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EFFECT OF *NIGELLA SATIVA* OIL ON *SCHISTOSOMA MANSONI* MATURE WORMS IN EXPERIMENTALLY INFECTED MICE

By

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Abstract

The chemical drugs are safe and effective being the main tool in controlling schistosomiasis. Referring to the possibility of appearance of drug resistant parasites especially with retreatment regimens in endemic areas, search for new schistosomicidal is essential. *Nigella sativa* has been extensively studied for its biological activities and therapeutic potentials and shown to possess wide spectrum of activities including antihelminthic and antiprotozoal activities. The present report aimed to investigate the prophylactic and therapeutic effect of *Nigella sativa* oil in treatment of *Schistosoma mansoni*. *Nigella sativa* oil was administrated orally to *S. mansoni* infected mice in two different doses, a prophylactic dose (1.14g/kg) every day for 2 weeks before infection and a therapeutic dose (1.14g/kg) daily for 4 weeks starting from 2nd day post infection aiming to investigate its potential therapeutic role. Therapeutic treatment with *Nigella sativa* oil had a high significant effect on mature worm burden, as the reduction percentage was (57.5%). *Nigella sativa* oil in prophylactic and therapeutic doses significantly increased the mean number of dead ova (14.75±6.5) and (21.43±3.64) respectively in comparison to control (5.25±1.71). *Nigella sativa* oil therapeutic dose had a high reduction effect on ova count in intestinal tissue (81.45%) and a moderate reduction on ova count in hepatic tissue (57.86%). Prophylactic dose gave a high reduction effect (78.76%) on ova count in intestinal tissue. As regard the size of hepatic granuloma, *Nigella sativa* oil treated group had the highest significant reduction on mean granuloma diameter; the reduction was 26.69% with marked improvement of hepatic pathology. *N. sativa* oil treated group showed obvious improvement of liver pathology with mild hydrobic degeneration and small sized fibrocellular granuloma accompanied by multiple malformations in both *S. mansoni* adult worms. These results showed that *N. sativa* oil has remarkable effect on mature *S. mansoni* which could be helpful for potentiating Praziquantel effect and thus reducing development of resistance.

Keywords: *Nigella sativa, Schistosoma mansoni*, mature worms.

Introduction

Schistosomiasis is a neglected disease that affects approximately 240 million people worldwide (WHO, 2012). The used drugs for treatment of schistosomisasis are Praziquantel (PZQ) and Oxamniquine. However, any parasitic treatment based on the use of a single drug possesses serious concerns regarding the onset of resistance (Castro *et al,* 2013).

There is an increasing awareness of the therapeutic potentials of natural products and medicinal plants which were considered to be less toxic and free from side effects than synthetic drugs in treating some diseases (Queiroz *et al,* 2009). Larger number of medicinal plants and their purified constituents were thoroughly investigated for medicinal properties, potentials, mechanism of action, safety evaluation and toxicological studies (Salem, 2005). The miracle herb, *Nigella sativa* seeds have been employed as food preservative and a spice (Al-Ali *et al,* 2008). The chemical composition of the seeds is oil 31-35.5%, protein 16-19.9% and various chemical composition of fixed and volatile oils (El-Tahir *et al,* 2006; Nickavar, 2003). Thymoquinone (TQ) is an active chemical component of *Nigella sativa* essential oil, of which many therapeutic properties

included (Abdel Hady *et al,* 2008). Historically, *Nigella sativa* seeds were prescribed by ancient Egyptian and Greek physician to treat many diseases (Goreja, 2003). Its oil exhibited a wide range of pharmacological activities including anti-inflammatory (Chakra-barty *et al,* 2003), anti-microbial (Bakathir and Abbas, 2011; Barakat *et al,* 2013) and anti-oxidant (Bourgou *et al,* 2012). It has anticestodal and antinematodal properties (Agrawal *et al,* 1979). *N. sativa* oil has antiparasitic effect against some protozoal infections as *Entamoeba histolytica* (Shariff *et al,* 2011), *Trichomonas vaginalis* (Tonkal, 2009) and *Plasmodium yoelii* (Okeola *et al,* 2011).

This present work aimed to explore the potential role of *Nigella sativa* oil either therapeutic or prophylactic in treatment of *Schistosoma mansoni*.

Materials and Methods

Herbal extract: *Nigella sativa* oil (Family Ranunculaceae) was obtained as soft gelatin capsules of 450mg (Pharco-Pharmaceutical, Alexandria). Capsules were opened and oil was dissolved in corn oil (Sharafeldin *et al,* 2015) to obtain a concentration of 1.14gm /kg (Soliman and El-Shenawy, 2003).

Parasites and animals: Cercariae of *Schistosoma mansoni* were obtained from infected *Biomphalaria alexandrina* reared and maintained at Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute. 120 laboratory-bred male Swiss albino mice, CD1 bred, were used. All mice were infected with 60±10 *S. mansoni* cercariae suspended in 0.2ml water via subcutaneous injection.

Experimental design: Infected mice were divided into 6 groups. G1: Non-infected non-treated. G2: Infected non-treated. G3: Infected and treated with *N. sativa* oil (1.14g/kg) daily for 2 weeks before infection, (prophylactic).G4: Infected and treated with Praziquantel (500mg/Kg) for 2 successive days at 6^{th} week of infection. G5: Infected and treated with *N. sativa* oil (1.14g/kg) daily for 4 weeks starting from 2^{nd} day post infection,(therapeutic group).Group 6: Infected and treated with *N. sativa* oil (1.14 g kg) daily for 4 weeks starting from 2^{nd} day post infection and Praziquantel (500mg/ Kg) for 2 successive days at 6^{th} week of infection. All mice were sacrificed after the 8^{th} week post infection.

