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IMMUNOMODULATORY AND AMELIORATIVE ROLE OF *NIGELLA SATIVA* OIL ON *SCHISTOSOMA MANSONI* INFECTED MICE

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ABSTRACT

Schistosomiasis is one of the world's most prevalent parasitic diseases and cause of morbidity and mortality. The present study was designed to investigate the immune mechanisms possibly involved in the amelioration of histopathological changes in livers of *Schistosoma mansoni* infected mice treated with *Nigella sativa* oil combined with artemether (ART) or praziquantel (PZQ). Hitological alterations of liver and granulomas diameter were recorded. Total serum immunoglobulin G (IgG) and cytokine profils; IL-2, IL-12 and TNF-α were studied. The levels of total serum IgG after treatment with *N. sativa* or ART or PZQ of non-infected mice recorded a significant decrease compared to normal control group. While *N. sativa* combined with ART or PZQ of infected groups showed significant increase in total IgG (684.24 ± 3.03, 647.42 ± 2.21 and 708.50 ± 18.06, respectively) compared to infected lab control group (570.84 ± 6.55). Effect of *N. sativa* oil on cytokines IL-2 and TNF-α of non-infected and infected mice showed significant increase (P<0.001, Kruskal wallis). While treatment with ART of non-infected showed significant decrease in IL-2 and 12 and increase in TNF-α. PZQ treatment showed significant increase in IL-2 and decrease in IL-12 compared to normal control group. Effect of *N. sativa* oil combined with ART or PZQ showed significant increase on cytokines (IL-2, IL-12 and TNF-α) compared to infected control group. Histological observation of liver tissue of infected and non-infected mice treated with *N. sativa* combined with ART or PZQ showed some improvement in histological damages in all treated groups. The present data recorded that *N. sativa* combined with ART or PZQ recorded a significant decrease in granuloma diameter (35.42 % and 32.23 %), when compared to infected control group. It can be concluded that *N. sativa* is a promising adjuvant with antihelminthic drugs in the treatment of schistosomiasis.

Keywords: *Nagella sativa*, immunomodulation, granuloma, schistosomiasis.

INTRODUCTION

Schistosomiasis is one of the world's most prevalent parasitic diseases, and is considered one important of the human helminthiases beside malaria in terms of morbidity and mortality (Hotez and Ferris 2006; Hotez *et al*., 2008; Mimche *et al*., 2014). In Egypt, schistosomiasis is one of the most important environmental diseases (WHO, 2010).

Medicinal plants could serve as safe therapeutic alternatives, or the only effective treatment. People in separate cultures are known to use plants for medical problems. Large number of these plants and their constituents have shown beneficial therapeutic properties, including anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial and immunomodulatory effects on the experimental animals (Salem and Hossain 2000; Huffman, 2003; Xuan *et al*., 2010). Mohamed *et al*. (2014) used the natural product, the Blue green algae (BGA, 100 mg/kg BW), alone or combined with praziquantel PZQ (250 mg/kg BW) as a therapeutic agent on *Schistosoma mansoni* infected mice. Among the promising medicinal plants, *Nigella sativa* (N. S.) is an amazing plant with loaded historical and religious backgrounds (Goreja, 2003) reflecting the medicinal influences of *Nigella sativa* L. on the Arabic folk medicine (Scholz *et al*., 2009).

Artemisia annua (ART, family: Asteraceae), commonly known as sweet Annie, is a weed that grows worldwide throughout temperate areas and is currently grown as a pharmaceutical crop in China, Vietnam, and North and East Africa (White, 1996; Ferreira *et al*., 2005; Haynes 2006). The constituent that present in *A. annua* is responsible for its antimalarial properties are artemisinin, a sesquiterpene lactone that contains an endoperoxide bridge. In addition, it is developed as chemoprophylactic agent against malaria and schistosomiasis (Liu *et al*., 2012).

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Praziquantel (PZQ) was first used for its anti-helminthic properties in the mid 1970's to treat veterinary trematode and cestode infections. The present preferred method of treatment and control is chemotherapy with praziquantel. However, concerns the drug resistance may take place has provoked the need for improvement of alternative chemotherapy options (Utzinger and Keiser, 2004).

Cytokines play important roles in immunomodulation during schistosomiasis. Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) and IL-6 probably cause the fever in acute schistosomiasis (Dela Rosa, 1992; Van Die *et al*., 2010). TNF-α, IL-1, IL-2 and IL-12 cause granuloma formation in acute murine schistosomiasis (Stadecker and Villanueva, 1994). Granuloma cells comprise of macrophages, lymphocytes, eosinophils and release of profibrotic lymphokines such as IL-4. That interleukin stimulates fibroblasts to secrete collagen and other matrix proteins (Cheever *et al*., 2000; Pearce, 2005 and Liang *et al*., 2011).

This study was aimed to evaluate the effect of *Nigella sativa* oil alone or combined with artemether and praziquantel on liver histology and some immune and inflammatory responses of the host (mice) infected with *Schistosoma mansoni*.

MATERIALS AND METHODS

Experimental animals

The adult freshwater snails, *Biomphalaria alexandrina* (8 ± 2 mm) and Female Swiss albino mice CD-1 strain (20 ± 2 gm) were obtained from Schistosome Biological Supply Program (SBSP), Unit of Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Mice were infected subcutaneously with *S. mansoni* cercariae 75 ± 5 cercariae/mouse (Peters and Warren, 1969) shed out from experimentally infected *B. alexanderina* snails according to each group of the experiment.

