



# Effectiveness of *Origanum syriacum* on *Schistosoma mansoni* Infected Mice: Parasitological, Histopathological and Scanning Electron Microscopical Studies

HEND M. TAG<sup>1,2\*</sup>, EMAN M. ABOUELHASSAN<sup>3</sup>, MONA M. HENEDAK<sup>4</sup>, EHSSAN A. HASSAN<sup>2,5</sup>

<sup>1</sup>Department of Biology, College of Science and Arts at Kulis, University of Jeddah, Jeddah, Saudi Arabia; <sup>2</sup>Department of Zoology, Faculty of Sciences, Suez Canal University, Ismailia, Egypt; <sup>3</sup>Department of Veterinary Parasitology, Suez Canal University, Ismailia, Egypt; <sup>4</sup>Zoology Department, Faculty of Science, El Arish University, Egypt; <sup>5</sup>Department of Biology, College of Science and Humanities, Prince Sattam Bin Abdulaziz University, Al-Kharj11942, Saudi Arabia.

**Abstract** | Schistosomiasis remains one of the most prevalent parasitic infections worldwide. Millions of people suffer severe illness related to schistosomiasis. The present studies aimed to evaluate the anti-parasitic effects of the crude, aqueous, and hexane fractions of *Origanum syriacum* against *Schistosoma mansoni* infected mice, the anti-schistosomal activity of the crude extracts of *O. syriacum*, their aqueous and hexane fractions were assessed by some parasitological and histopathological studies as worm load, liver egg load, intestine egg load, oogram pattern and also the SEM studies of the ultrastructure of *schistosoma* tegument. Sixty male albino mice were used in the studies; twenty mice were observed for the acute toxicity of the *Origanum syriacum* extract and its fractions, for the evaluation of the anti-parasitic effects, twenty mice were infected with *S. mansoni* cercariae via subcutaneous route by a rate of by  $80 \pm 10$  cercariae per mouse and randomly divided into 4 groups (5 mice in each cage). The other uninfected 20 mice were randomly divided into 4 groups, after 6 weeks post-infection all experiment trials were conducted for 14 consecutive days. The results revealed that the groups treated with crude extracts of *Origanum syriacum*, and their aqueous fractions might be considered a promising anti-inflammatory and anti-schistosomal agent as they have schistosomicidal, ovicidal, histopathological effects and they reduced the total worm burden as compared with the untreated infected group.

**Keywords** | *Origanum syriacum*, *Schistosoma mansoni*, Parasitological, Histopathological, Scanning

**Received** | September 24, 2020; **Accepted** | September 30, 2020; **Published** | January 01, 2021

**\*Correspondence** | Hend M Tag, Department of Zoology, Faculty of Sciences, Suez Canal University, Ismailia 41522, Egypt; **Email:** hend\_taha@science.suez.edu.eg

**Citation** | Tag HM, Abouelhassan EM, Henedak MM, Hassan EA (2021). Effectiveness of *origanum syriacum* on *schistosoma mansoni* infected mice: parasitological, histopathological and scanning electron microscopical studies. Adv. Anim. Vet. Sci. 9(2): 221-229.

**DOI** | <http://dx.doi.org/10.17582/journal.aavs/2021/9.2.221.229>

**ISSN (Online)** | 2307-8316; **ISSN (Print)** | 2309-3331

**Copyright** © 2021 Tag et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## INTRODUCTION

Schistosomiasis is one of the most important public health problems affecting particularly people of the Nile Delta, the most widespread type of schistosomiasis in this region is *S. mansoni* (Chitsulo et al., 2000). Schistosomal infection is prevalent in Upper Egypt, along the Nile river due to agricultural purposes from 70 or 80 years ago (Chitsulo et al., 2000; EL-Khoby et al., 2000; EL-Sisi et

al., 2011).

In Egypt, the beginning of schistosomiasis control programs has enhanced the decline of *Schistosoma* infections (El-Khoby et al., 1998; El-Khoby et al., 2000; Youssef and Uga, 2014). Although the use of various controls programs, *Schistosoma* infections are still recorded with a relatively high prevalence of *S. mansoni* (Youssef and Uga, 2014). Several medications are used in the treatment of schisto-

somiasis as praziquantel, oxfamniquine, metrifonate, antimonials, hycanthone, and niridazole (Fahmy et al., 2014; Tag et al., 2019) but developing resistances against drugs is a serious problem.

Phytomedicines play a vital role in the human health care system (Prakash et al., 2012). Among the medicinal plants and aromatic, *Origanum sp.* is an important genus belonging to the family *Lamiaceae* (Chishti et al., 2013). In folk medicine, *Origanum* species are used as, flavoring agents and powerful disinfectants (Chishti et al., 2013). Dried *Origanum* species are used as antitussives, analgesics, expectorants, sedatives, antirheumatics, antiparasitics, and gastrointestinal complaints in folk medicine (El-Beyrouthy et al., 2008; Loizzo et al., 2009). The present study aimed to evaluate the effects of *Origanum syriacum*, and their fractions against *Schistosoma mansoni* infected mice.

## MATERIAL AND METHODS

### PLANTS EXTRACTION

Plant materials were collected from Arish, North Sinai, Egypt, in March 2015 and were identified and authenticated by Botany Department, Faculty of Science, Suez Canal University based on taxonomic characters and by direct comparison with the herbarium specimens available at the herbarium of Botany department. The plant extracts were prepared as described by (Azwanida, 2015). Fractionation of ethanolic plant extracts was prepared as described by (Tag et al., 2020).

### EXPERIMENTAL DESIGN (FIGURE 1)

Sixty male albino mice were used in the studies, the studies divided in to:

- Experiment 1:** Acute Toxicity Study of *Origanum syriacum* extract and its fractions in mice.
- Experiment 2:** Evaluation of the anti-parasitic effects of the crude, aqueous, and hexane fractions of *Origanum syriacum* against *Schistosoma mansoni* infected mice.