Evaluation of *N. sativa* oil schistosomicidal effect: Worm Burden: Adult schistosome recovery was assessed by porto-mesenteric perfusion technique according to the method of Duvall and DeWitt (Duvall and Dewitte, 1967).

Oogram pattern: The pattern (Pellegrino *et al*, 1962) evaluated degree of ova maturity and viability reflected the drug action affecting oviposition and maturation. After perfusion, three fragments (each 1cm in length) of small intestine were cut longitudinally, rinsed in saline, slightly dried on filter paper and then compressed between two glass slides .One hundred eggs were counted as a rule in each fragment and this was repeated with other fragments until a total 300eggs were obtained and classified into three types: immature, mature and dead ones.

Histopathological examination: Mice livers were fixed for 48hr in 10% buffered formalin and then embedded in paraffin. Five sections (5 microns in thickness) were taken from each liver specimen, each section being at a distance of at least 500µm from the preceding one (Von Lichtenberg, 1962). Sections were stained with hematoxylin and eosin (Harris, 1900) for granuloma counting and Masson trichrome stains (Masson, 1929) for collagen fibers. Lesions with a single ovum in their centers were selected for measurement (Botros *et al,* 1986). The greatest diameter of the lesion was obtained, then the ocular micrometer was rotated 90 degrees and diameter perpendicular to 1^{st} one was measured. Size of each liver granuloma was calculated from mean diameter of each lesion on assumption they were spherical (Mahmoud and Warren, 1974). For each section, granulomas were counted in five successive fields (10x10).

SEM: Perfused hepatic & porto-mesentric *S. mansoni* were collected in glutaraldehyde buffer solution (25%) as a fixative and left overnight at 4°C. Worms were washed off from traces of fixative by kept overnight at 4°C in phosphate buffer. They were passed in increasing concentrations of alcohol (30, 40, & 50%) each for 15 min. Worms were left in 70% ethanol, washed twice for 30min in 80 & 90% ethanol, mounted on stainless steel holders, placed in a drier for about 30 min, and then subjected to a sputter coat of gold. Worms' parts were examined using Joel JEM-1200 SEM (Hassan *et al*, 2003).

Statistical analysis: Data were recorded on an investigation report form, tabulated, coded then analyzed using computer program SPSS version 16. ANOVA (analysis of variance) test used to compare between more than two groups of numerical (parametric) data and Kruskal Wallis test between more than two groups of numerical (non- parametric) data. Student's t-test used to compare between mean of two groups of numerical (parametric) data. For continuous non- parametric data; Mann-Whitney U-test were used for inter-group analysis. P value <0.05 was statistically significant & P value <0.01 was highly significant in all analyses.

Results

The results showed that *Nigella sativa* oil treated group has a high significant effect on mean number of total mature worm burden (R 57.51%), also prophylactic group showed significant effect on mean number of mature worms compared with the control group (R 40.91%). The highest significant decrease on mean number of total mature worm burden was observed in PZQ treated group (R 93.96%) followed by the combination group (R93.74%).

N. sativa oil showed high significant effect on reduction rate of couples whether given alone or combined with PZQ (R 68.67% & R 91.67% respectively). Male worms were more sensitive (R 97.5%) to combined therapy than females (R89.28%).

Nigella sativa oil treated group showed a high significant effect on mean number of female worms (R65.92%) more than males (R 50%),while Non-significant reduction was observed in male and female worm burden in group received prophylactic treatment in comparison with control group, as it reached (R47.57%)&(R 33.28%) respectively (Tab. 1, Fig. 1).

In oogram pattern, *N. sativa* oil treated group gave a high significant increase on mean of dead ova (21.43±3.64)(which is 4.08 times as the control)and a high significant decrease on percentage of immature ova (31.0±8.49), while it has no effect on mature ova (47.57±10.7). In group treated with combined therapy, *Nigella sativa* oil proved to potentiate effect of PZQ, with the complete disappearance in immature ova (0.0±0.0), highest significant increased % of dead ova (98.2±4.02) and significantly decreased % of mature ova (1.8±4.02). Prophylactic group showed significantly increased percentage of dead ova (14.75±6.5) 2.8 times as control but, without significant effect on mature (30.5±5.26) and immature ova (54.75±7.8) (Tab. 2, Fig. 2).

The most evident reduction in the ova count ova/gm of both intestinal and hepatic tissues was in group treated with combined therapy (R91.76%&R 75.76%respectively). In *N. sativa* oil treated group, there was a high reduction in ova count in intestinal tissue (R 81.45%) and a moderate reduction in hepatic tissue (R57.86%) as compared with control. In the present work, lowest reduction% in ova count/gram of hepatic tissue was in pro-phylactic group (R 51.46%) and reached (R 78.76%) in intestinal tissue (Tab. 3, Fig 3).

The highest significant reduction in mean granuloma diameter was in combined therapy (R 27.06%). *N. sativa* oil group exerted a high significant reduction of granuloma size compared with control (R 26.69%). Least decrease in granuloma diameter was in pro phylactic group (R 14.85%)(Tab.4, Fig 4).

In infected untreated mice, histopathology of liver sections showed inflammatory cellular infiltrate mainly lymphocytes, with hydropic degeneration of hepatocytes and dilated sinusoids that lined by hyperplastic pigmented Von Kupffer cells. Sections of *S. mansoni* infected mice treated with therapeutic and prophylactic doses of *N. sativa* oil and scarified 8 weeks post-infection showed inflammatory cellular infiltrate mainly lymphocytes with improvement of hydropic degeneration of hepatocytes in the therapeutic group with dilated sinusoids lined by hyperplastic pigmented Von Kupffer cells. But, infected mice treated with combined therapy showed slight improvement of cloudy swelling and hydropic degeneration, fibrocellular granulomas formed mainly of monocytes and plasma cells.