Tested drugs

Nigella sativa oil (CAP pharm, Egypt) was administered to mice orally with 0.2 mg⁄kg of body weight (three times per week) for 4 weeks starting from 1st day post infection (EL Shenawy *et al*., 2008).

Artemether (Ipca Laboratories Ltd India) was intramuscularly injected as a single dose of 300 mg⁄kg of body weight; after 49 days post infection (Jiraungkoorskul *et al*., 2005).

Praziquantel (SEDICO, Egyptian Pharmaceutical Industries Company) was administered orally as a suspension in distilled water each tablet of Praziquantel contains 600 mg. PZQ was freshly prepared and used as a single oral dose 300 mg⁄kg of body weight; after 49 days post infection (Chaiworaporn *et al*., 2005).

Experimental design

In the present study, ten groups of female CD-1 mice, ten mice each were divided as the following: **Group 1:** normal control, **Group 2:** orally administered with 0.2 mg/kg *Nigella sativa* oil (three times per week) for 28 days, **Group 3:** injected intramuscularly with 300 mg/kg of artemether as a single dose, **Group 4:** orally administered with 300 mg/kg PZQ as a single dose, **Group 5:** infected with *S. mansoni*, **Group 6:** infected and orally administered with 0.2 mg/kg *Nigella sativa* oil (three times per week) for 28 days, **Group 7:** infected and injected intramuscularly with 300 mg/kg artemether as a single dose after 49 days of infection, **Group 8:** infected and orally administered with 300 mg/kg PZQ as a single dose after 49 days of infection, **Group 9:** infected and orally administered with 0.2 mg/kg *Nigella sativa* oil (three times per week) for 28 days combined with injected intramuscularly with 300 mg/kg artemether as a single dose after 49 days of infection and **Group 10:** infected and orally administered with 0.2 mg/kg *Nigella sativa* oil (three times per week) for 28 days combined with orally administered with 300 mg/kg PZQ as a single dose after 49 days of infection. At the end of the experiment, all animals were sacrificed.

Liver histology and Granuloma measurements

The liver from mice of each experimental group was excised and washed with ice-cold saline solution. After fixation in 10% formaline, specimens were dehydrated in an ascending series of alcohol (70, 80, 90 and 100%). The specimens were cleared in xylene and embedded in molten paraplast at 60ºC. Serial sections were cut at 5 µ thickness and stained with Ehrlich's haematoxylin and counterstained eosin (Romeis, 1989). Histological sections were photographed using Olympus b x. 41, Japans microscope, photo automated camera.

Granulomas containing a single egg (with intact or degenerated miracidia) were selected for measurements. Granulomas were measured by ocular micrometer. The mean granuloma diameter (MGD, 40-50/mouse) was calculated (Mahmoud and Warren, 1974). The reduction percentage in granuloma diameter relative to the infected controls was calculated.

Immunological studies

Blood samples were collected and left at room temperature to clot. Sera were separated by centrifugation at 3000 rpm for 5 minutes and kept at -20 ºC until use for the immunological studies.

Determination of IgG in serum

The level of serum IgG was determined according to the method of Wilson *et al*. (2006) using a commercial ELIZA kit (Mouse IgG GenWay Biotech, Cat. No. CA 92121). Absorbance was measured on ELIZA plate reader at 450 nm.

Determination of Interleukin-2 in serum

The level of serum IL-2 was determined according to the method of Kaufman *et al*. (2002) using a commercial ELIZA kit (Mouse Interleukin-2 –Thermo scientific, Cat. No. EMI3747). Absorbance was measured as pg/ml on ELIZA plate reader at 450 nm for 30 mins from stopping the reaction.

Determination of Interleukin-12 in serum

The level of serum nterleukin IL-12 was determined according to the method of Huang *et al*. (1999) using a commercial ELIZA kit (Mouse Interleukin-12 p70-Biosource, Cat. No. KMC9121). Absorbance was read as pg/ml at 450 nm.

Determination of tumor necrosis factor TNF-α in serum:

The level of serum TNF- α was determined according to the method of Chan and Perlstein (1987) using a commercial ELIZA kit (Thermo-Scientific, Cat. No. K1347). Absorbance was measured as pg/ml on ELISA reader at 450 nm.

STATISTICAL ANALYSIS

Data were presented as mean ± standard error (Mean ± SE). All numerical data were analyzed using Statgraphics 4.1 plus software, using a rejection level of $P < 0.05$ using one–way ANOVA. Bartlett's test was performed for variance check. Where ANOVA could not be applied, a non-parametric ranking test was used (Kruskal Wallis test).

RESULTS

Effect of *Nigella sativa* oil combined with artemether or praziquantel on liver histology and hepatic granuloma diameter of *Schistosoma mansoni* infected mice

Liver of laboratory control animals consists of hepatic lobules; each lobule consists of hepatocytes organized into strands that radically arranged around a central vein. The spaces between these strands are called sinusoids that extend along the liver lobules. Sinusoids are irregularly dilated blood vessels that composed of discontinuous layer of fenestrated endothelial cells. Sinusoids contain phagocytic cells of the mononuclear phagocytes system called Von Kupffer cells derived from blood monocytes. These cells arrange on the luminal surface of the endothelial cells. Hepatocytes are polyhedral, round cells having eosinophilic cytoplasm. A portal vein is found in portal space that is wide in size (Fig. 1A).