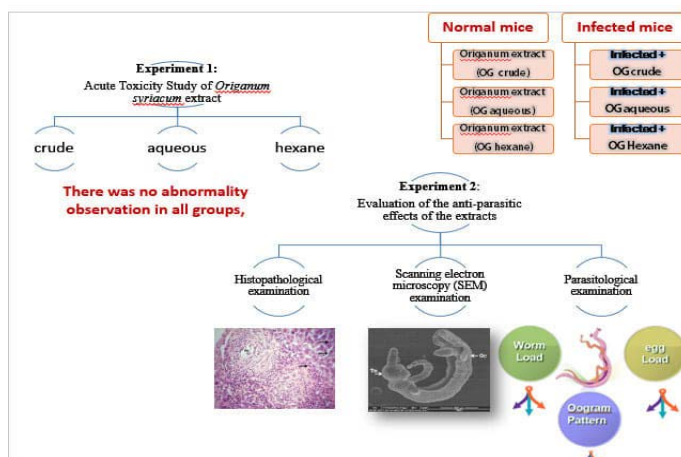


Figure 1: The experimental design of the two experiments

### Experiment 1: Acute Toxicity Study of *Origanum syriacum* extract and its fractions in mice

Twenty male albino mice of average weight (20-22g) were acclimatized for a week in cleaned cages and randomly divided into 4 groups of 5 animals each for each extract. Groups 1, 2, and 3 were intraperitoneally injected with crude, aqueous, and hexane extracts either for *Origanum syriacum* extracts (10, 100 and 1000 mg/kg body weight) following the method of (Lorke, 1983). The last group was the control group which was administered saline intraperitoneally. Immediately after dosing, the mice were continuously observed for at least 4 h, and occasionally up to 6 h. They were then observed for up to 14 days (frequency of 12 h/day) for signs of toxicity and mortality (Tag et al., 2019).

### Experiment 2: Evaluation of the anti-parasitic effects of the crude, aqueous and hexane fractions of *Origanum syriacum* against *Schistosoma mansoni* infected mice

Forty male albino mice were used in this study; they were purchased from the animal unit from the Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI) (Giza, Egypt) where they had been bred under conventional conditions for research purposes. Mice were selected and individually marked. The animals were matched, as closely as possible with an average weight (20-22g) to reduce the variability of their responses according to guidelines of the Organization for Economic Co-operation and Development (OECD) (OECD, 1998; 2000), they were fed on a standard commercial pelleted diet (El-Kahira Company for oils and soap) in an air-conditioned animal house at 20-22°C, humidity 65±5% and light/dark cycles (12/12hrs). The animal experiments were conducted in the animal house at the Department of Zoology Faculty of Science, Suez Canal University. Each of the used cages has a card with the data of the experiment (Tag et al., 2019).

*S. mansoni* cercariae were obtained from laboratory-bred infected *Biomphalaria alexandrina* snails in SBSP at TBRI, Giza, Egypt. Following the methodologies previously described by (Wasilewski et al., 1996). Twenty mice were infected with *S. mansoni* cercariae via a subcutaneous route by a rate of 80±10 cercariae per mouse (He et al., 2005) and randomly divided into 4 groups (5 mice in each cage). The other uninfected 20 mice were randomly divided into 4 groups (Table 1) (Tag et al., 2019).

### SAMPLING AND EXAMINATION

**Parasitological examination:** The experimental design was prepared as in (Table 1), Mice were decapitated and underwent Porto-mesenteric perfusion for recovery of the adult schistosome worms for the Parasitological examination, which includes:

**Table 1:** The experimental groups design

Group 1	negative control group
Group 2	Uninfected and treated with ethanolic extract of <i>O.syriacum</i> , (200 mg/kg,), intraperitoneal (i.p.) administration daily.
Group 3	Uninfected and treated with aqueous extract of <i>O.syriacum</i> , (200 mg/kg,), intraperitoneal (i.p.) administration daily.
Group 4	Uninfected and treated with hexane extract of <i>O.syriacum</i> , (40 mg/kg,), intraperitoneal (i.p.) administration daily.
Group 5	Infected and untreated
Group 6	Infected and treated with ethanolic extract of <i>O.syriacum</i> , (200 mg/kg,), intraperitoneal (i.p.) administration daily.
Group 7	Infected and treated with aqueous extract of <i>O.syriacum</i> , (200 mg/kg,), intraperitoneal (i.p.) administration daily.
Group 8	Infected and treated with hexane extract of <i>O.syriacum</i> , (40 mg/kg,), intraperitoneal (i.p.) administration daily.

**Table 2:** Worm burden in *Schistosoma mansoni* infected mice treated with *O.syriacum*, and their fractions after 8 weeks post-infection

Groups	Mean worm burden			Total worm burden	Worm % reduction	
	Worm % reduction	Paired	Single			
Infected non-treated		4.00±1.5	1.00±0.53	5.00±0.10		
Infected +OG crude extract	79.25	0.83*±0.83	160	2.60*±0.55	3.30*±0.60	34
Infected +OG aqueous extract	75	1.00*±0.88	230	3.30*±1.33	4.30*±0.30	8
Infected +OG hexane extract	66.75	1.33*±0.66	100	2.00*±1.00	3.30*±0.30	14

Values are means ± SE., One Way ANOVA followed by Values are means ± SE. n=6, One Way ANOVA followed by Duncan multiple comparison tests \*p<0.05 compared with *Schistosoma mansoni* infected mice.