SEM of *S. mansoni* untreated male showed intact tegument with normal tubercles covering its surface. Tubercles were covered with numerous intact apically directed spines with normal intertubercular spaces in between, also oral sucker and ventral sucker were intact.

SEM of the dorsal surface of adult male *S. mansoni* of prophylactic group showed extensive swelling of tubercles with complete loss of spines, wrinkles and vesicles on dorsal tegumental surface. The oral sucker was swollen and ventral one was normal. SEM of dorsal surface of treated adult male *S. mansoni* from mice infected and treated with praziquantel showed severe reduction in number and size of tubercles, being swollen and destructed in other parts with complete loss of spines. Multiple vesicles, peeling, erosions and destruction of intertegmental spaces were seen. In adult male of *N. sativa* oil treated group, there were severe reduction in number and size of tubercles being swollen in some parts and collapsed or destructed in others with complete loss of spines and destruction of inter-tegumental spaces. There were multiple pits in tegument.

The oral and ventral suckers were retracted in both sexes with swelling of anterior part of male and constriction of worm posterior end. Female tegument showed extensive peeling and blabbing. The combined therapy changed tegumental of adults. The SEM indicated severe reduction in number and size of tubercles being swollen, collapsed and destructed in other parts of the dorsal tegumental surface of the worms with complete loss of spines and presence of multiple vesicles plus retraction in oral sucker and ventral sucker.

Table 1: Effect of *Nigella sativa* oil, PZQ and combination of both on mature worm burden in *S. mansoni* infected mice.

Groups	Mean mature worm burden (%reduction) P value			
	Couple (R%)	Male (R%)	Female (R%)	Total worms (R%)
Control	6.0±1.83	7.0±2.16	6.25±1.26	13.25±2.5
Prophylactic	3.17±1.6 (47.17%) (0.032*)	3.67±1.03 (47.57%) (0.011*)	4.17±1.83 (33.28%)	7.83±2.23 (40.91%) (0.007**)
Praziquantel treated	0.1±0.32 (98.33%) (0.001**)	0.30±0.48 (95.7%) (0.001**)	0.50±0.53 (92%) (0.001**)	0.8±0.92 (93.96%) (0.001**)
Nigella sativa treated	1.88±0.83 (68.67%) (0.001**)	3.5±1.2 (50%) (0.004**)	2.13±0.99 (65.92%) # (0.001**)	5.63±1.69 (57.51%) (0.001**)
Combined	0.50±0.55 (91.67%) (0.009**)	0.17±0.41 (97.5%) (0.006**)	0.67±0.52 (89.28%) (0.008**)	0.83±0.75 (93.74%) (0.009**)

N. sativa oil prophylactic dose: (1.14 g /kg) daily for 2 weeks before infection.
Nigella sativa oil therapeutic dose: (1.14 g /kg) daily for 4weeks starting from 2nd day post infection.
Praziquantel dose: (500mg/ Kg) for 2 successive days at 6th week of infection.
Combined therapy: *Nigella sativa* oil therapeutic dose + praziquantel dose.
*P value <0.05: Significant difference between treated groups versus control group.
**P value <0.01: High significant difference between treated groups versus control group.
Nigella sativa group showed significant difference versus prophylactic group (P value = 0.037*)

Table 2: Effect of *N. sativa* oil, PZQ and both on oogram pattern in intestinal segments of *S. mansoni* infected mice.

Groups	Egg types Mean ± SD P value		
	Mature	Dead	Immature
Control	32.25±10.05	5.25±1.71	62.5±9.43
Prophylactic	30.5±5.26	14.75±6.5 (0.03*)	54.75±7.8
Praziquantel treated	1.8±1.93 (0.004**)	98.1±2.13 (0.001**)	0.10±0.32 (0.001**)
Nigella sativa oil treated	47.57±10.7 (0.045*)	21.43±3.6# (0.001**)	31.0±8.49 (0.001**)
combined group	1.8±4.02 (0.011*)	98.2±4.02 (0.001**)	0.0±0.0 (0.007**)

#N. sativa group showed significant difference versus prophylactic group (P value = 0.008**).

Table 3: Effect of *N. sativa* oil, PZQ and both drugs on mean number of (ova/gram) on hepatic tissue and intestinal tissue of *S. mansoni* infected mice.

Groups	No of ova/gram hepatic tissue (R%) P value	No of ova/gram intestinal tissue (R%) P value
Control	8250.0±5881.89	25990.0±22291.55
Prophylactic	4004.83±1613.44(51.46%)	5520.0±2131.5(78.76%)
Praziquantel treated	2024.0±845.7(75.47%) 0.005**	2339.0±1036.78(91%) 0.004**
Nigella sativa oil treated	3476.33±2396.61(57.86%)	4820.0±2427.15(81.45%) 0.019*
Combined	1999.83±1184.12(75.76%) 0.032*	2141.67±633.19(91.76%) 0.027*

Table 4: Effect of *N.sativa* oil, PZQ and combination of both drugs on mean number and diameter of hepatic granuloma in *S. mansoni* infected mice.