Non-infected group treated with *N. sativa* showed abnormal hepatic cells which has pyknotic nuclei with a cytoplasmic vacuolization (Fig. 1B). On the other hand, examination of hepatic tissues of non-infected mice treated with artemether showed loss of their normal shape, the cytoplasm of the hepatocytes had a space on one direction. The nucleus of hepatocytes appeared enlarged and the central veins congested with blood cells (Fig. 1C). Administration of PZQ in non-infected mice showed hepatocytes lost their normal shape with enlarged sinusoid spaces (Fig. 1D).

Granuloma formation with different sizes occurred in all *S. mansoni* infected mice. *Schistosoma* ova were randomly lodged in liver tissue, although clustering of eggs was seen some times. Granulomas composed of numerous macrophages, eosinophilic granulocytes, lymphocytes and fibroblasts. The hepatic tissue around the granuloma revealed many histopathological changes such as inflammation, leucocytes infiltration and congestion of intra hepatic vein (central and portal veins, Fig. 2A). *S. mansoni* –infected mice liver treated with *N. sativa* showed fibrocellular granuloma with more inflammatory cells near granuloma. In addition, the fibrocelluar granuloma becomes smaller than control-infected group (Fig. 2B). Treatment with artemether of *S. mansoni*-infected mice showed different size of granulomas. Also sever leucocytes inflammatory cells were noticed (Fig. 2C). Combination of *N. sativa* and artemether and *S. mansoni* –infection caused size reduction of the developing liver granuloma. In addition, it reduced the inflammatory cells (Fig. 2D). Liver section of *S. mansoni* infected mice treated with PZQ showed granulomas with different sizes. It characterized by damaged liver structure and severe leucocytes inflammatory cells (Fig. 2E). Combination of *N. sativa* and PZQ treatments of *S. mansoni* –infected mice showed reduction of fibrocellular granuloma when compared to control-infected liver groups. In addition, increased leucocytes inflammatory cells and abnormal hepatocytes were observed (Fig. 2F).

The results indicated a significant reduction ($P < 0.001$, Kruskal wallis, Figs. 7, 1 and 2) in the mean diameter of hepatic granuloma in all treated groups when compared to infected control group. Combination of *N. sativa* and artemether and/or PZQ treatments recorded marked reduction of hepatic granuloma diameter by 35.42 % and 32.23 %, respectively.

Effect of *Nigella sativa* oil combined with artemether/ praziquantel on immunological parameters in serum of *Schistosoma mansoni*-infected mice

Serum IgG

The level of total serum (IgG) antibodies of non-infected mice treated with *N. sativa* or artemether or PZQ alone recorded significant decrease (Fig. 3, $P < 0.001$, Kruskal wallis) than normal control group. Combined treatment with *N. sativa*, artemether or PZQ of *S. mansoni*-infected groups recorded marked increase in the levels of serum IgG antibodies (684.24 ± 3.03, 647.42 ± 2.21 and 708.50 ± 18.06, respectively) when compared to infected control (570.84 ± 6.55).

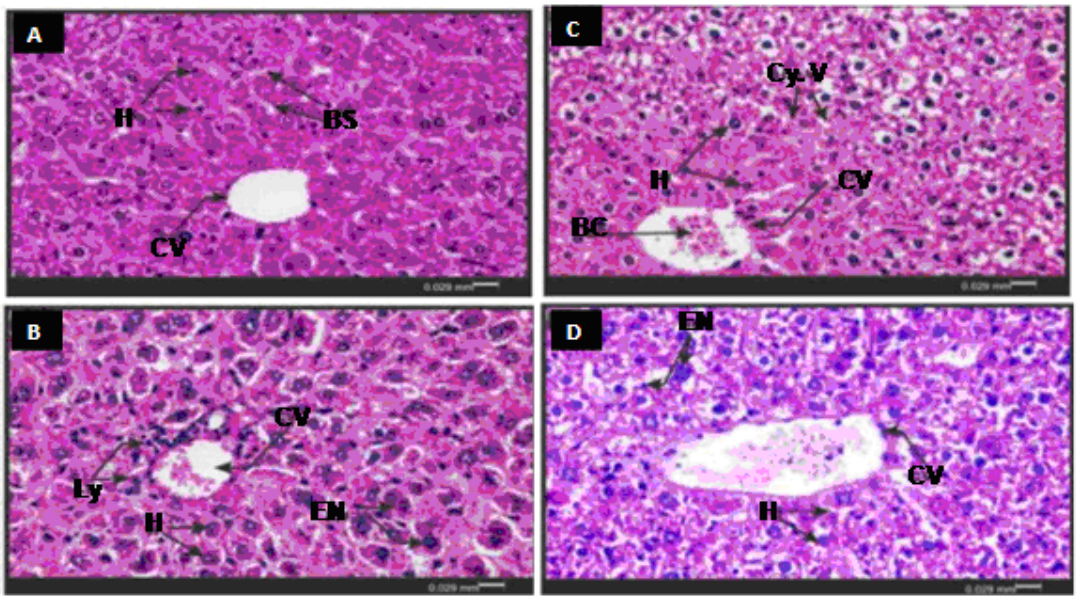

Fig. 1. Light photomicrographs of mice liver stained with Hematoxylin and Eosin. (A). Normal control liver, showing cords of hepatocytes (H) radiating from the central vein (CV) and separated by blood sinusoids (BS), (B). Control treated liver with *N. sativa*, (C). Control treated liver with ART, (D). Control treated liver with PZQ.

