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

Gehan L. El-Enain1,2*, Sharaf, H.M3 and Abd El-Atti M. S3

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

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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...
disease transmission in some of these countries.

Schistosomal pathology is a direct consequence of the immunological response to oviposition 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 schistosomiasis 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 Albenza (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 Albenza sulphoxide per kg bodyweight. Although ABZ is only effective against adult flukes, it is a broad-spectrum nematocidal compound with well-known ovicidal activity (Alvarez et al., 2009).

Nitazoxanide (NTZ) is a new thiazolide antiparasitic agent that shows excellent 

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 LC50 ppm values.

Effect of LC25 from Albenza 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 LC25 from Albenza and miracidia (10 freshly hatched miracidia/snail). The second group was exposed for 24 h to LC25 from Nitazode, miracidia (10 freshly hatched miracidia/snail).

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).
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 LC25 from Albendazole and Nitazode on histological aspects of Biomphalaria alexandrina

The first group of _B. alexandrina_ snails (8-10 mm) were continuously exposed to LC25 of Albendazole for one week. The second group was continuously exposed to LC25 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 Bruni'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:

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

**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 LC25 from Albendazole and Nitazode on infectivity of _S. mansoni_ miracidia to _B. alexandrina_ snails**

The molluscidal 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 LC50 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 LC25 of Albendazole and Nitazode during exposure were less than that of control ones. The rates for snails exposed to LC25 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 LC25 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 LC25 of
Table 1. Molluscicidal activity of Albendazole and Nitazode against Biomphalaria alexandrina snails.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LC25 (ppm)</th>
<th>LC50 (ppm)</th>
<th>LC90 (ppm)</th>
<th>Slope function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albendazole</td>
<td>4.66 (3.8 - 5.52)</td>
<td>6.8 (5.7 - 8.16)</td>
<td>11.2</td>
<td>1.5</td>
</tr>
<tr>
<td>Nitazode</td>
<td>8.9 (6.35 - 12.46)</td>
<td>13.5 (9.6 - 18.9)</td>
<td>21.6</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 2. Effect of LC25 from Albendazole and Nitazode on the infectivity of S. mansoni miracidia to Biomphalaria alexandrina snails.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Survival at 1st shedding</th>
<th>Infection of snails</th>
<th>Prepatent period (Days)</th>
<th>Cercarial production/infected snail</th>
<th>Shedding duration (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>Range</td>
</tr>
<tr>
<td>Albendazole</td>
<td>30</td>
<td>60</td>
<td>12</td>
<td>40</td>
<td>32 - 46</td>
</tr>
<tr>
<td>Nitazode</td>
<td>40</td>
<td>80</td>
<td>20</td>
<td>50</td>
<td>36 - 48</td>
</tr>
<tr>
<td>Control</td>
<td>46</td>
<td>92</td>
<td>38</td>
<td>82</td>
<td>24 - 49</td>
</tr>
</tbody>
</table>

*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 LC25 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 LC25 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
results (Figures 2 and 3) of snails treated with Albendazole and Nitazode showed great histological changes in hermaphrodite gland.

Exposure of snails to LC25 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 LC25 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
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.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Oogram pattern (% ova)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immature</td>
<td>Mature</td>
<td>Dead</td>
</tr>
<tr>
<td>Infected control</td>
<td>62.1 ± 5</td>
<td>37.1 ± 2.6</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>Immunized infected control</td>
<td>58 ± 2.4</td>
<td>36.1 ± 3.5</td>
<td>5.9 ± 0.2*</td>
</tr>
<tr>
<td>Albendazole</td>
<td>42.3 ± 0.2**</td>
<td>22.6 ± 0.8**</td>
<td>35.1 ± 1.1***</td>
</tr>
<tr>
<td>Nitazode</td>
<td>41.1 ± 1.4**</td>
<td>28.5 ± 3.1**</td>
<td>30.4 ± 1.1***</td>
</tr>
</tbody>
</table>

***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.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Hepatic granuloma diameter(a)</th>
<th>% Reduction (b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean μm ± SEM</td>
<td></td>
</tr>
<tr>
<td>Infected control</td>
<td>234.2 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>Immunized infected control</td>
<td>*212 ± 5.6</td>
<td>9.48</td>
</tr>
<tr>
<td>Albendazole</td>
<td>** 124 ± 14.4</td>
<td>41.5</td>
</tr>
<tr>
<td>Nitazode</td>
<td>** 136 ± 8.1</td>
<td>35.84</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>X' O.D ± SEM (Ig G1)</th>
<th>X' O.D ± SEM (Ig G2)</th>
<th>X' O.D ± SEM (Ig G4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.323 ± 0.4</td>
<td>0.51 ± 0.4</td>
<td>0.39 ± 0.2</td>
</tr>
<tr>
<td>Infected control</td>
<td>0.44 ± 0.18</td>
<td>0.6 ± 0.3</td>
<td>0.51 ± 0.17</td>
</tr>
<tr>
<td>Immunized infected control</td>
<td>0.99 ± 0.6**</td>
<td>0.65 ± 0.4**</td>
<td>0.98 ± 0.14**</td>
</tr>
<tr>
<td>Albendazole</td>
<td>0.88 ± 0.7**</td>
<td>1.12 ± 0.4***</td>
<td>1.02 ± 0.3**</td>
</tr>
<tr>
<td>Nitazode</td>
<td>0.77 ± 0.3**</td>
<td>1.2 ± 0.2***</td>
<td>1.10 ± 0.3**</td>
</tr>
</tbody>
</table>

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

imunized infected control (Table 7).

DISCUSSION

The infectivity of S. mansoni miracidia to B. alexandrina was greatly reduced by LC25 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 LC25 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 styloa 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,
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 hermaphroditic 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 hermaphroditic gland of B. alexandrina post two weeks of exposure to LC25 of plant molluscicides. 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-

<table>
<thead>
<tr>
<th>Animal group</th>
<th>IFN - γ Pg/ml ± SEM</th>
<th>IL - 4 Pg/ml ± SEM</th>
<th>IL - 10 Pg/ml ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>184 ± 44.3</td>
<td>24 ± 0.57</td>
<td>83 ± 4.3</td>
</tr>
<tr>
<td>Infected control</td>
<td>56 ± 31</td>
<td>74 ± 3</td>
<td>433 ± 15.6</td>
</tr>
<tr>
<td>Immunized infected control</td>
<td>*396 ± 15</td>
<td>**31 ± 1.3</td>
<td>**567 ± 18.7</td>
</tr>
<tr>
<td><strong>Treated groups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albendazole</td>
<td>***128 ± 33</td>
<td>**23 ± 3.2</td>
<td>**644 ± 27.7</td>
</tr>
<tr>
<td>Nitazode</td>
<td>**197 ± 54</td>
<td>*34 ± 6.3</td>
<td>**622 ± 34.1</td>
</tr>
</tbody>
</table>

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

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
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 Nitazoxanide 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 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 Nitazoxanide as it has a potent anti-inflammatory role. In conclusion, treatment with Albendazole and Nitazoxanide with immunization resulted in significant reduction of parasitological parameters and rise of specific immunoglobulins. The addition of antifibrotic drugs Albendazole and Nitazoxanide, potentiated an antiparasitological 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.

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