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EFFECT OF *ARTEMISIA SANTONICA* ON *SCHISTOSOMA MANSONI* INFECTED MICE: PARASITOLOGIOCAL, HISTOPATHOLOGICAL AND SCANNING ELECTRON MICROSCOPICAL STUDIES

By

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Abstract

Schistosomiasis is one of the most wide spread tropical diseases. Millions of people suffer severe illness related to schistosomiasis. This study evaluated the anti-parasitic effects of crude *Artemisia santonica* and their aqueous and hexane fractions against *Schistosoma mansoni* infected mice by some parasitologiocal and histopathological studies as worm load, liver egg load, intestine egg load, oogram pattern and also by surface ultrastructure of recovered worms using SEM. The result reflected that mice treated with crude extracts of *A. santonica* and their aqueous fractions gave a promise anti-inflammatory and anti-schistosomal agent.

Key words: *Artemisia santonica*, *Schistosoma mansoni*, Parasitologic, SEM

Introduction

Schistosomiasis, a worldwide concern, affects more than 200 million people worldwide, and another 500-779 million people are at risk, especially children (Steinmann *et al,* 2006). Globally, it was estimated to cause more than 200,000 deaths per year and at least 90% of people requiring treatment in Africa (Clements *et al,* 2009). Among the different known schistosome species, zoonotic *Schistosoma mansoni* was the most endemic one in Egypt (Helmy *et al,* 2009). Schistosomiasis was reported in others Arabian Countries as for example Saudi Arabia (Mohammad, 2014), Yemen (Khalil *et al*, 2018), Sudan (Amin and Abubaker, 2017), and Libya (Amara *et al*, 2018),

Pathology associated with *S. mansoni* results primarily from the accumulation of parasite eggs, giving rise to hepatomegaly that may be superseded by extensive liver fibrosis (Gryseels *et al*, 2006). It has also been shown that the granulomatous inflammatory response to *S. mansoni* eggs entrapped in the liver induces oxidative stress.

Several medications were used in treatment of schistosomiasis including the Praziquantel, Oxamniquine, Metrifonate, Antimonials, Hycanthone and Niridazole (Fahmy *et al*, 2014).

Current treatment relies on praziquantel (PZQ). But, El-Hawy *et al.* (1990) among the Egyptian school children with active urinary hematobiasis treated with praziquantel orally 40mg/kg b.w. every 6 months, reported adverse reactions as nausea, abdominal colic, vomiting, diarrhea, dizziness, pyrexia and headache. Experimental in mice, praziquantel showed to be a hepatotoxic, genotoxic and carcinogenic drug (Omar *et al,* 2005). Besides, the PZQ has stage-dependent susceptibility, showed only poor efficacy to the immature schistosome stages (Keiser *et al,* 2009). Also, many lines of evidence indicated to increasing the emergence of strains of *S. mansoni* resistant to PZQ (Zhang and Coultas, 2013). So, for controlling schistosomiasis, there was an urgent need to develop a new effective drug. Phytomedicine play a major role in human health care system

(Panossian, 2014). There is a considerable interest in elucidating the mechanism of their action to develop better medicines. Plants contain many free radical scavenging molecules such as the phenolic compounds, the nitrogen compounds, the vitamins, terpenoids…etc. **(**Nalini *et al,* 2015).

In traditional medicine, *Artemsia* sp. was used for expelling of rheumatism, paralyzing and killing parasites in the digestive tract in addition to controlling their eggs (Mehdi and Farshid, 2015). *Artemisia* sp. is frequently utilized for the treatment of diseases such as malaria, hepatitis, cancer, inflammation, and infections by bacteria, fungi and viruses (Abad *et al,* 2012). Recently, some *Artemisia* species essential oils proved to be friend insecticides for myiasis producing dipterous flies (Bedini *et al,* 2017), against the stored product insects (Liang *et al,* 2017) and allopathic chemicals (Gholami *et al,* 2011). Its compounds are also active towards viral, bacterial and protozoa diseases in vivo and in vitro (Efferth *et al,* 2011) as well as for cancer therapy (Efferth, 2017).

Generally, a total of 3000 essential oils were recorded, only about 300 of which are significant in the fields of pharmaceutical, agricultural and food (Calo *et al,* 2015).