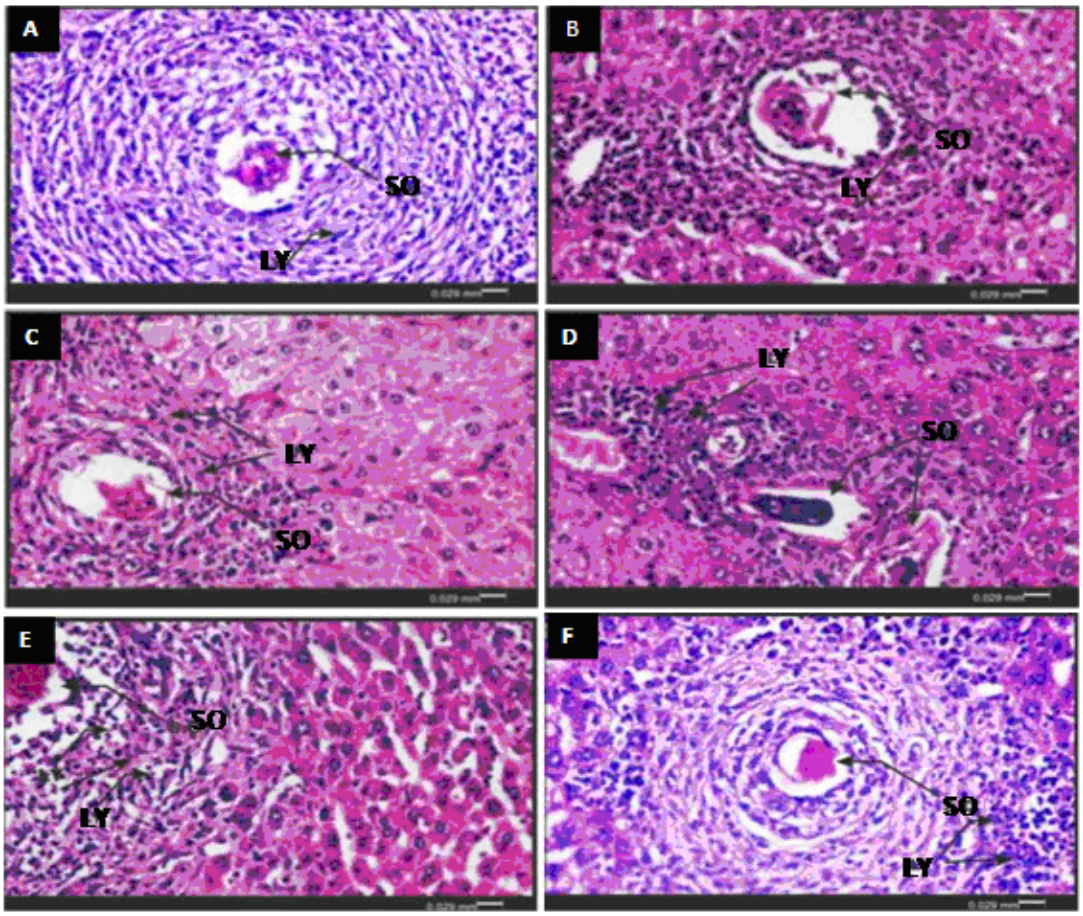

Fig. 2. Light photomicrographs of mice liver stained with Hematoxylin and Eosin. (A). *S. mansoni* infected liver, (B). *S. mansoni* infected treated liver with *N. sativa*, (C). *S. mansoni* infected and treated liver with ART, (D). *S. mansoni* infected treated liver with *N. sativa* combined with ART, (E). *S. mansoni* infected treated liver with PZQ and (F). *S. mansoni* infected treated liver with *N. sativa* combined with PZQ. cytoplasmic vacuolization (Cy. V), blood cells (BC), enlarged nucleus (EN), lymphocytes (LY), granulomas (G) and schistosomal ovum (SO).