Groups	Mean hepatic granuloma (P value)	
	Mean n. of granuloma(R%)	Mean diameter of granuloma in (µm) (R%)
Control group	10.5±1.19	246.82±37.65
Prophylactic group	6.53±2.0 (37.81%) 0.008**	210.16±33.34 (14.85%)
Praziquantel treated group	4.13±2.75 (60.67%) 0.001**	197.7±25.44 (19.9%) 0.022*
Nigella sativa oil treated group	5.14±2.29 (51.05%) 0.002*	180.94±22.57 (26.69%) 0.003**
combined group	3.17±1.17 (69.81%) 0.001**	180.04±43.16 (27.06%) 0.036*

Discussion

Nigella sativa oil is one of the promising drugs of herbaceous plant with an anti-*Schistosoma* effect (Ali *et al*, 2016). Substances having antioxidant, antimicrobial or cytotoxic other than genotoxic and mutagenic potentials are safe for human consumption (Islam, 2016).

The oil contains significant (10%) amounts of fatty acid ethyl esters. On storage, thymoquinone turns into the dithymoquinonene and higher oligocondensation products. Fatty oil rich in unsaturated fatty acids, such as linoleic acid (50 60%), oleic acid (20%), eicodadienoic acid (3%) and dihomolinoleic acid (10%) are also find in the seeds. Saturated fatty acids (stearic acid and palmitic acid) amount is approximately 30% or less. Also, parts of the essential oil, mostly thymoquinone that is responsible for an aromatic flavor. The oil contains d -limonene, carvone, and a carbonyl compound, nigellone (Ziaee *et al*, 2012). In the present study, *N. sativa* oil proved to have a non-antagonistic effect on PZQ. This was evident in group treated by combined therapy as reduction rate of total mature worm burden more or less the same as group treated by PZQ. These data were in agreement with Mahmoud *et al.* (2002) who reported more reduction in mean number of total worm from 98% to 99% when treated with increased dose of *N. sativa* oil in combination with PZQ. Also, *N. sativa* oil treated group gave a high significant effect on mean number of total mature worm burden. These data more or less went with Ali *et al.* (2016) who reported that the total worm burden reduction percentage increased from 45 to 57 in group treated with *N. sativa* oil at a dose of 250µl/kg/body wright/day after day from the 5^{th} week to 7^{th} week post-infection. This agreed with the reports of El shenawy *et al.* (2008) and Adamu and Dukku (2009). *N. sativa* oil affected the schitosome worms by altering their level of various eicosanoids that in turn enhanced the immune system to cause disintegration of the worms or at least damaged by stop maturation and hence prevent egg-laying (Mahmoud *et al*, 2002).

Moreover, *N. sativa* oil showed high significant effect on reduction rate of couples whether given alone or in combination with PZQ. This also agreed with Yarnell and

Abascal (2011) who noticed separation of the couple after incubation with black seed extract *in vitro* while the control untreated paired worms remain coupled with vigorous activity. Moreover, *Nigella sativa* oil treated group showed more reduction on mean number of female worm burden than reduction in male worm burden. However, Ali *et al.* (2016) who reported more decrease on male worm burden than female one. This difference in sensitivity of both sexes might be due to differences in *S. mansoni* strain used in each study (El-Lakkany *et al,* 2011). Also, these last authors reported that female worms alone were significantly more susceptible than male worms to goyazensolide killing. The oogram pattern for enumeration of the various egg types prove to be an easy and reliable method of evaluating the therapeutic value of anti-schistosomal drugs (Pellegrino *et al,* 1977).

In the current study, *N. sativa* oil treated group showed a high significant increase in percentage of dead ova and a high significant decrease on percentage of immature ova. This result greed with Muriel (2009) who found that *N. sativa* oil alone significantly increased the number of dead ova while hardly affecting the immature ova.

In the present study, Praziquantel gave a high significant decrease on percentage of immature (0.10±0.32) and mature ova (1.8±1.93).

This result agreed with Khalil (2000) who stated that PZQ treatment showed a complete disappearance of all immature ova from the wall of the intestine, a reduction in the number of mature ova and a four folds increase in dead ova .In the group treated with combined therapy, *Nigella sativa* oil proved to potentiate the effect of PZQ, as there was complete disappearance in the immature ova ,also, it has the highest significant increase in the percentage of dead ova and it significantly decreases the percentage of mature ova.

Also, combination of *Nigella sativa* oil with aqueous garlic extract led to increase the number of dead eggs and enhanced maturation of *Schistosoma* ova (ElShenawy *et al,* 2008). Also, they recorded a significant reduction in number of eggs/g liver in groups treated with *Nigella sativa* oil or its combination with garlic extract. Ali *et al.* (2016) stated that use of *Nigella sativa* oil alone exerted a significant effect on ova count in liver and intestinal tissue.

In the present study, the most evident reduction in the mean number of hepatic granuloma per 5/low power fields was in group treated with combined therapy. In *N. sativa* oil, treated group there was a moderate reduction on mean number of hepatic granuloma while, the lowest percentage of reduction was in prophylactic group. The number of granuloma coincided with their effect in increasing dead ova count compared to positive control group.

Mahmoud *et al.* (2002) reported that *N. sativa* oil suppressed the size of the developing liver granuloma in a dose dependent manner, being reduced 15.8 % and 24.3% for the doses 2.5 ml & 5ml respectively. Also, Sheir *et al.* (2015) found that combination of black seed oil and Artemether and/or PZQ treatments recorded marked reduction of hepatic granuloma diameter 35.42% & 32.23%, respectively. Soliman and El-Shenawy (2003) who reported that *Nigella sativa* oil produced less pathological lesions in the liver of *S. mansoni* infected mice, especially the inflammatory reactions that mediate both granuloma diameter and number.

No doubt, black seed oil and thymoquinie (TQ) have strong anti-inflammatory effect, as they were found to reduce the synthesis of nitric oxide, interleukin-1, cyclooxygenase (COX)-1, COX-2 and histone deacetylase along with other pro-inflammatory mediators such as interleukin-1β, interleukin-6, tumor necrosis factor-α, interferon-γ and prostaglandin (Ahmad *et al,* 2013).