The present study aimed to evaluate the antiparasitic effects of *Artemisia santonica*, and their fractions against *Schistosoma mansoni* infected mice.

Material and Methods

Schistosoma mansoni cercariae were purchased from laboratory bred infected *Biomphalaria alexandrina* in SBSP at TBRI, Giza, Egypt. Following the methodologies as described (Wasilewski *et al,* 1996). Twenty Albino mice were infected with *S. mansoni* cercariae via the subcutaneous route by 80±10 cercariae per mouse (Stirewalt and Dorsey, 1974) and randomly divided into 4 groups (5 mice in each cage). The other uninfected 25 mice were randomly divided into 5 groups (Tab. 1).

Selection of animal species: Male Albino mice were used in the study. They were purchased from the animal unit from the Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI) where they had been bred under conventional conditions for research purposes. Mice were selected and individually marked. Animals were matched, as closely as possible with an average weight (20-22g) in order to reduce the variability of their responses according to guidelines of Organization for Economic Co-operation and Development (OECD, 1998; 2000). All animals were conducted in accordance with the lines Guided for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council and in accordance with the guidelines of the international guidelines for animal experimentation.

Housing and feeding conditions: The mice 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 Department of Zoology Faculty of Science, Suez Canal University. Each of the used cages has a card with the data of the experiment.

Preparation of animals: Before use, the selected individual animals were weighed and identified through marks made on their body with permanent ink pens. Mice were housed in plastic cages with stainless steel mesh lids. The animals were kept in their cages for at least 5 days prior to dosing, to allow for their acclimatization to the laboratory conditions. During acclimatization, the animals were observed for ill health or abnormality.

Plants extraction Plant materials were collected from Arish, North Sinai, Egypt, in March 2015 and were identified and authenticated by Department of Botany, Faculty of Science, Suez Canal University on the basis of taxonomic characters and by direct comparison with the herbarium specimens available at the herbarium of Botany department. The plant extract was prepared (Azwanida, 2015) with minor modifications. Briefly, the

leaves of *Artemisia santonica* were washed, air-dried and powdered. The dried powder was extracted with 70% hydro-ethanol solution for 48 h. The marc was further extracted by 70% hydro-ethanol for 48 h. to obtain the extract. The extract was then filtered and evaporated to dry under reduced pressure on a rotary evaporator (Yamato, Rotary Evaporator, model-RE 801) at the Dewedar's Lab. The yield of crude ethanolic extract of *A. santonica* leaves was found to be 7.1 &4 % w/w, respectively

Fractionation of ethanolic plant extract: Ethanol crude extracts from *A. santonica* were partitioned between 2 volumes, then one of them suspended in water (60 mL). It was extracted with hexane organic solvent by using the separator funnel the residue left in separator funnel was re-extracted twice to separate the aqueous and the hexane fractions of the ethanolic extract then filtered using Whatman No. 41 filter paper to remove particles. Extracts were evaporated to dry under reduced pressure on a rotary evaporator (Yamato, Rotary Evaporator, model-RE 801) at Dewedar's Lab., yield of aqueous and hexane extracts of *A. santonica* leaves were 48.5 & 20.5% w/w, respectively.

Phytochemical screening of plant extracts and its fractions: Air-dried powdered plants materials which collected in flowering stage and (1gm) of the aqueous & hexane fractions of both plants were subjected to the steam distillation for volatile oil (Egyptian Pharmacopeia, 1953), carbohydrate and/or glycoside (Trease and Evans, 1978), flavonoids (Mabry *et al*, 1970), tannins (Balbaa, *et al* 1976), Saponin (Farnsworth, 1996), alkaloids and/or basic nitrogenous substances and mucilage (Trease and Evans, 1978), sterols and/or triterpenes (Libermann's 1885; Kebede *et al,* 2015).

Acute toxicity plant extracts study and their fractions in mice: Fifty seven male Albino mice with average weight (20-22g) were acclimatized for a week in cleaned cages and randomly divided into 3 groups of 3 animals each for each extract. Gs 1, 2 & 3 were intraperitoneal injected with the crude, aqueous and hexane extracts either for *A. santonica* extracts (10, 100 & 1000 mg/kg body/wt) following the method of (Lorke, 1983). The control group was administered saline intraperitoneal. Immediately after dosing, the mice were continuously observed for at least 4hr, and occasionally up to 6hr. They were kept under observation for up to 14 days (frequency of 12hr/day) for signs of toxicity and mortality.