**a- Estimation of worm burden**

All mice were sacrificed at the end of the 8<sup>th</sup> week after infection by decapitation. The sacrificed mouse was placed in the supine position, a longitudinal midline incision was made in the skin of the abdomen and the abdominal skin was removed, the peritoneal cavity was opened through another longitudinal midline incision. An incision was made in the portal vein near its entry to the liver, then a fine needle (20 gauge) connected to a perfusion pump containing citrated saline was introduced in the descending aorta. Worms emerging from the portal vein incision were sucked into a 20 µ sieve fitted to the proximal end of a rubber tube connected to a suction pump. The worms were retained in the sieve. Perfusion continued until the fluid coming from the animal was free of blood and the liver became pale. The liver of each mouse was removed, washed by a saline solution to remove blood, dried by blotting between filter papers, weighted and 0.5 mg of it was used to prepare liver homogenate and 1mg for egg load counting, and then the rest of the liver was stored in an aluminum foil paper to be preserved in the deep freezer for future requirements. The loops of the intestine were lifted and washed down to dislodge any adherent worms. The sieve was inverted into a Petri-dish and the worms washed off with normal saline and counted (Tag et al., 2019).

**b- Calculation of the percentage of mature and dead ova in tissues (oogram study).**

After perfusion, the small intestine for each sacrificed mouse was wholly separated and transferred to a Petri-dish, three fragments of the small intestine were cut longitudi-

nally (1 cm in length), rinsed in saline, slightly dried on filter paper and then placed between 2 slides and compressed carefully, The preparations were examined under the low power of the light microscope. (Morales et al., 2014).

**c- Estimation of the egg load by counting the number of eggs in infected tissues (intestine and liver)**

The whole small intestine was removed, and then 3 fragments were taken for oogram study, The rest of the intestine was slit opened and washed with saline to remove any faecal matter present in the lumen, a representative portion of the liver was also taken after perfusion.

One gram of each liver and each fragment of intestine was weighed and then plotted between two filter papers and then placed each in a test tube containing 5 ml 5% KOH solution. The tubes were incubated at 37°C for 24 hours until the tissues were hydrolyzed. The digest was well shaken on a magnetic mixer for at least one minute, 3 samples (0.1 ml each) were pipetted by the Eppendorf micro-pipette, placed on a slide, and examined under the low power of the microscope, one sample at a time (Tag et al., 2019).

**SCANNING ELECTRON MICROSCOPY (SEM) EXAMINATION:**

Scanning electron microscope examination by (Inspect S; FEI, Holland) at Electron Microscopy Unite of Theodor Bilharz Research Institute (TBRI). It was performed according to (Kevin and Winfried, 2006) to determine the damaging effects on the surface of the treated worms in comparison to the untreated one. The samples were cen-

trifuged then the pellets were immediately processed (Tag et al., 2019).

### HISTOPATHOLOGICAL EXAMINATION

Control and treated groups were anesthetized and dissected immediately. The tissues of the liver were fixed and prepared for histopathological examination according to (Drury and Wallington, 1980), measuring the size of hepatic granulomas in *S. mansoni*-infected mice utilizing the method previously described (Jacobs et al., 1997).

The classification of evolutionary stages of granulomas was performed according to previous studies (Costa-Silva et al., 2002; Lins et al., 2008), and for the morphometric analysis, Image J software was used to measure granuloma diameter using spatial calibration. The process of spatial calibration involves calibrating a single image against known values, then applying that calibration to the uncalibrated image, both images are at the same magnification. For each treatment granuloma size was measured and the mean values from 5 mice for each group were used for statistical analysis.

### STATISTICAL ANALYSIS

The collected data were tabulated and analyzed using IBM's personal computer using SPSS 16 microstate software package. ANOVA (analysis of variance) was used as the test of significance. P-value was considered significant if it was <0.05 (Field, 2000). Duncan test was used as the multiple comparison tests after obtaining significant results by i.e. post-hoc comparison to determine which pair was significantly different (Abdi and Sidak, 2007).

### ETHICS:

This study was conducted following legal ethical guidelines of the Medical Ethical Committee of the National Research Center, Dokki, Egypt (approval no. 09210).

## RESULTS

### Experiment 1: Acute Toxicity Study of *Origanum syriacum* extract and its fractions in mice

The acute toxicity study was conducted in mice weighting 20-22g. The whole plant ethanolic extract, their aqueous and hexane fractions were given by intraperitoneal injection. Initially, the animals were administrated with a single dose of 10,100 mg/kg of body weight, and the dose was increased to 1000 mg. All animals were observed for clinical signs and pre-terminal deaths for the first 4 hours after dose administration on the first day of treatment and once daily on days 2 to 14 for changes in skin and fur, eyes, mucous membrane, musculature, and respiratory, symptoms. There was no abnormality observation in all groups, all extracts had no significant toxic effect in mice and the plant

materials were found to be nontoxic.

### Experiment 2: Evaluation of the anti-parasitic effects of the crude, aqueous, and hexane fractions of *Origanum syriacum* against *Schistosoma mansoni* infected mice.

#### Parasitological examinations

Worm burden:

The present result showed significant differences between the total worm load of mice treated with *O. syriacum* or their fractions as compared to the untreated animals (Table 2). The treatment with crude, aqueous and the hexane fractions of *O. syriacum* significantly decreased ( $P < 0.05$ ) the total worm load as compared to the untreated ones. In addition to there was a reduction in the number of paired worms in all the infected treated mice compared to the infected untreated one and the number of single worms showed a significant increase in the treated groups compared to the untreated one (Table 2).