In the current work, hepatic tissue from mice infected treated with *N. sativa* oil post infection, showed mild hydrobic degeneration and slightly dilated sinusoids. The two

types of granulomas were observed, well-formed cellular granulomas were present in hepatic lobules and the fibrocellular granulomas with less inflammatory cells formed mainly of lymphocytes, eosinophils and plasma cells that becomes smaller than control-infected group.

These results coincided with Alenzi *et al.* (2010) who recorded that the Nigella sativa seed are reported to possess potent antioxidant effects. The effect of *Nigella sativa* on *S. mansoni* infected liver may be due to its important role of antioxidant effect of the oxidative stress in mediating liver injury in schistosomiasis, which increases the production of reactive oxygen intermediates by eosinophils and macrophages at the site of the granulomatous inflammation (McCormick *et al*, 1996).

The hepatic schistosomiasis, or schistosomal hepatopathology, was the commonest form of chronic disease and usually resulted from heavy *S. mansoni* infection (Lambertucci *et al*, 2001). The use of *N. sativa* oil, an immunomodulating agent, as an assistant to chemotherapy may be effective in augmenting the reduction of immunopathology, PZQ is active against all schistosome species infecting humans, an important feature, especially in those areas where more than a single species is present, typically in Africa where *S. mansoni* and *S. haematobium* are often co-endemic (Cioli *et al*, 2012). In the present study, combined therapy of *Nigella sativa* oil and PZQ, Liver cells showed slight improvement of cloudy swelling and hydropic degeneration, fibrocellular granulomas formed mainly of monocytes and plasma cells were observed. SEM played an important role in elucidating the detailed morphology and different alterations of the *S. mansoni* tegument allowing the interpretation of its functionality (El-Shabasy *et al*, 2015). The surface topography of schistosomes have been investigated to evaluate the antischistosomal activities of several compounds, since the tegument of schistosomes is an important target for such drugs (Jiraungkoorskul *et al*, 2005; Mostafa, 2005). The current study proved alterations in the tegumental surface of mature *S. mansoni* following *N. sativa* oil treatment in vivo by using SEM. Treatment with *N. sativa* oil alone or combined with PZQ showed structural changes in tegument of the adult treated worms by reduction of number and size of tubercles being swollen in some parts and collapsed in other parts and retraction of both oral and ventral suckers. The edema also involved the tubercles, which appeared thickened with raised knobs. These results agreed with Mostafa (2005) who found that the tegument of worms developed in mice treated with black-seed oil showed moderate structural changes, since the tubercles on the dorsal surface of the male showed partial loss of spines. The dorsal surface of the tegument of the adult worm in the prophylactic group showed extensive swelling of tubercles with complete loss of spines, wrinkles and vesicles as well as obliteration and swollen suckers which coincided with Mostafa and Soliman (2002) who found that treatment with black seed oil from day zero of infection showed extensive loss of spines with small sized tubercles and edema of the inter-tubercular regions. Tubercle distortions caused inability of worm to adhere to host's walls of blood vessels that almost led schistosome to be dislodged and transported in blood stream from mesenteric veins to portal vein and intravenous hepatic capillaries and lodged in liver (Mehlhorn *et al*, 1981).

Conclusion

Based on the outcome of this study, *Nigella sativa* oil proved to have a potential bioactivity against *S. mansoni* adult stages and its potentiality in improving hepatic pathology. Efficacy of *N. sativa* to postpone progression in chronic liver diseases must be considered as preventive medicine in patients with hepatic disorders. Antioxidant and anti-inflammatory properties are features of preventing and protected liver from injury

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Explanation of figures

Fig.1: Effect of *Nigella sativa* oil, PZQ and combination of both drugs on mature worm burden in *S. mansoni* infected mice.

Fig. 2: Effect of *N. sativa* oil, PZQ and combination of both drugs on oogram pattern in intestinal segments of *S. mansoni* infected mice.

Fig. 3: Effect of *N. sativa* oil, PZQ and both drugs on mean ova/gram in both hepatic tissue and intestinal tissue of *S. mansoni* infected mice.

Fig. 4: Effect of *N. sativa* oil, PZQ and combination of both drugs on number (A) and diameter (B) of hepatic granuloma in *S. mansoni* infected mice

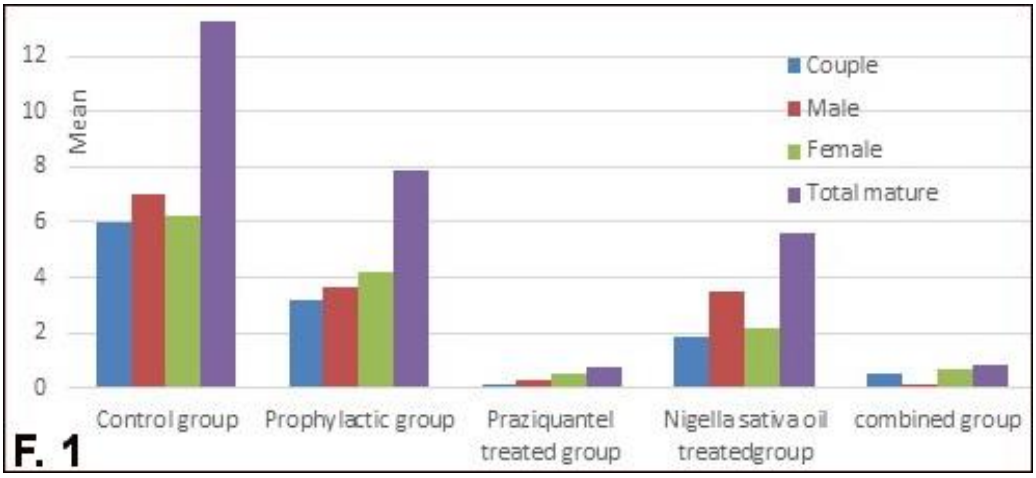
Fig. 5: Liver section of Praziquantel treated group. a- Mild infiltrate of hepatic parenchyma with inflammatory cells mainly lymphocytes (yellow arrow) and slightly dilated sinusoids that lined by hyperplastic pigmented *Von kupffer* cells (red arrow), hepatocytes show slight hydropic degeneration (H & E, X400), b- Well-demarcated medium sized fibrocellular granuloma with several layers of concentrically arranged collagenous fibrous tissue (green arrow) surrounding degenerated ovum (red arrow) with rim of inflammatory cells, mainly eosinophils and lymphocytes (yellow arrow) (H & E, X100).