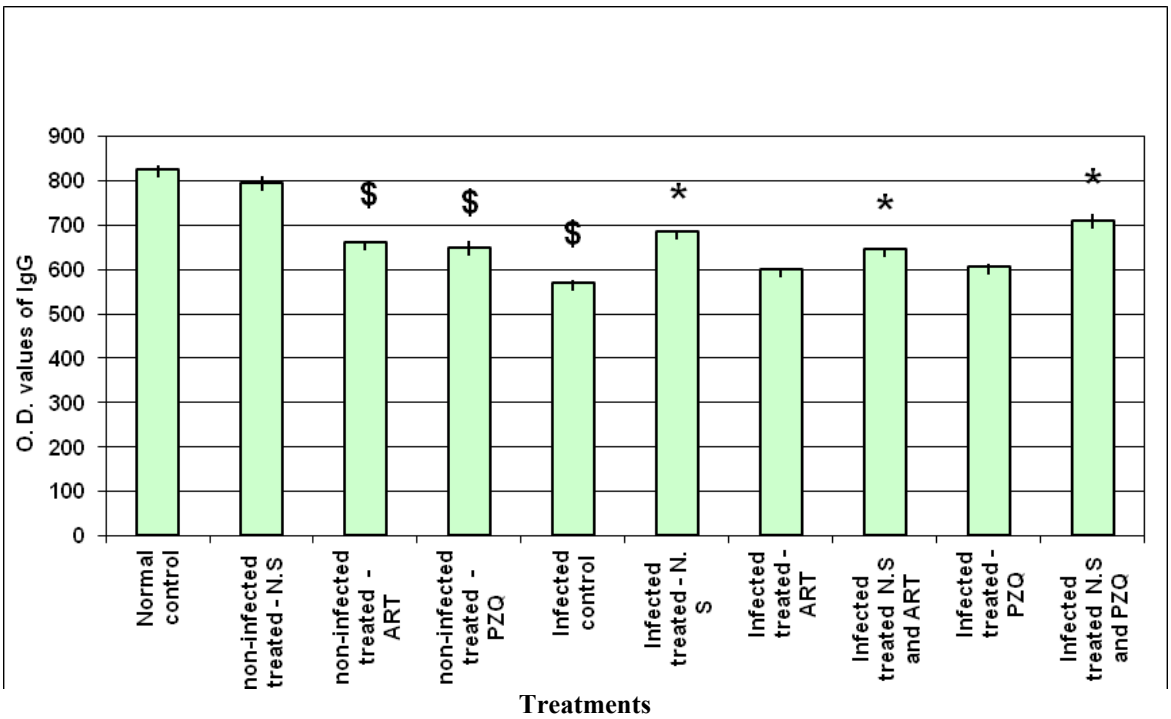

Fig. 3. Level of serum IgG of *S. mansoni*-infected mice treated with *N. sativa* oil combined with ART or PZQ. (*) represents significant differences compared to control infected group, ($) represents significant differences compared to normal control group when $P < 0.001$, (Kruskal- wallis).

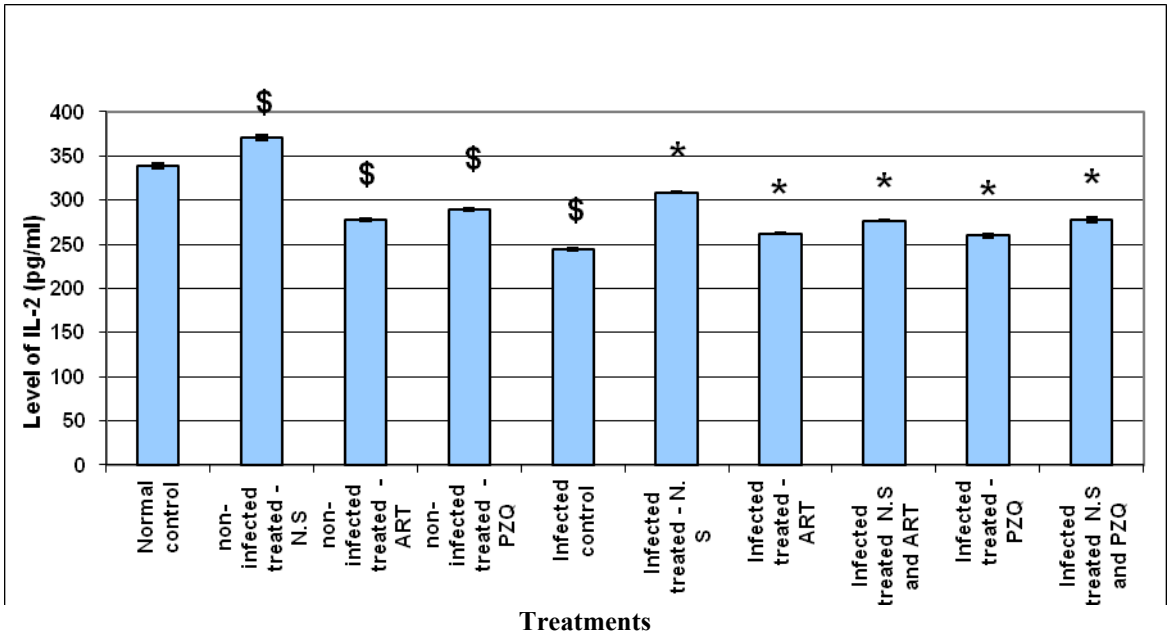

Fig. 4. Level of serum interleukin-2 in *S. mansoni*-infected mice treated with *N. sativa* oil combined with ART or PZQ. (*) represents significant differences compared to control infected group, ($) represents significant differences compared to normal control group when $P < 0.001$, (Kruskal- wallis).

