal properties of the plants *Cestrum parqui* (Fam. Solanaceae and *Hedra canariensis* (Fam. Araliaceae). Egypt. J. Bilh. 12:185-195.

El-Khoby T, Galal N, Fenwick A, (1998). The USAID/ Government of Egypt Schistosomiasis Research Project (SRP). Parasitol. Today. 14:92.

El-Sayed K, El-Sherbini SA (2006). Impact of hematoporphyrin and different laser sources on *Biomphalaria alexandrina* snails and their infection with *Schistosoma mansoni* J. Biol. Chem. Environ. Sci. 1:320-340.

Engvall E, Perlman P (1971). Enzyme linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. J. Immunochem. 8:871-874.

Finny DJ (1971). Probit Analysis, third ed., Cambridge University Press, London.

Fox LM, Saravolatz LD (2005). Nitazoxanide: A New Thiazolide Antiparasitic Agent. Clin. Infect. Dis. 40: 1173–80.

Friedman SL (2003). Liver fibrosis –from bench to bedside. J. Hepatol. 38 (238):538-53.

Garcia TC, Fonseca CT, Pacifico LG, Duraes Fdo, V, Marinho FA, Penido ML, Caliari MV, de Melo AL, Pinto HA, Barsante MM, Cunha-Neto E, Oliveia SC (2008). Peptides containing T cell epitopes drived from Sm 14, but not from paramyosin, induce a Th1 type of immune response, reduction in liver pathology and partial protection against *Schistosoma mansoni* infection in nice. Acta. Trop. 106:182-7.

Gawish FA (2008). Activity of the plant *Syzygium jambos* against *Biomphalaria alexandrina* snails' reproduction and infection with *Schistosoma mansoni*, N. Egypt. J. Med. 39:103-110.

Gawish FA, El-Sherbini SA, Hanan FA (2009). Effect of Photosensitization Process of Carbamide Perhydrate on *Biomphalaria alexandrina* Snails and Their Infection with *Schistosoma mansoni*. J. App. Sci. Res. 5: 46-56,

Gilles HM, Hoffman PS (2002). Treatment of intestinal parasitic infections: a review of nitazoxanide. Trends Parasitology 18: 95-97.

Gryseels B, Polman K, Clerinx J, Kestens L (2006). Human schistosomiasis, Lancet 368: 1106–1118.

Guirguis FR (2003). Efficacy of praziquantel and Ro 15 - 5458, a q-acridanonchydrazone derivative, against *Schistosoma haematobium.* Arzeim, Forsch, Drug Res. 53 (1): 57-61.

Ibrahim AM, El-Emam MA, El-Dafrawy SM, Mossalem HS(2004).Impact of certain plant species on *Schistosoma mansoni-Biomphalaria alexandrina* system. Proc. 3 Int. Conf. Sci. 3: 390-413.

Keyel PA (2014). How is inflammation initiated? Individual influences of IL-1, IL-18 and HMGB1. Cytokine 69(1):136-145.

Kim JJ, Aggvoo V, Bagarazzi ML, CHattergoon MA, Dang K, Wang B, Boyer J D and Weiner DB (1997). In vivo Engineering of a cellular immune response by co-administration of IL-12 expression vector with a DNA immunogn. J. Immunol. 158:816-826.

Litchfield JT, Wilcoxon F (1949). A simplified method of evaluating dose effect experiments, J. Pharmacol. Exp. 96:99-113.

Nessim NG, Hassan SI, William S, El-Baz H (2000). Effect of the broad spectrum anthelmintic drug flubendazole upon *Schistosoma mansoni* experimentally infected mice. Arzneimitted-Forrschung/Drug Res. 50:1129-1133.

Nelson DR, Tu 2, soldevila-Pico C (2003). long-term interleulin 10 therapy in chronic hepatitis C patients has a proviral and anti-inflammatory effect. Hepatology 38:859-868.

Njenga SM, Ng'ang'a PM, Mwanje MT, Bendera FS, Bockarie MJ (2014). A school-based cross-sectional survey of adverse events following co-administration of albendazole and praziquantel for preventive chemotherapy against urogenital schistosomiasis and soil-transmitted helminthiasis in Kwale County, Kenya, MJ. PLoS One. 9(2):e88315.

Njenga SM, Mutungi FM, Wamae CN, Mwanje MT, Njiru KK, Bockarie (2014). Once a year school-based deworming with praziquantel and albendazole combination may not be adequate for control of urogenital schistosomiasis and hookworm infection in Matuga District, Kwale County, Kenya. MJ. Parasit Vectors. 19:7:74.

Maher KM, El- Shennawy AM, Ibrahim R B, Aly E, Mahmoud S (2003). Protective and antipathology potential of immunization with *S. mansoni* Schistosomula-secretory / excretory product. New Egypt. J. Med. 28:173-182.

Mann J, Mann DA (2009). Transcriptional regulation of hepatic stellate cells. Advanced drug delivery reviews 61:497-512.

Mohamed SH, Saad AA (1990). Histological studies on the hermaphrodite gland of *Lymnaea caillaudi* and *Biomphalaria alexandrina* upon infection with certain larval trematodes. Egypt. J. Histolol. 13: 47-53.

Oliver L, Haskins WT, Gurian J (1962). Action of very low concentration of Na pentachlorophenate on freshly laid eggs of Australorbis glabratus, Bull. World Health Org. 27:87-94.

Pellegrino J, Oliveria CA, Faria J, Cunha AS (1962). New approach to the screening of drugs in experimental *schistosoma mansoni* in mice. Am. J. Trop. Med. Hyg. 11:201-215.

Romeih MH, Hassan HM, Shousha TS, Saber MA (2008). Immunization against Egyptian *Schistosoma mansoni* infection by multivalent DNA vaccine: Acta. Biochim. Biophys. Sin. (Shanghai). 40:327-38.

Rossignol JF, Ayoub A, Ayers M (2001). Treatment of diarrhea caused by *C. parvum*: a randomized, double-blind, placebo-controlled study of nitazoxanide. J. Infect. Dis. 184:103-106.

Soren K, Monard J, Johnsen MV, Lindberg R (2009). Persistent immune responses in late infection and after treatment in experimental *Schistosoma bovis* infections in goats. Res. Vet. Sci. 86:472-478.

Stadecker MJ, Ashai H, Finger E, Hernandez HJ, Rutitzky, Sun J (2004).The immunobiology of Th1 polarizaiton in high-pathology Schistosoniasis. Immunol. Rev. 201:168-179.

Suleiman MI, Akarim EI, Ibrahi KE, Saad AM, Mohammed AE, Ahmed BM, Sulaiman SM (2004). Antischistosomal effects of praziquantel, its alkaline hydrolysis and sun decomposed products on experimentally *S. mansoni* infected albino mice. (A) Efficacy assesament based on clinicopathological findings. J. Egypt. Soc. Parasitol. 34:131-42.

Tendler M, Brito CA, Vilar MM, Serra-Freire N, Diago CM, Almedin MS, Delbem AC, da Silva JF, Savino W, Garratt RC, Katz N, Simpson AS (1996). A *Schistosoma mansoni* fatty acid- binding protein, Sm14, is the potential basis of a dual-purpose anti-helminth vaccine. Proc. Nath. Acad. Sci. USA 93:269-273.