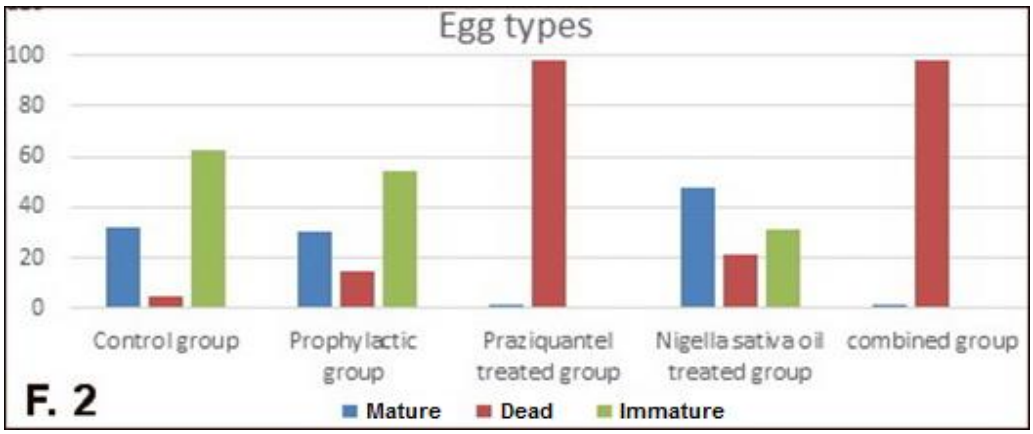
Fig. 6: Photomicrograph of liver section of *Nigella sativa* oil treated group. c- Scanty inflammatory cellular infiltrate of the liver parenchyma mainly lymphocytes (black arrow), mild hydrobic degeneration (yellow arrow) and slightly dilated sinusoids. (H&X stain) (X400), d- Small sized cellular granuloma (yellow arrow) consisting of inflammatory cells (mainly lymphocytes) surrounding single egg with degenerated miracidium (red arrow) (H & E, X100).

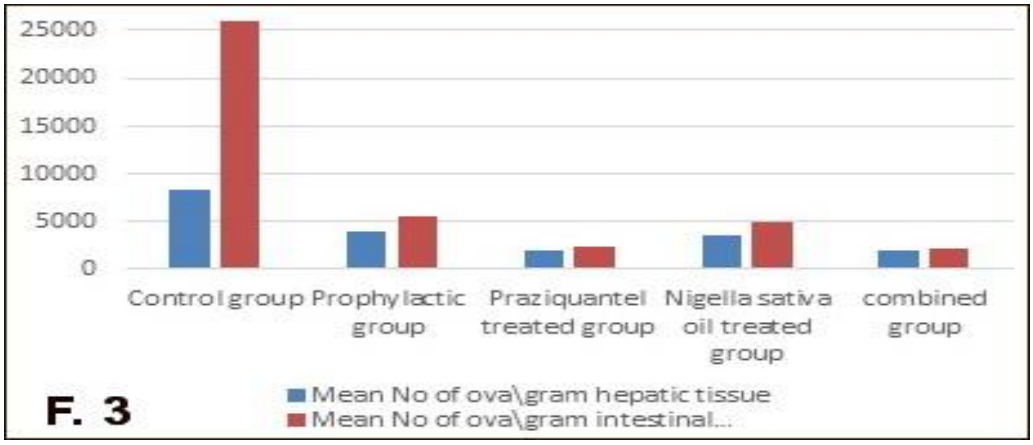
Fig. 7: Liver section of prophylactic group, e- Hydropic degeneration and moderate dilatation of sinusoids with moderate inflammatory infiltrate (red arrow) (H & E, X400), f- Cellular granuloma (yellow arrow) around single ova (red arrow) (H & E, X100).

Fig. 8: SEM of praziquantel treated group: dorsal surface of treated adult male *S. mansoni* with severe reduction in number and size of tubercles being swollen, collapsed and destructed in other parts of dorsal tegumental surface of worms with complete loss of spines (SL), multiple vesicles (V). (x 2000).

Fig. 9: SEM of *N. sativa* oil treated group, a-dorsal surface of treated adult male *S. mansoni* showed severe reduction in number and size of tubercles being swollen in some parts and collapsed or destructed (TD) in other parts of dorsal tegumental surface with complete loss of spines (SL), b- dorsal surface showed extensive destruction of the tubercles (TD) with complete loss of spines in some, multiple vesicles (V) of dorsal tegumental surface (x2000).