Parasitological examination: The experimental design was prepared (Tab. 1). The mice were decapitated and underwent portomesentric perfusion for recovery of the adult schistosome worms for the Parasitological examination, which include estimation of worm burden (Smithers and Terry, 1965), calculation of the percentage of immature, mature and dead ova in tissues according to (Pellegrino *et al,* 1962), Estimation of the egg load by counting number of eggs in infected intestine and liver tissues (Cheever, 1970).

SEM examination was done at Electron Microscopy Unite of Theodor Bilharz Research Institute (Glauert, 1974) to determine the damaging effects on the surface of the treated worms in comparison to the untreated one. The samples were centrifuged then the pellets were immediately processed.

Histopathological examination: Both control & treated mice groups were anaesthetized and immediately dissected. The tissues of liver were fixed and prepared for histopathological examination (Drury and Wallington, 1980). The size of hepatic granulomas in *S. mansoni*-infected was measured in mice as described by Jacobs *et al.* (1997).

The classification of evolutionary stages of granulomas was performed according to the previous studies (Costa-Silva *et al,* 2002; Lins *et al,* 2008), and for the morphometric analysis. Image J software was measured granuloma diameter using spatial calibration. The process of spatial calibration involves calibrating a single image against known values, then applying that calibration to the

un-calibrated image, both images are at the same magnification. For each treatment granuloma size were measured and the mean values from 5 mice for each group were used for statistical analysis.

Statistical analysis: Data were tabulated and analyzed using IBM 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 result by post-hoc comparison to determine significantly different pair (Abdi *et al*, 2007).

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

Results

In the preliminary test (test for primary metabolites) volatile oil, sterols and triterpens were detected in the crude extracts and the hexane fractions of both plants extracts and not detected in their aqueous fractions. Glycoside was detected in the crude extracts and the aqueous fractions of both plants extracts but not detected in their hexane fractions. Notably, tanins, saponins, alkaloids and mucilage were not detected in all tested plant extract (Tab. 2).

Acute toxicity study was conducted in mice weighting 20-22g. The whole plants 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 the groups animals (n=5) all animals were observed for clinical signs and preterminal deaths for the first 4hrs after dose administration on the 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 plants materials were found to be nontoxic.

Worm burden: The result showed significant differences between the total worm load of mice treated with *A. santonica* or their fractions as compared to the untreated animals (Tab. 3). The treatment with crude and aqueous fractions of *A. santonica* significantly decreased ($P < 0.05$) the total worm load as compared to the untreated ones and mean values were (4.26±0.40 & 4.13±0.50) for crude and aqueous fractions of *A. santonica* respectively. Worm reduction rates for the treated groups with crude, aqueous and hexane *A. santonica* were (32%, 24% and 20% respectively), the highest reduction rates in the number of paired worms were also recorded in the treated groups with crude, aqueous and hexane *A. santonica* (50%, 79.25 & 35%, respectively). Also, the highest reduction rates in the number of single worms were recorded in the treated groups comparing to untreated one. Crude extract, aqueous and hexane fractions of *A. santonica*, significantly affect the worm burden with a reduction rate of 160%, 230% & 100%, respectively.

Oogram study: showed the percentage of the different developmental stages of *S. mansoni* eggs. The immature ova of treated groups with crude extracts and aqueous fractions of plants showed significant differences ($P<0.05$) (71.2±3.07 & 88.20±1.02, respectively). Similarly, significant reductions were found ($P< 0.05$) in the mature ova in same treated groups with aqueous fractions and crude extracts of plants (334.4± 24.75 & 443.60±31.66 respectively) as compared to the untreated group. There were significant (Tab. 4) increase ($P< 0.05$) in numbers of dead ova in groups treated with the crude extracts and the aqueous fraction of the plants as compared to untreated group (5.20±0.48 & 7.00±0.44 respectively)

Tissue egg load in intestine and liver: Effect of crude *A. santonica* and their fractions on the worm ability for oviposition was assessed by counting *S. mansoni* eggs/g ret-

ained in both intestine and liver tissues (Tab. 4). As to egg load in intestine tissue, 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 (2.20±0.40, 4.40± 1.50 & 9.26±4.56, respectively) when compared to untreated one (24.05±6.10) ($P < 0.05$). As to liver egg load, significant reductions were recorded in mean number of eggs/g liver in infected mice treated with the crude and aqueous and hexane fractions of *A. santonica* (1.50±0.14 & 1.70±0.7 respectively) compared to the untreated infected group (9.50±3.60) ($P< 0.05$).