Serum IL-2

The serum levels of interleukin **IL-2** after administration of *N. sativa* of non-infected groups recorded significant increase (Fig. 4). Treatment with artemether or PZQ of non-infected mice showed a significant decrease ($P <$ 0.001, Kruskal wallis) when compared to normal control group. Meanwhile, a significant increase in IL-2 serum levels ($P < 0.001$, Kruskal wallis) was recorded in all animals after treatment with *N. sativa* combined with artemether or PZQ (276.95 ± 1.22 and 277.63 ± 3.14

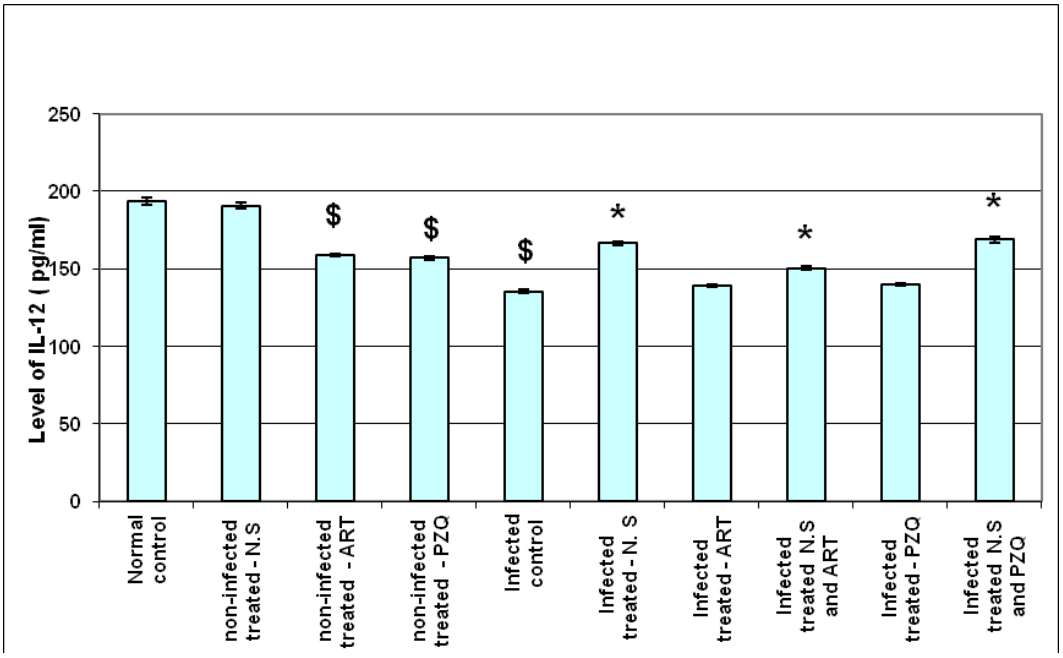

Fig. 5. Level of interleukin-12 in serum of *S. mansoni*-infected mice treated with *N. sativa* oil combined with ART or PZQ. (*) represents significant differences compared to control infected group, ($) represents significant differences compared to normal control group when $P < 0.001$, (Kruskal-wallis).

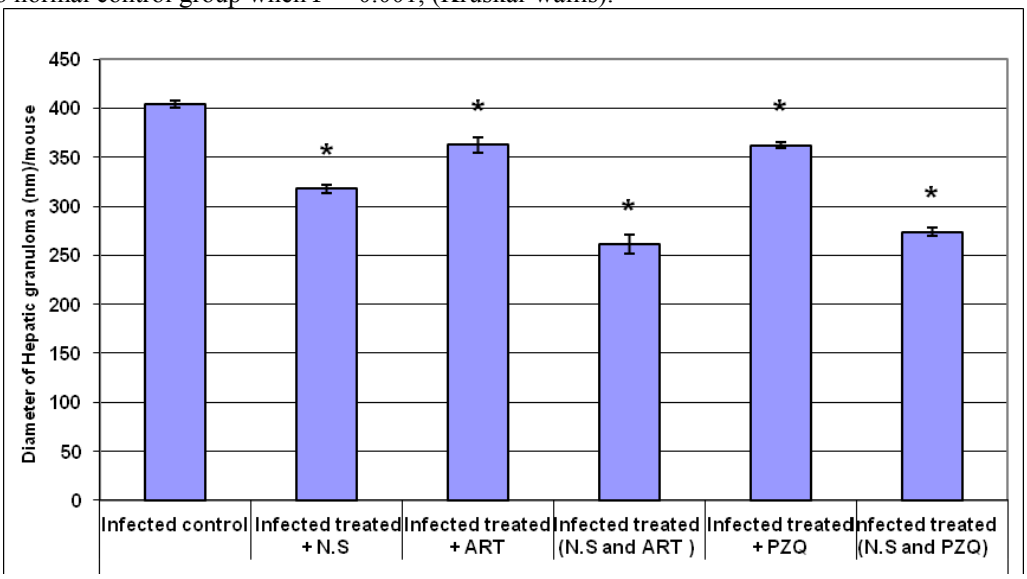

Fig. 6. Level of tumor necrosis factor-alpha (TNFα) in serum of *S. mansoni*-infected mice treated with *N. sativa* oil combined with ART or PZQ. (*) represents significant differences compared to control infected group, ($) represents significant differences compared to normal control group when $P < 0.001$, (Kruskal-wallis).

pg/ml, respectively) when compared to infected control (243.76 ± 1.49 pg/ml).