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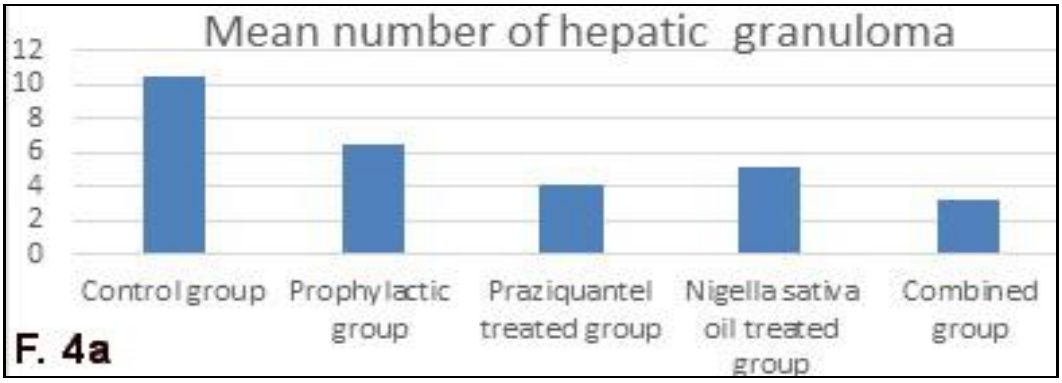

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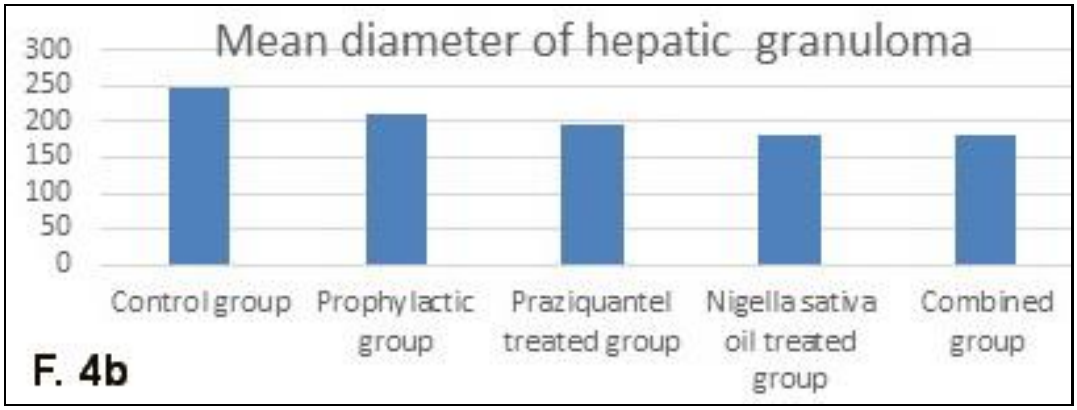

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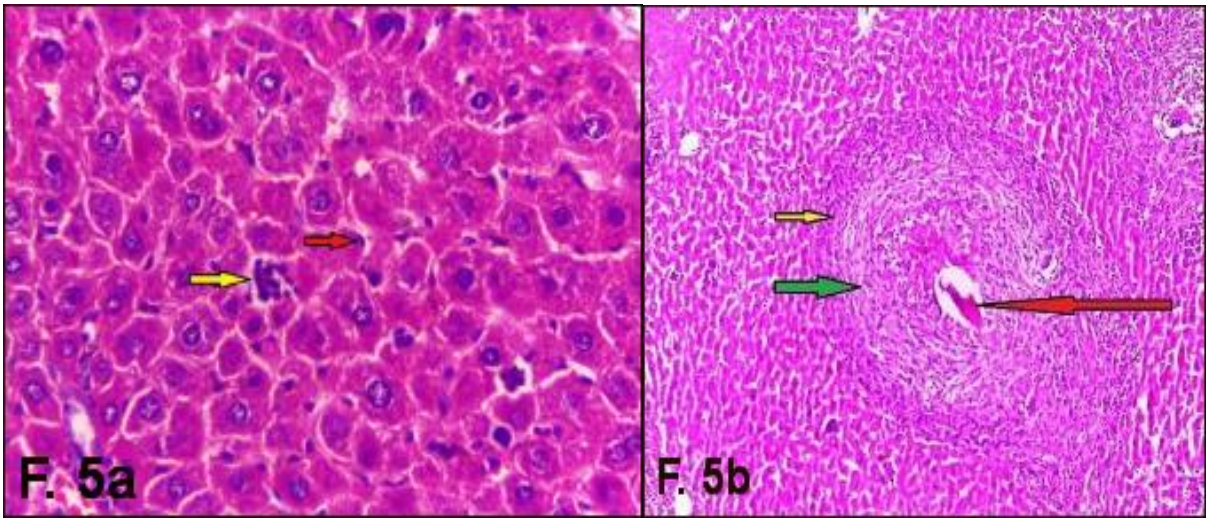