SEM: After treatment with crude *A. santonica* extract, the adult male worms showed obvious swelling in the anterior part of the tegument in addition, pronounced alterations in gynaecophoric canal (Fig. 1A) collapsed and reduced tubercles with shortened or diminished spines (Fig. 1B). Furthermore, vesicles were near damaged tubercles (Fig. 1C), while after aqueous fraction treatment showing mild to severe obvious collapsed and reduced tubercles with shortened or diminished spines. Pronounced alterations, peeling in tegument and ruptured tubercles pronounced vesicles were near damaged tubercles (Figs. 2A & B), and to hexane treatment Adult males showed pronounced alterations in tegument in terms of swelling of worm anterior part, wrinkling and pronounced vesicles, generally collapsed and reduced tubercles with shortened or diminished spines (Figs. 2C & D).

Histopathological studies: After 8 weeks post infection, liver of infected mice treated with the crude *A. santonica* extract showed normal hepatocytes away from granulomas (Fig. 3A). In granulomatous areas hydropic degeneration was recorded. Dark brown pigment wasn't observed. Kupffer cells were minor than those of untreated infected ones, with narrowed sinusoids exudative-productive granuloma (Fig. 3B). Liver of mice treated with aqueous fraction showed pre-granulomatous exudative lesions were common with less fibrotic granulomas (Figs. 3C & D) and foreign body, lesser hydropic degeneration as compared with untreated infected mice, also narrowed sinusoids, Kupffer cells were minor than those of untreated infected group. Liver at this dose showed minor pyknotic hepatocytes near granulomas; dark brown pigmentation was minor or not observed totally in some mice. With hexane fraction extract caused productive granuloma (Fig. 4A). Exudative productive granuloma stages (Fig. 4B). Pregranulomatous stages of granuloma, liver at this dose showed minor pyknotic hepatocytes near granulomas, dark brown pigmentation minor or not seen in some mice and kupffer cells hyperplasia near granulomas.

Measuring hepatic granulomas size: Granulomas diameter was assessed in livers of untreated infected mice and infected treated mice after 8 weeks post infection showed different diameters among different groups (Tab. 5). Granulomas diameter showed highly significant differences ($P < 0.05$) between untreated infected group (645.01±67.86) and those treated with crude extracts, aqueous fractions & hexane fraction (451.33±20.26, 363.33±15.07 & 446.66± 94.98, respectively). Details were given in tables (1, 2, 3, 4 & 5) and in figures (1, 2 & 3).

Table 1: Experimental groups design

G1	negative control group
G2	Uninfected and treated with *A. santonica* ethanolic extract (200mg/kg,), intraperitoneal (i.p.)daily.
G3	Uninfected and treated with *A. santonica* aqueous extract (200mg/kg,), intraperitoneal (i.p.) daily.
G4	Uninfected and treated with *A. santonica* hexane extract (40 mg/kg,), intraperitoneal (i.p.) daily.
G5	Infected and untreated
G6	Infected and treated with *A. santonica* ethanolic extract (200mg/kg,), intraperitoneal (i.p.) daily.
G7	Infected and treated with *A. santonica* aqueous extract (200mg/kg,), intraperitoneal (i.p.) daily.
G8	Infected and treated with *A. santonica* hexane extract (40 mg/kg,), intraperitoneal (i.p.) daily.
G9	Uninfected mice treated with DMSO solution (10 mg/kg,), intraperitoneal (i.p.) daily.

Table 2: Phytochemical analysis of crude, aqueous and hexane *A. santonica* leaf extract

Test	Crude	Aqueous	Hexane
Volatile oil	+	-	+
Glycoside	+	+	-
Flavonoids	+	+	-
Tannins	-	-	-
Saponin	-	-	-
Alkaloids	-	-	-
Mucilage	-	-	-
Sterols and triterpenes.	+	-	+

+ = indicates presence - = indicates absent

Table 3: Worm burden in *S. mansoni* infected mice treated with *A. santonica* and its fractions after 8 weeks post-infection.