#### OGRAM STUDY

The viable mature ova of the treated groups with crude, aqueous, and the hexane fractions of *O. syriacum* showed significant reductions ( $335.2 \pm 36.08$ ,  $318.2 \pm 26.16$  and  $374.0 \pm 25.80$ , respectively) ( $P < 0.05$ ) as compared to the untreated group ( $681.60 \pm 56.85$ ) (Table 3). Also, there were significant increases ( $3.6 \pm 0.67$  and  $7.0 \pm 0.00$  respectively) ( $P < 0.05$ ) in numbers of dead ova found in groups treated with the crude extracts and the aqueous fraction of the plants as compared to the untreated group ( $1.80 \pm 0.48$ ) (Table 3).

#### TISSUE EGG LOAD IN INTESTINE AND LIVER

The effect of crude *O. syriacum* and their fractions on the worm ability for oviposition was assessed by counting *S. mansoni* eggs /g retained in both intestine and liver tissues (Table 3). The result revealed significant reductions were recorded in the mean number of eggs/g intestine in groups treated with the aqueous, crude extracts and hexane fractions of the plants ( $3.4 \pm 1.40$ ,  $4.46 \pm 1.40$  &  $4.45 \pm 1.52$  respectively) when compared to the untreated one ( $24.05 \pm 6.10$ ) ( $P < 0.05$ ). Regarding the liver egg load, significant reductions were also recorded in the mean number of eggs/g liver in infected mice treated with the crude and hexane fractions of *O. syriacum* ( $2.20 \pm 0.53$ ,  $1.40 \pm 0.46$  respectively) as compared with the untreated infected group ( $9.50 \pm 3.60$ ) ( $P < 0.05$ ). While there was a highly significant decrease in the mean number of eggs/g liver in infected mice treated with an aqueous fraction of *O. syriacum* ( $1.34 \pm 0.60$ ) as compared with untreated infected mice ( $P < 0.05$ ).

#### SCANNING ELECTRON MICROSCOPY (SEM) EXAMINATION:

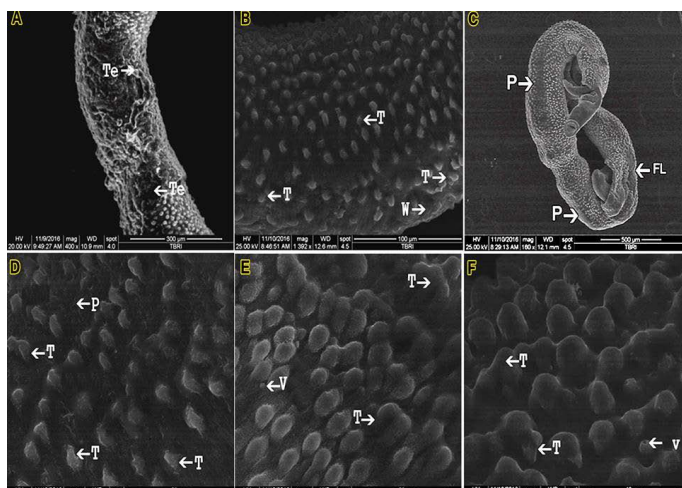
Treating infected mice with crude *O. syriacum* extract led to mild to severe alterations in the tegument of adult worms,

**Table 3:** Effect of crude *O.syriacum* , and their fractions on oogram and tissue egg load(8 weeks post-infection).

Groups	Oogram			Egg load		
	% of change	Dead	% of change	Mature	Intestine	Liver
Infected non-treated		1.80±0.48		681.60±56.85	24.05 ±6.10	9.50 ± 3.60
infected + OG crude extract	-128	3.60±0.67*	+53.3	335.20±36.08*	4.46 ± 1.40*	2.20±0.53*
Infected + OG aqueous extract	-28	7.00±0.00*	+45.1	318.20±26.17*	3.41 ± 1.40*	1.34±0.66*
Infected + OG hexane extract	-188	1.40±0.50	+35	374.0±25.80*	4.45 ± 1.52*	2.40±1.11*

Values are means ± SE., One Way ANOVA followed by Duncan multiple comparison tests. Duncan multiple comparison tests \*p<0.05 compared with *Schistosoma mansoni* infected mice

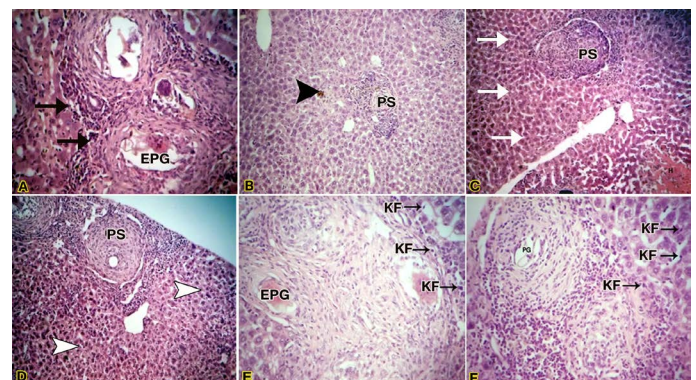
the male worms showed variable alterations in their tegument that increased with increasing the administrated dose. Tubercles appeared ruptured, collapsed, disrupted, or reduced in size with obviously diminished spines and sensory papillae. Besides, grooves between tubercles showed diminished spines (figure 2.A). While treating with an aqueous fraction of *O.syriacum* extracted to severe alterations in the tegument of adult worms. Showed variable alterations, accompanied by focal lesions on the tegument, disruption, tegumental peeling on its dorsal body surface, wrinkling that increased with increasing the administrated dose in addition to the formation of vesicles. Tubercles appeared ruptured, collapsed, disrupted, or reduced in size with obviously diminished spines and sensory papillae. Also, grooves between tubercles showed diminished spines (figure 2.B, C, D &E), treating with hexane fraction of *O.syriacum* extract leads to mild to severe alterations in the tegument of adult worms. The tubercles appeared ruptured, collapsed, disrupted, or reduced in size with obviously diminished spines and numerous membrane-bound vesicles (figure 2.F).