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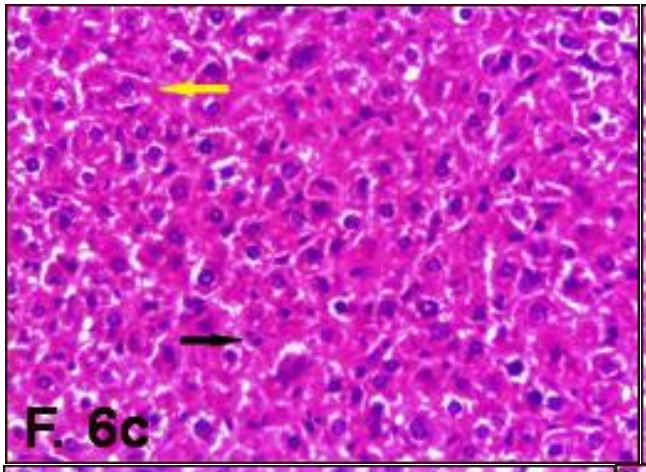
F. 5b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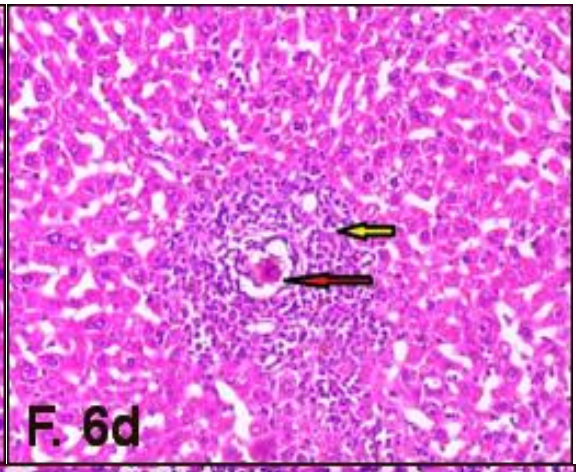
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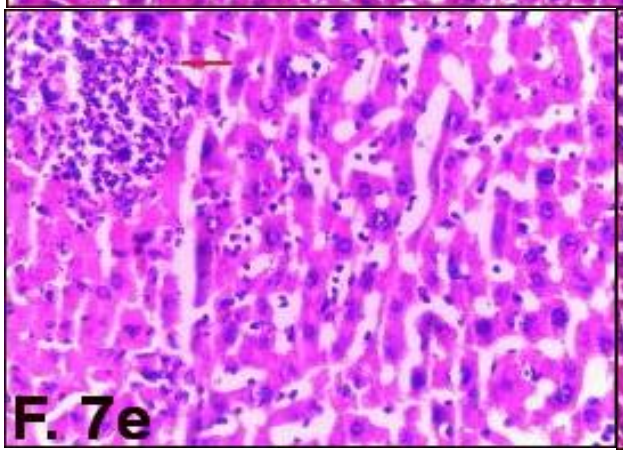
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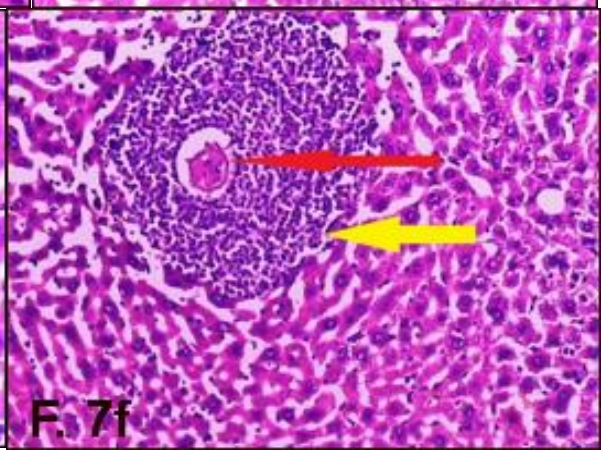
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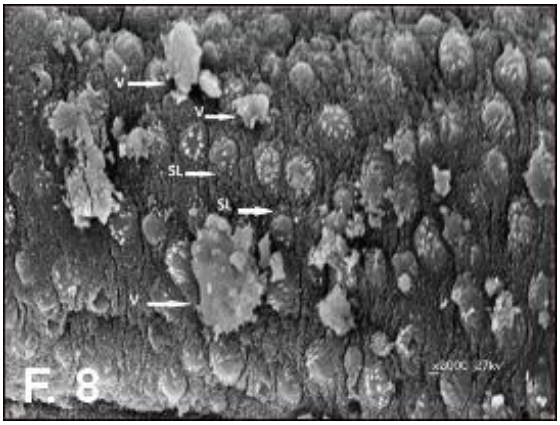
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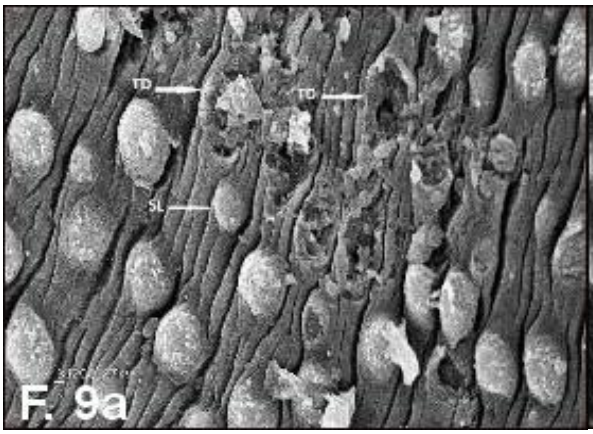
F. 8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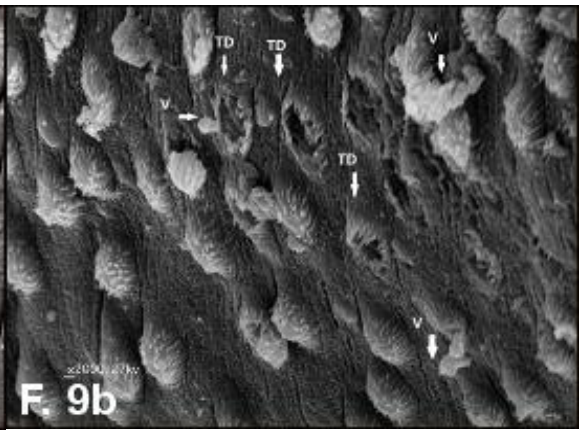
F. 9a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F. 9b