Groups	Mean worm burden			% Re-duction	Total worm burden	Worm % reduction
	Single	% Reduction	Paired			
Infected non-treated	1.00±0.53		4.00±1.5		5.00±0.10	
Infected +AT crude extract	2.26*±1.20	160	2.00*±1.00	50	4.26*±0.40	32
Infected+AT aqueous extract	3.30*±1.40	230	0.83*±0.47	79.25	4.13*±0.50	24
Infected +AT hexane extract	2.00*±1.40	100	2.6*±0.66	35	4.60±1.00	20

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

Table 4: Effect of crude *A. santonica* and its fractions on oogram & tissue egg load, 8 weeks post-infection.

Groups	Oogram						Egg load	
	Mature	Change %	Immature	Change %	Dead	Change %	Liver	Intestine
Infected non-treated	681.60± 56.85		40.60± 3.14		1.80± 0.48		9.50 ± 3.60	24.05 ± 6.10
Infected + AT crude extract	443.60± 31.66*	+51	71.20± 3.07*	-117	5.20± 0.48*	-128	1.40 ±0.46*	4.40 ± 1.50*
Infected+ AT aqueous extract	334.40± 24.75*	+35	88.20± 1.02*	11.3	7.00± 0.44*	-55.5	1.50± 0.14*	2. 20 ± 0.40*
Infected + AT hexane	511.80± 52.59	+25	36.0± 1.67	11.3	0.80± 0.20	55.5	1.70± 0.70*	9.26 ± 4.56

Table 5: Differences in hepatic granulomas mean diameter (µm ± SE) in the liver of *S. mansoni* infected mice treated with crude *A. santonica* and their fractions after 8 weeks post-infection.

Groups	Granuloma size (µm ± SE)
Infected non-treated	645.01 ± 67.86
Infected +AT crude extract	451.33 ±20.26*
Infected+ AT aqueous extract	363.33 ±15.07*
Infected +AT hexane extract	446.66 ±94.98*

Discussion

Egypt is considered one of the highest countries in schistosome's infection rates in the world (Barakat, 2013). Chemotherapy plays a very important role in reducing mortality in highly infected areas. However, recent drug-resistance strains of schistosomes have been recorded (Li *et al,* 2011; Huang *et al,* 2012). Results of phytochemical screening tests in the present study showed that the main constituent of crude, aqueous and hexane extracts of *A. santonica* was glycosides, flavonoids and triterpenes (Badae *et al,* 2017).

Also, the present results agreed with Alwahibi *et al.* (2016) who reported that natural products Flavonoids isolated from the bark Artemisia species exhibited promising growth inhibitory action of some cancer cells displayed an antioxidant activity. Fereira (2009) reported that *Artemisia* sp. have high content of flavonoid and phenolic compounds that are associated with their high antioxidant capacity. The present results showed a significant decrease in the paired worms' burden and increase the single worms' burden in all treated groups, indicating that *A. santonica* crude extracts and their

fractions negatively affected the coupling of the worms.

In the present study, the oogram pattern showed significant reduction in the number of viable mature eggs at groups treated with the crude and aqueous extracts of *A. santonica* while hexane fraction didn't affect number of either mature and immature eggs. The number of immature ova increased in groups treated with the crude and aqueous extracts of *A. santonica*, while there wasn't any significant difference in groups treated with the hexane fraction when compared with untreated infected mice. This reduction in viable egg numbers may be to the death of adult worms. The number of dead ova increased in groups treated with the crude and aqueous extracts of *A. santonica* while there wasn't a significant difference in groups treated with the hexane fraction compared with untreated infected mice. Such changes indicate possible lethal effect of the crude and aqueous extracts of *A. santonica* on released eggs in the lumen of the intestines. Concerning effect of crude extract and their fractions on egg load in the liver and intestine tissues, there were reductions in egg count in these tissues. It is clear from the results that there was the 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 females (Abdel-Ghaffar, 2004; Zhang *et al,* 2009; Rabia *et al,* 2010). Moreover, other factors may also explain such reduction in schistosomal egg count. These factors are a probable 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 the treated groups of *A. santonica* and their fractions was assessed by the SEM studies of the ultrastructure of schistosome tegument. The 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 *A. santonica* and their fractions, as vesicle formation was indicator of stress and swelling of tegument and focal lysis of worm muscles (Manneck *et al,* 2011; Zhang *et al,* 2009). This agreed with Frezza *et al.* (2013) who pointed out that treatment of mice experimentally infected with 500mg/kg of *A. 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 worm surface to host immune system that result subsequently in worm death (Brindley *et al,* 1989; Soliman and Ibrahim, 2005).