**Figure 2:** SEM micrograph (A) male *S. mansoni* after exposure to the crude *Origanum syriacum*; (B, C, D &E) male *S. mansoni* after exposure to the aqueous fraction of *O.syriacum* extracts; (F): male *S. mansoni* after exposure to the hexane fraction of *O.syriacum* extract Tubercles (T); Wrinkling (W); Peeling (P) Vesicles (V); Focal lesions (FL).

**HISTOPATHOLOGICAL STUDIES**

After 8 weeks post-infection, the liver of infected mice treated with crude *O.syriacum* extract showed less hydropic degeneration as compared with the untreated infected mice (figure 3.B). Kupffer cells were lesser than those of the untreated infected group. Pyknotic necrosis (an irreversibly damaged cell characterized by condensation and increased basophilia of its nucleus) is associated with granulomas lesions (figure 3.A). Moderate periportal inflammatory infiltration was observed. Also, dark brown pigments of hemozoin were observed (figure 3A). Yellowish pigments of bilirubin (figure 2.B). Exudative productive granuloma stages were observed (figure 3.A). Pre-granulomatous stages were common and less fibrotic granulomas were observed (Figure 3.B).



**Figure 3:** (A) Light micrograph of liver of infected mouse with *S. mansoni* treated with crude *Origanum syriacum* extract H&E. Magnification (400x); (B): Light micrograph of liver of infected mouse with *S. mansoni* treated with crude *O.syriacum* extract Magnification (100x); (C & D) Light micrograph of liver of infected mouse with *S. mansoni* treated with aqueous fraction of *O.syriacum* extract H&E. Magnification (100x); (E&F): Light micrograph of liver of infected mouse with *S. mansoni* treated with hexane fraction of *O.syriacum* extract H&E. Magnification (200x). Productive granuloma (PG); Exudative-productive granuloma (EPG); Kupffer cells (KF).

Regarding the liver of infected mice treated with an aqueous fraction of *O.syriacum* extract showed normal hepatocytes away from granulomas (figure 3.C). At granuloma-

tous areas, hydropic degeneration and pyctonic nuclei of some hepatocytes were recorded (figure 2.D). Pre-granulomatous stages were common (figure 3.C) as well as productive granuloma (figure 3.D), besides, Kupffer cells were lesser than those of the untreated infected group. Dark brown pigmentation was minor or not observed totally in some mice.

Concerning, the liver of infected mice treated with hexane fraction of *O. syriacum* extract showed relatively normal hepatocytes and dilated sinusoids (figure 3.F). The Kupffer cells were lesser than untreated infected mice (figures 3.E & F). Also, some pyknotic hepatocytes were observed near granulomas. However, necrosis in this group was minor when compared with untreated infected liver. Productive granuloma (Figure 3F) and exudative productive granuloma were observed.

**MEASURING THE SIZE OF HEPATIC GRANULOMAS:**

The diameter of granulomas showed highly significant differences (P < 0.05) between the untreated infected group (645.01±67.86) and groups treated with the crude and aqueous fractions while The diameter changed in granulomas of groups treated with the hexane fraction of *O. syriacum* extract was not significantly different (P > 0.05) from the untreated infected group (Table 4).

**Table 4:** Differences in hepatic granulomas mean diameter (µm ± SE) in the liver of *Schistosoma mansoni* infected mice treated with crude *O. syriacum*, and their fractions after 8 weeks post-infection.

Groups	Granuloma size (µm ± SE)
Infected non-treated	645.01 ± 67.86
Infected +OG crude extract	301.00 ±39.55*
Infected+ OG aqueous extract	264.33 ±52.717*
Infected +OG hexane extract	502.33 ±50.23

Values are means ± SE., One Way ANOVA followed by Duncan multiple comparison tests \*p<0.05 compared with *Schistosoma mansoni* infected mice.

**DISCUSSION**

Schistosomiasis remains one of the most prevalent parasitic infections in the world and is endemic in more than 75 countries (World Health Organization, 2010). Medical plant extracts have been used since ancient times and they considered the first anthelmintic products in traditional medicine (Ndjonka et al., 2013). Previous attempts have been done to combat schistosomiasis using traditional medicinal plants (Soliman and El-Shenawy, 2003; Tag et al., 2019).

The present study demonstrated that the administration

of crude *O. syriacum* extracts and their aqueous fractions to infected mice revealed less pathological features than untreated ones. Granuloma size is the most variable frequently used to evaluate the immunopathogenesis of schistosome infections (Shaha et al., 2017). The size of the granuloma is controlled by many immune factors related to the persistence of the egg in the lesion and the ability of the host cells to destroy antigens (Zuim et al., 2012). In the present study, the size of hepatic granulomas in infected mice treated with all extracts showed a significant decrease except the group treated with the hexane fraction of *O. syriacum* did not show a significant decrease as compared with untreated infected mice. The variation in the activity of different extracts might be due to the different nature and amount of active components released with the solvent used in the extraction processes (Mansour et al., 2002; Tag et al., 2019). The studies showed that there was a significant decrease in the paired worms' burden and an increase the single worms' burden in all treated groups. This indicates that *O. syriacum* crude extracts and their fractions negatively affected the coupling of the worms (Tag et al., 2020). Besides, the treatments of the infected animals with crude and aqueous extracts of *O. syriacum* were effective at reducing the total worm burden with untreated infected mice, indicating its useful antischistosomal action. Some authors have demonstrated that the death of worms upon treatment with antischistosomal drugs resulted from metabolic disorders, mechanical destruction, or muscular contraction of treated worms (Doenhoff et al., 2002; El Ridi and Tallima, 2013). The efficacy of *O. syriacum* and their aqueous fractions as an antischistosomal agent was not studied before but some authors studied their efficacy as anti-inflammatory and antioxidant agents and found that low concentration of crude *O. syriacum* had anti-inflammatory and antioxidant properties due to its content of flavonoids, triterpenoids, glycoside, and steroids (Soliman et al., 2007; Tag et al., 2019).