Serum IL-12

Treatment with *N. sativa* or artemether or PZQ of non-infected mice showed significant decrease in production of IL-12 ($P < 0.001$, Kruskal wallis, Fig. 5) when compared to normal control. On the other hand, the effect of *N. sativa* alone or combined with artemether or PZQ treatment of *S. mansoni*-infected group showed significant increase ($P < 0.001$, Kruskal wallis) in IL-12 production (150.44 ± 1.04 and 169.01 ± 1.95 pg/ml, respectively) when compared to infected control group (135.36 ± 1.04 pg/ml**)**. While, infected-treated animals

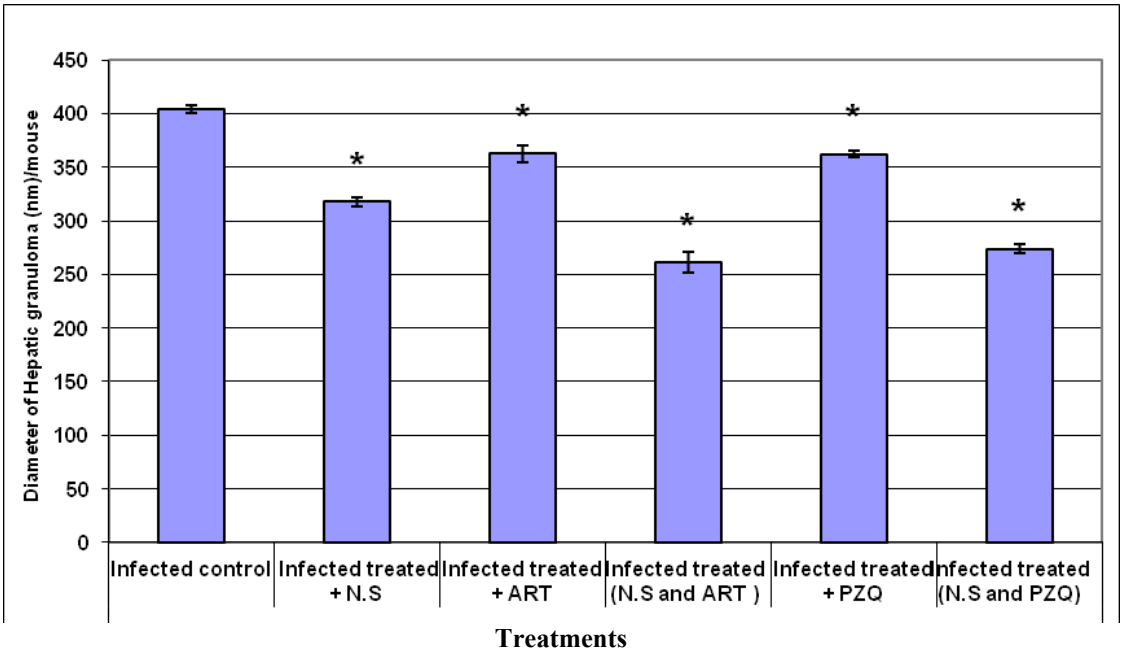

Fig. 7. Effect of *N. sativa* oil combined with ART or PZQ on hepatic granulomas of *S. mansoni* infected mice. (*) represents significant differences compared to control infected group, when $P < 0.001$, (Kruskal- wallis).

with artemether or PZQ showed non-significant differences when compared to infected control group.

Serum TNF-α

The serum levels of tumor necrosis factor-alpha (**TNF-α**) after treatment with *N. sativa* or artemether of non-infected mice recorded significant increase (Fig. 6, $P<0.001$, Kruskal wallis) when compared to normal control group. In the same pattern, treatment with *N. sativa* alone or combined with artemether or PZQ in *S. mansoni* infected mice showed significant increase ($P < 0.001$, Kruskal wallis) with values 1053.9 ± 3.35, 940 ± 1.31 and 987.15 ± 13.52 pg/ml, respectively when compared to infected control (751.57 ± 3.62 pg/ml).

DISCUSSION

In the present study, histological examination of the liver of treated mice with *N. sativa*, artemether or PZQ showed tissue abnormalities. The liver of *S. mansoni* – infected mice showed many lesions of granulomatous inflammation and fibrosis around eggs. However, the pathological alterations observed, especially granuloma diameter in the liver of *S. mansoni*-infected mice were remarkably reduced after treatment with *N. sativa* combined with artemether or PZQ. Treatment with *N. sativa* significantly reduced number of eggs in liver and intestine and increased dead and immature eggs (Mohamed *et al*., 2015). In the liver, the parasite-egg antigens induced granulomatous inflammatory reactions, involving macrophages, eosinophils, lymphocytes and hepatic satellite cells. Activated hepatic satellite cells synthesize collagens I and III, and alpha-smooth muscle. These collagens were fibril forming in nature, contributing to a significant part of the scar tissue in liver fibrosis (Schuppan, 1990, Chatterjee *et al*., 2002; Mourra *et al*., 2006). Pearce and McDonald (2002) stated that granuloma formation requires the recruitment of inflammatory cells from bloodstream, via up-regulation of adhesion molecules on activated vascular endothelial cells, induced by cytokines and chemokines released at the site of inflammation. Mahmoud *et al*. (2002) recorded smaller fibrocellular granuloma with less inflammatory cells after treatment with *N. sativa* oil alone or in combination with PZQ for 2 weeks starting from week 7 post infections. Soliman and El-Shenawy (2003) reported that treatment of schistosomiasis in mice with *N. sativa* oil induced less sever pathological changes in the liver, particularly the frequency of the inflammatory reactions; mediating both granuloma size and numbers. The effect of *N. sativa* on *S. mansoni* infected liver may be due to its important role of anti-oxidant processes of the oxidative stress in mediating liver injury in schistosomiasis which increased production of reactive oxygen intermediates by eosinophils and macrophages at the site of granulomatous inflammation (McCormick *et al*., 1996). PZQ treatment reduced the hepatic granuloma with small fibrocellular granuloma, few inflammatory cells and excess fibrous collagen tissue (El-Banhawey, 2007; Botros *et al*., 2008). Administration of a combination more than one drug like PZQ and artemether (Botros *et al*., 2010), pentoxifylline and PZQ (El-Lakkany *et al*., 2011b), PZQ and alpha lipoic acid (Abdel-Hafeez *et al*.,

2012) silymarin or PZQ (Tousson *et al*., 2013) and N-acetyl-L-cysteine and PZQ (De-Lima *et al*., 2012) inhibited and improved the histopathological effects of granuloma in the liver of *S. mansoni* infected mice.