Granuloma formation and fibrosis are the major causes of morbidity and mortality in association with schistosomiasis (Melo *et al,* 2011). Where, killing eggs by granuloma inhibit its maturation and lead to damage of its embryo rich in immunopathologic antigens (Lin *et al,* 2003). Zuim *et al*. (2012) reported that the greater number of eggs and worms the more number of granulomas are recorded in tissues. Such low granulomas intensities in this study indicate low number of eggs per liver and consequently less severe pathological responses.

In the current results, after 8 weeks post infection most granulomas were in the exudative-productive and productive stages for both untreated infected mice and infected treated mice. These results agreed with Lenzi *et al.* (2006). Also, the untreated infected mice showed heavy chronic inflammatory infiltration at portal areas that considered an important factor in the development of severe schistosomiasis forms, this result agreed with Zuim *et al*. (2012). The intensity of schistosomal infection increased the degree of liver fibrosis and granulomatous reaction (El-Lakkany *et al,* 2004). This agreed with the present histopathological results of infected untreated mice liver, which showed an increased granuloma diameter, total infection area and extensive fibrous tissue ac-

cumulation. The present study showed that the crude *A. santonica* extracts and their aqueous fractions to infect mice showed less pathological features than untreated ones. The hepatocellular necrosis and hemorrhage diminished greatly around granulomas area. Granuloma size is the most variable frequently used to evaluate the immunopathogenesis of schistosomiasis (Shaha *et al*, 2017). Granuloma size was controlled by many immune factors related to the egg persistence in lesion and ability of host cells to destroy antigens (Zuim *et al*, 2012).

In the present study, size of hepatic granulomas in infected mice treated with all extracts showed a significant decrease as compared with untreated infected mice. Variation in activity of different extracts might be due to different nature and amount of active components released with the extraction processes solvent (Mansour *et al*, 2002).

The present study showed that crude and aqueous extracts of *A. santonica* were effective in reducing worm burden and egg count compared with untreated infected mice, indicating its useful antischistosomal action. Doenhoff *et al.* (2002) and El Ridi and Tallima (2013) showed that the death of worms after treatment with antischistosomal drugs due to metabolic disorders, mechanical destruction or muscular contraction of them. Moreover, antioxidant supplementation supported the immune response through the antioxidative mechanisms, reduced infectious morbidity and protected from pathogens (Actor *et al*, 2009). Aly *et al.* (2007) reported that the importance of antioxidants in the treatment of schistosomal infection and the reduction of worm load as well as ova count.

In the present study, *A. santonica* and its' aqueous fractions have anti-schistosomal efficacy. Soliman *et al.* (2007), Abd-Alla *et al.* (2007); Mehdi and Farshid (2015) found that low concentration of crude *A. santonica* had anti-inflammatory and antioxidant properties due to flavonoids, triterpenoids, glycoside and steroids.

Conclusion

The mice treated with crude extracts of *A. santonica* and their aqueous fractions gave a promise anti-inflammatory and anti-schistosomal actions, and safe in animal model. Whereas they improve the alterations of hematological, biochemical, antioxidants parameters in *S. mansoni* infected mice.

Future studies must determine suitable dosage, synergistic effects, and isolation of active compound in all extracts and to clarify mechanism of action. It was recommended to use a cocktail of plant's crude and aqueous extracts to increase for complete elimination of worms and to improve host anti-inflammatory and antioxidant properties.