Concerning, the oogram pattern, they showed a significant reduction in the number of viable mature eggs at groups treated with the crude and aqueous extracts of *O. syriacum* as well as hexane fraction of *O. syriacum*. This reduction in viable egg numbers may be to the death of adult worms. Additionally, the number of dead ova increased in groups treated with the crude and aqueous extracts of *O. syriacum* while there wasn't a significant difference in groups treated with the hexane fraction of *O. syriacum* compared with untreated infected mice. Such changes may indicate the possible lethal effects of the crude and aqueous extracts of *O. syriacum* on released eggs in the lumen of the intestines. Concerning effects of *Origanum syriacum* crude extract and their fractions on egg load in the liver and intestine tissues, the present data revealed a reduction in egg count in these tissues It is clear from the results that there was a suppression of egg-laying capacity; which may be attributed

to a reduced worm burden and/or the extracts may affect the ability of the worms to copulate, thereby affecting egg production by the female adult worms (Abdel-Ghaffar, 2004; Zhang et al., 2009; Rabia et al., 2010). Moreover, other factors may also explain such a reduction in schistosomal egg count. These factors are probably diminished fecundity of the worm pairs and an increased rate of egg excretion due to the egg death (Riad et al., 2009). The anti-schistosomal activity of treated groups of *O. syriacum* and their fractions was also assessed by the SEM studies of the ultrastructure of schistosome tegument. The present results showed alteration in male worm tegument including tubercles collapsing, tubercles with reduced spines, tegument swelling or tearing, and presences of vesicles. These changes confirm the anti-schistosomal activities of treated groups of *O. syriacum* and their fractions as vesicle formation are an indicator of stress and swelling of tegument and focal lysis of worm muscles (Manneck et al., 2011; Zhang et al., 2009).

Our results are in agreement with Frezza et al. (2013) who pointed out that the treatment of experimentally infected mice with 500 mg/kg of *Artemisia annua*, showed significant erosion, peeling, sensory structure damage, and vesicle formation on the tegument of *S. mansoni* 30 days post-infection. Moreover, worm tegument tearing increases antigen exposure on the worm surface to the host immune system that results subsequently in worm death (Brindley et al., 1989; Soliman and Ibrahim, 2005). Tegumental changes recorded in this study were observed by other anti-schistosomal drugs and compounds (Munro and McLaren, 1990; Xiong et al., 2000; Xiao et al., 2002; Jiraungkoorskula et al., 2005; Soliman and Ibrahim, 2005; Tag et al., 2019).

In conclusion, Schistosomiasis is one of the important wide spread tropical parasitic diseases, the present study reflected that the groups treated with crude extracts of *O. syriacum* and their aqueous fractions might be considered a promising anti-inflammatory and anti-schistosomal agent especially after the findings of the safe use of them in animals in this study, they have schistosomicidal and ovicidal effects besides they reduced the histopathological effects and the size of granulomas induced by *S. mansoni*, Further studies are required to increase the efficacy of complete the elimination of worms and ova and to improve the anti-inflammatory and antioxidant properties of the infected host.

## CONFLICT OF INTEREST

The authors have no conflicts of interest relevant to this article.

All the authors contributed to the design and the performance of the experiments, the analysis of the results and to the writing of the manuscript.

## REFERENCES

- Abdel G (2004). Assessment of the efficacy of Ro 16-2308 against the Egyptian strain of *S. mansoni* in mice: parasitological, hematological and biochemical criteria Egypt. J. Zool. 42:173-203.
- Abdi H, Bonferroni A, Sidak Z (2007). Corrections for multiple comparisons. In: Salkind NJ, editor. Encyclopedia of measurement and statistics. Thousand Oaks, CA: Sage. (103-107).
- Azwanida NN (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. Aromat. Plants. 4:1-6.
- Brindley PJ, Strand M, Norden AP, Sher A (1989). Role of host antibody in the chemotherapeutic action of praziquantel against *Schistosoma mansoni*: identification of target antigens Mol. Biochem. Parasitol. 34(2):99-108. [https://doi.org/10.1016/0166-6851\(89\)90001-7](https://doi.org/10.1016/0166-6851(89)90001-7)
- Chishti S, Zahoor A, Kaloo and Phalestine S (2013). Medicinal importance of genus *Origanum*: A review. J. Pharmacog. Phytother. 5(10): 170-177.
- Chitsulo L, Engels D, Montresor A, Salvioli L (2000). The global status of schistosomiasis and its control. Acta. Tropica. 77: 41-51. [https://doi.org/10.1016/S0001-706X\(00\)00122-4](https://doi.org/10.1016/S0001-706X(00)00122-4)
- Costa-Silva M, Rodrigues SR, Hulstijn M, Neves RH, De Souza PM, Lenzi HL (2002). Natural *Schistosoma mansoni* infection in *Nectomys squamipes*: histopathological and morphometric analysis in comparison to experimentally infected *N. squamipes* and C3H/He mice. Mem Inst Oswaldo Cruz.; 97 Suppl 1:129-42. Pmid: 12426608. <https://doi.org/10.1590/S0074-02762002000900026>
- Drury RB, Wallington EA (1980). Carleton's Histological Technique. Oxford University Press, 520.
- Doenhoff MJ, Kusel JR, Coles GC, Cioli D (2002). Resistance of *Schistosoma mansoni* to praziquantel: is there a problem? Trans R Soc Trop Med Hyg.; 96(5):465-9. [https://doi.org/10.1016/S0035-9203\(02\)90405-0](https://doi.org/10.1016/S0035-9203(02)90405-0)
- El-Beyrouthy M, Arnold N, Delelis-Dusollier A, Dupont F (2008). Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon J Ethnopharmacol. 120:315-334. <https://doi.org/10.1016/j.jep.2008.08.024>
- El Ridi, RAF, Tallima HAM (2013). Novel therapeutic and prevention approaches for Schistosomiasis: Review. J. Adv. Res. 4 (5): 467-78. <https://doi.org/10.1016/j.jare.2012.05.002>
- El-Khoby T, Ghalal N, Fenwick A (1998). The USAID/ Government of Egypt's Schistosomiasis Research Project (SRP). Parasito.Today. 14: 92-96. [https://doi.org/10.1016/S0169-4758\(97\)01206-4](https://doi.org/10.1016/S0169-4758(97)01206-4)
- El-Khoby T, Hussein MH, Galal N, Miller FD (2000). Epidemiology 1, 2, 3: origins, objectives, organization, and implementation. Am. J. Trop. Med. Hyg., 62(2S):2-7. <https://doi.org/10.4269/ajtmh.2000.62.2>
- El-Sisi A, Awara W, El-Masry T, El-Kowrany S, El-Gharbawy R (2011). Effects and mechanism of action of