In this study, the level of total serum (IgG) antibodies significantly decreased under the effect of infection with *S. mansoni* and treatment with *N. sativa*, artemether or PZQ of non-infected mice. However, treatment of *S. mansoni* infected mice with *N. sativa* as well as the combination of artemether or PZQ resulted in a significant increase in the total serum (IgG). These results are in agreement with Abbas *et al*. (2005) who showed that *Nigella sativa* was significantly reduced peripheral blood eosinophil count, IgG1 and IgG2a levels in lung tissue in mice. Hanallah *et al*. (2003) showed that serum immunoglobulins, in mice infected with PZQ-insensitive *S. mansoni* isolate have significantly lowered IgG and IgG1 after 8 and 10 weeks post infection. Moreover, Allam (2009) showed that curcumin treatment of *S. mansoni* infected mice augmented both IgG and IgG1 responses against both soluble adult worm and egg antigen (SWAP) and (SEA), respectively. In addition, Mantawy *et al*. (2011) showed that the level of IgG increased after treatment of *S. mansoni*-infected mice with dry extract of onion or garlic, individually or mixed, with or without PZQ compared to the untreated infected mice. They added that PZQ treatment in normal healthy mice decreased the level of IgG, while, the level of IgG increased in *S. mansoni* infected mice treated with PZQ. In a recent study Allam and Abuelsaad (2013) studied the effect of hesperidin (a flavanone glycoside found abundantly in citrus fruits) as an antioxidant of *S. mansoni* infected mice and showed significant increased in specific IgG level.

In the present study, serum levels of IL-2, IL-12 and TNF-α significantly decreased in *S. mansoni* infected mice compared to normal control as well as treatment with artemether or PZQ of non-infected mice. However, treatment of *S. mansoni* infected mice with *N. sativa* as well as the combination of artemether or PZQ resulted in a significant increase in IL-2, IL-12 and TNF-α activities as well as treatment with *N. sativa* of non-infected mice. These results are in disagreement with El-Lakkany *et al*. (2011a) who showed that *S. mansoni* infection produced remarkable elevations in the serum level of TNF-α. This dissimilarity of results is due to sampling time of our experiment, i.e., after granuloma formation. Mosmann and Coffman (1989) reported that the initial CD4T helper 1 (Th1) type is associated with stimulation of TNF and IL-2. It causes vigorous granuloma formation in acute schistosomiasis (Van-Die *et al*., 2010). So, Th1 cells are present at 3-5 week's early granuloma development (Hassan *et al*., 2000). Furthermore, once granuloma size decreases during schistosomiasis, the infection stage switches from acute to chronic phase (Stadecker and Villanueva, 1994). Alterations in the cytokine network could be related to the physiopathology of schistosomiasis (Yu *et al*., 2012). Another study, Umar *et al*. (2012) showed that oral administration of thymoquinone (TQ) caused reduction of the pro-inflammatory mediators levels (IL-1β, IL-6, TNF-α and IFN-γ) and increased level of IL-10 in the rats by collagen induced arthritis. So, that explains the increase in the present study cytokines, which may be due to the suppressive effect of *N. sativa* on blood cytokines in mice liver inflammation. In 1999, Romagnani studied thymoquinone regulation of T_H1 and T_H2 balance, it was shown that the inflammatory condition of an asthmatic lung is regulated by the balance of these two mutually, T_H1 cells produce IL-2 and TNF-α, whereas T_H2 cells produce IL-4 and IL-10. T_H2 cells promote the activity of macrophages and regulate the pro-inflammatory response, whereas T_H1 cells inhibit the activity of macrophages directly or indirectly by inhibiting T_H2 activity and thus regulate the anti-inflammatory response. Another study, Wang *et al*. (2007) reported that female mice treated with artemether in vitro or in vivo suppressed production of the cytokines IL-2 and IFN- γ. Also, Liang *et al*. (2011) showed that anti-fibrosis treatment with PZQ significantly inhibited gene expression of both Th1 and Th2 cytokines, and suggested that the alteration of Th1/Th2 cytokines in hepatic microenvironment after PZQ treatment. Mantawy *et al*. (2011) showed that the level of TNF-α and IL-2 increased after treatment of *S. mansoni*-infected mice with dry extract of onion or garlic, individually or mixed, with or without PZQ. Aly and Mantawy (2013) concluded that oral administration of ginger extract (daily for 45 days beginning at 2nd day) against infection with *S. mansoni* in mice caused reduction in TNF-α and IL-12 compared with control group.

CONCLUSION

It can be concluded that *N. sativa* combination with artemether or PZQ accelerated healing of the pathological granulomatous lesions of the liver architecture and improved host immunity by stimulating its cytokines.

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