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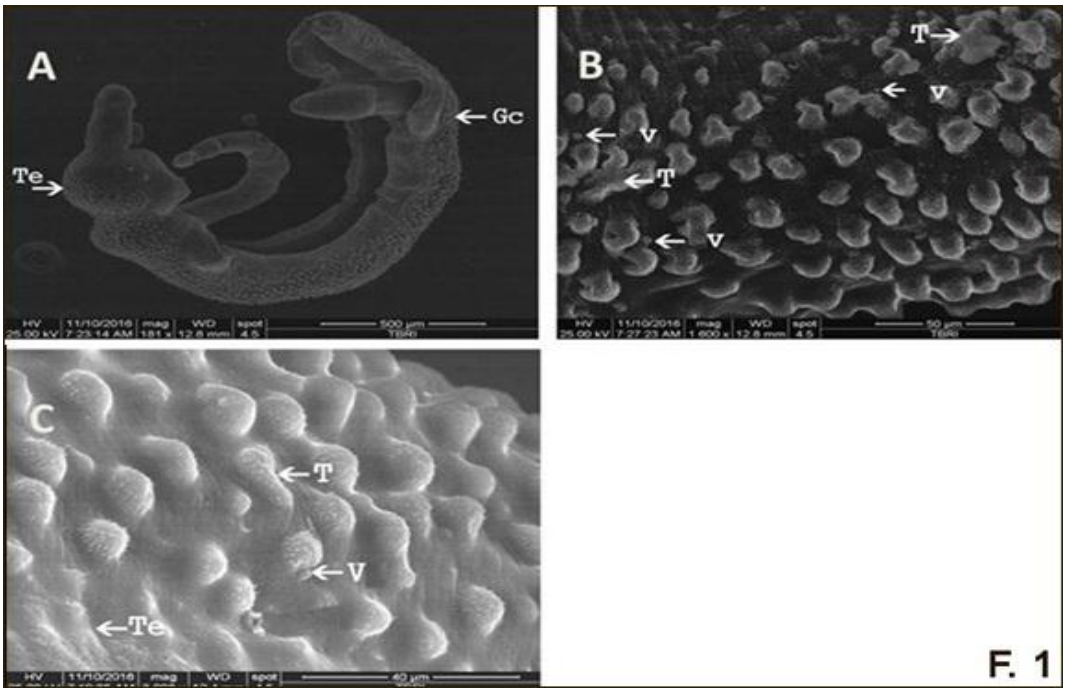
Explanation of figures

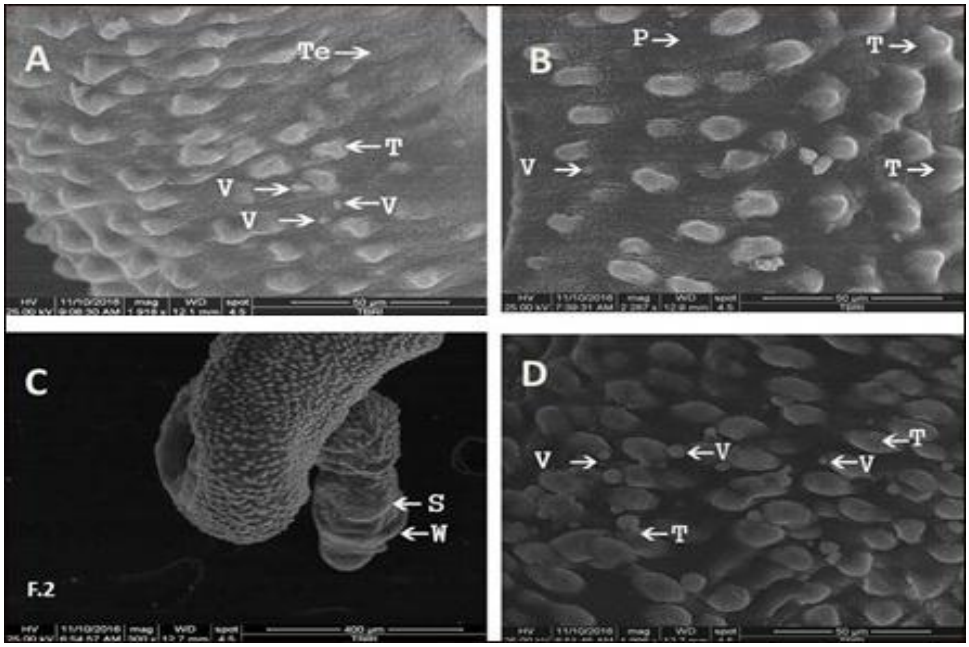
Fig. 1: SEM (A) *Schistosoma mansoni* couple after exposure to crude *A. santonica*, (B) and (C) *S. mansoni* after exposure to crude *A. santonica* extract

Fig. 2: SEM (A & B) male *S. mansoni* after exposure to aqueous fraction of *A. santonica*, (C & D) male *S. mansoni* after exposure to hexane fraction of *A. santonica.*

Fig. 3: Light micrograph of liver of *S. mansoni* infected mouse H&E (A) treated with crude *A. santonica* extract (100x), (B) treated with crude *A. santonica* extract (400x), (C) treated with aqueous fraction of A. santonica extract Magnification (200x), (D) treated with aqueous fraction of *A. santonica* extract (100x).