- immunomodulating agents against schistosomiasis-induced hepatic inflammation and fibrosis in mice. *Res. Pharm. Biotech.*, 3:32-45.
- Fahmy RS, Ibrahim R, Mansour EM (2014). The potential role of mefloquine against *Schistosoma mansoni* infection by prohibition of hepatic oxidative stress in mice. *JOBAZ.*, 67: 40–47. <https://doi.org/10.1016/j.jobaz.2014.09.002>
  - Field A (2000). *Discovering Statistics using SPSS for Windows*. SAGE publications, Thousand Oaks – New Delhi, London. 496 pp, ISBN: 0-7619-5755-3.
  - Frezza TF, Fernandes de OCN, Banin TM, Garcia Rehder, VL, Boaventura SJ, Allegretti SM (2013). Tegumentary changes in two different strains of *Schistosoma mansoni* treated with artemisinin and artesunic acid. *Rev. Patol. Trop.*, 42: 309–321. <https://doi.org/10.5216/rpt.v42i3.26930>
  - Gryseels B, Polman K, Clerinx J, Kestens L (2006). Human schistosomiasis. *Lancet*. 368: 1106–1118. [https://doi.org/10.1016/S0140-6736\(06\)69440-3](https://doi.org/10.1016/S0140-6736(06)69440-3)
  - Jacobs W, Bogers J, Deelder A, Wéry M, Van ME (1997). Adult *Schistosoma mansoni* worms positively modulate soluble egg antigen-induced inflammatory hepatic granuloma formation in vivo. Stereological analysis and immunophenotyping of extracellular matrix proteins, adhesion molecules, and chemokines. *Am. J. Pathol.*, 150(6): 2033–2045.
  - Jiraungkoorskula W, Sahaphong S, Sobhonc P, Riengrojpitaka, S, Kangwanrangsana N (2005). Effects of praziquantel and artesunate on the tegument of adult *Schistosoma mekongi* harboured in mice. *Parasitol. Int.*, 54: 177 – 183. <https://doi.org/10.1016/j.parint.2005.04.001>
  - Kevin LB, Winfried D (2006). Towards neural circuit reconstruction with volume electron microscopy techniques. (2006). *Neurobiology*. 16 (5): 562-570. <https://doi.org/10.1016/j.conb.2006.08.010>
  - Lins RA, Cavalcanti CB, Araujo-Filho JL, Melo-Junior MR, Chaves ME (2008). Distribution of eosinophils at different stages of hepatic granuloma evolution in mice infected with *Schistosoma mansoni*. *Rev. Soc. Bras. Med. Trop.* 41(2):173–8. Pmid: 18545839. <https://doi.org/10.1590/S0037-86822008000200008>
  - Loizzo MR, Menichini F, Conforti F, Tundis R, Bonesi M, Saab AM, Statti GA, Cindio B, Houghton PJ, Menichini FR, Frega NG (2009). Chemical analysis, antioxidant, anti-inflammatory and anticholinesterase activities of *Origanum ehrenbergii* Boiss and *Origanum syriacum* L. essential oils. *Food Chem.* 117: 174-180. <https://doi.org/10.1016/j.foodchem.2009.03.095>
  - Lorke DA, (1983). new approach to practical acute toxicity testing. *Arch. Toxicol.* 54:275–87. <https://doi.org/10.1007/BF01234480>
  - Manneck T, Braissant O, Ellis W, Keiser J (2011). *Schistosoma mansoni*: Antischistosomal activity of the four optical isomers and the two racemates of mefloquine on schistosomula and adult worms *in vitro* and *in vivo*. *Exp. Parasitol.* 127: 260–269. <https://doi.org/10.1016/j.exppara.2010.08.011>
  - Mansour SA, Ibrahim G, Abdel-Hamid H (2002). Botanical biocides, 8. Impact of some plant extracts on *Biomphalaria alexandrina* snails and on *Schistosoma mansoni* miracidia and cercariae. *Egypt J. Sch. Infect. Dis.*, 24: 81-99.
  - Moraes J, de Oliveira RN, Costa JP, Junior ALG, de Sousa DP, Freitas RM (2014). Phytol, a Diterpene Alcohol from Chlorophyll, as a Drug against Neglected Tropical Disease Schistosomiasis *Mansoni*. *PLoS Negl Trop Dis* 8(1): e2617.
  - Munro GH, McLaren DJ (1990). *Schistosoma mansoni*: morphology and ultrastructure of adult worms recovered from cyclosporin A-treated mice. *Parasitology*. 100 (1):19-28. <https://doi.org/10.1017/S0031182000060078>
  - Ndjonka D, Rapado LN, Silber AM, Liebau E, Wrenger C (2013). Natural Products as a Source for Treating Neglected Parasitic Diseases. *Int. J. Mol. Sci.* 14: 3395-3439. <https://doi.org/10.3390/ijms14023395>
  - OECD (Organization for Economic Co-operation and Development) (1998). *Acute oral toxicity— Modified up and down procedure*, No. 425. Paris: OECD.
  - OECD (Organization for Economic Co-operation and development) (2000). *Guideline for the Testing of Chemicals, Up-and-Down Procedure, Peer Panel Report, Appendix G, The Up-and-Down Procedure for Acute Oral Toxicity*. Paris: OECD.
  - Prakash HS, Sampath KK, Shailasree S (2012). Antioxidant, Antibacterial And DNA Protecting Activity Of Selected Medicinally Important Asteraceae Plants *Int. J. Pharm. Sci.* 4: 257-261
  - Rabia F, Nagy E, Aly A, Mohamed F, EL-Assal A, El- Amir A (2010). Effect of treatment with antifibrotic drugs in combination with Pzq in immunized *Schistosoma mansoni* infected murine model *J. Am. Sci.* 6 (5): 208-216.
  - Riad NHA, Taha HA, Mahmoud YI (2009). Effects of garlic on albino mice experimentally infected with *Schistosoma mansoni*: A parasitological and ultrastructural study. *Trop. Biomed.* 26 (1): 40–50.
  - Shaha K, Bobbi P, Mariam A (2017). Histopathologic review of granulomatous inflammation. *J. Clin. Tuberculosis Other Mycobact. Dis.* 7:1–12. <https://doi.org/10.1016/j.jctube.2017.02.001>
  - Soliman MFM, El-Shenawy NS (2003). Evaluation of the Protective Effect of Two Antioxidative Agents in Mice Experimentally Infected with *Schistosoma mansoni*: Haematological and Histopathological Aspects. *Pakistan J. Biolog. Sci.* 6:887- 897. <https://doi.org/10.3923/pjbs.2003.887.897>
  - Soliman MFM, Ibrahim MM (2005). Antischistosomal action of atorvastatin alone and concurrently with medroxyprogesterone acetate on *Schistosoma haematobium* harboured in hamster: surface ultrastructure and parasitological study. *Acta Trop.* 93 (1): 1-9. <https://doi.org/10.1016/j.actatropica.2004.08.006>
  - Soliman MFM, Yousif MF, Zaghloul SS, Okba MM (2007). Seasonal variation in the essential oil composition of *Origanum majorana* L. cultivated in Egypt. *Z Naturforsch. C.* 64(9-10):611-614. <https://doi.org/10.1515/znc-2009-9-1001>
  - Tag HM, Henedak MM, Sallam NH, Hassan EH, Zayed RA, Mohammed ST (2019). Effect of artemisia santonica on schistosoma mansoni infected mice; parasitological, histopathological and scanning electron microscopical studies. *J. E. Soci. Parasito.* 49 ( 2)321-332.
  - Tag HM, Henedak MM, AbouelhassanEM, Hassan EH, Zayed, RA, Mohammed, ST (2020). Antioxidative and hepatoprotective effects of *Artemisia santonica* and *Origanum syriacum* against *Schistosoma mansoni* infection. *J. Appl. Biolog. Sci.* 14 (1): 86-97.
  - Wasilewski MM, Lim KC, Philips J, McKerrow JM (1996). Cysteine protease inhibitors block schistosome haemoglobin degradation *in vitro* and decrease worm burdens and egg



- production *in vivo*. Mol. Biochem. Parasitol. 81:179-189. [https://doi.org/10.1016/0166-6851\(96\)02703-X](https://doi.org/10.1016/0166-6851(96)02703-X)
- World Health Organization (2010). Schistosomiasis, Fact Sheet No 115. Prevention And Control Of Schistosomiasis And Soil-Transmitted Helminthiasis: First Report Of The Joint Who Expert Committees. Who Tech. Rep. Ser. No. Geneva: World Health Organization.
  - Xiao S, Shen B, Utzinger J, Chollet J, Tanner M (2002). Ultrastructural alterations in adult *Schistosoma mansoni* caused by artemether. Memórias do Instituto Oswaldo Cruz. 97(5):717- 724. <https://doi.org/10.1590/S0074-02762002000500023>
  - Xiong K, Li C, Weight FF (2000). Inhibition by ethanol of rat P2X 4 receptors expressed in *Xenopus* oocytes. Br. J. Pharmacol. 130:1394-1398.
  - He YX, Salafsky B, Ramaswamy K (2005). Comparison of skin invasion among three major species of *Schistosoma*. TRENDS in Parasitology. 21 (5) 201-203. <https://doi.org/10.1016/j.pt.2005.03.003>
  - Youssef AI, Uga S (2014). Review of Parasitic Zoonoses in Egypt. Trop. Med. Int. Health. 42 (1): 3-14. <https://doi.org/10.2149/tmh.2013-23>
  - Zhang CW, Xiao SH, Utzinger J, Chollet J, Keiser J, Tanner M (2009). Histopathological changes in adult *Schistosoma japonicum* harbored in mice treated with a single dose of mefloquine Parasitol. Res. 104 (6): 1407-1416. <https://doi.org/10.1007/s00436-009-1341-0>
  - Zuim NRB, Allegretti SM, Linhares AX, Magalhaes LA, Zanotti-Magalhaes EM (2012). A Study of the Granulomatous Responses Induced by Different Strains of *Schistosoma mansoni*. Interdisciplinary Perspectives on Infect. Dis. 9 (1): 1-8. <https://doi.org/10.1155/2012/953524>