Fig. 4: (A) Light micrograph of liver of *S. mansoni* infected mouse treated with hexane fraction of A. *A. santonica* extract H&E. Magnification (400x), (B) and (C) Light micrograph of liver of *S. mansoni* infected mouse treated with hexane fraction (100x), (C): H&E. (100x).

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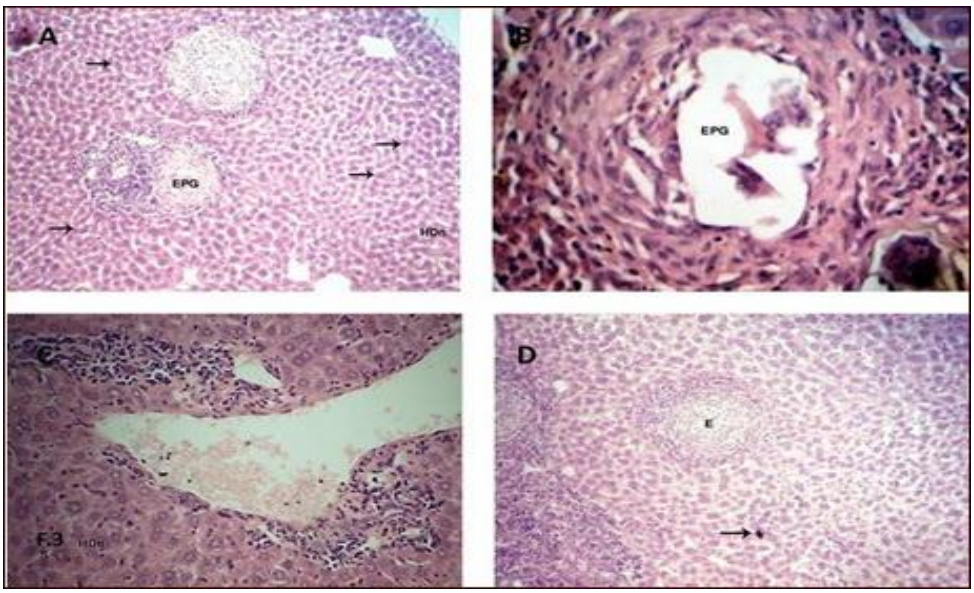
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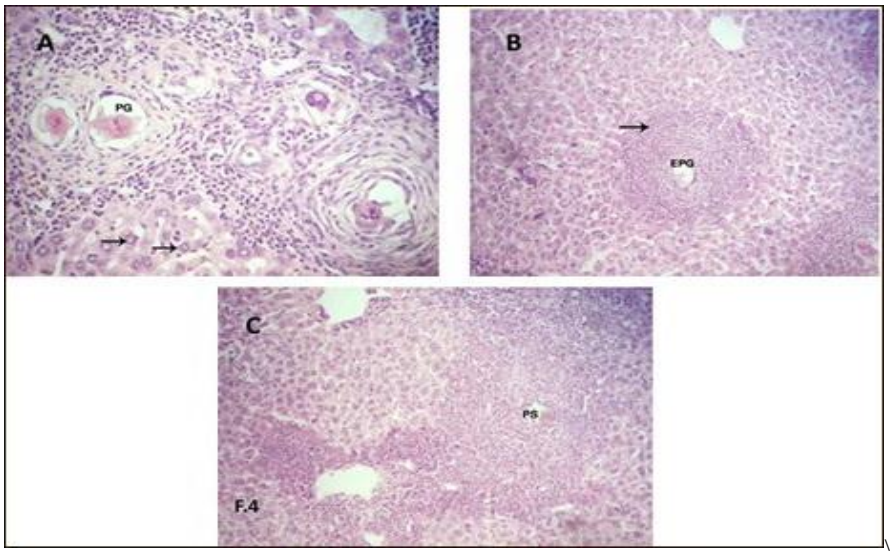
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