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Effects of *Dodonaea viscosa* Ethanolic Extract on Experimental Schistosomiasis in Mice

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ABSTRACT

Helminthic infections were among the most common infections in human beings, affected a large proportion of the world's population. In developing countries, they posed a large threat to public health and contributed to the prevalence of anaemia, malnutrition, eosinophilia, and pneumonia. The emergence of resistance to anthelmintic drugs, which is now a worldwide phenomenon and the increased awareness of consumers about drug residues that potentially enter the food chain have stimulated investigation into alternatives to commercially available anthelmintic, such as medicinal plants. *Dodonaea viscosa* is a species of flowering plant in the soapberry family, Sapindaceae, that has a cosmopolitan distribution in tropical, subtropical and warm temperate. The present study will be conducted to evaluate the antiparasitic effects of the aerial plant part (leaves) extracts of *Dodonaea viscosa* against *Schistosoma mansoni* infected mice. Mice infected with *S. mansoni* were orally treated with plant extract for 14 consecutive days. After the last dose, all animals were sacrificed to evaluate the efficacy of plant extract in the treatment of the infection through Parasitological and histological studies as well as Biochemical Assays. The results of the present study indicated that *D. viscosa* reduces the number of eggs in hepatic tissues of experimentally infected mice besides attenuates the increments of ALT and AST. Also, histopathological investigation of hepatic tissue indicated a reduction in the granuloma size.

INTRODUCTION

Schistosomiasis is a parasitic disease caused by the digenetic trematodes of the genus *Schistosoma* members which are commonly known as blood flukes. There are two major forms of schistosomiasis –intestinal and urogenital-caused by five species of the parasite. Intestinal schistosomiasis is caused by four species namely: *Schistosoma mansoni* (*S.mansoni*), *S. japonicum, S. mekongi and S. intercalatum. S. mansoni* is the most prevalent species being endemic in 55 countries e.g. Arabian Peninsula, Egypt, Libya, Sudan, Sub-saharan Africa, Brazil, some Caribbean islands, Suriname and Venzuela (Chitsulo, *et al.*, 2000).

Three compounds are currently in use, that is, metrifonate, oxamniquine, and praziquantel (PZQ) for treatment of schistosomiasis and all are included in the World Health Organization list of essential drugs. PZQ is currently the drug of choice for the treatment of schistosomiasis and is rapidly becoming the only commercially available antischistosomal drug. It is widely preferred owing to its safety, present low cost, accepted single dose with improved patient compliance, and efficacy against all five schistosome species (Bergquist *et al.*, 2017). Since the mid-1980s, along with a significant cost reduction, PZQ has become the drug of choice for morbidity control due to schistosomiasis (WHO, 2002).

Since PZQ helps as the only antischistosomal treatment in widespread use, there might be the possibility of emerging drug resistance. The first alarming reports of possible PZQ resistance came from an intensive focus in Northern Senegal, where the drug had produced very low cure rates (18 to 39%) (Doenhoff *et al.*, 2002; Deribew and Petros, 2013).

Plants synthesize many compounds called primary metabolites that are critical to their existence. These include proteins, fats, and carbohydrates that serve a variety of purposes indispensable for sustenance and reproduction, not only for the plants themselves but also for animals that feed on them. Plants also synthesize a dazzling array of additional components, called secondary metabolites, whose function has been debated. Many secondary metabolites are "antibiotic" in a broad sense, protecting the plants against fungi, bacteria, animals, and even other plants (Ramawat et al., 2009). Every plant species contains chemicals that can affect some animals or microorganisms negatively, strongly supporting the interpretation that secondary metabolites play a vital role in combating diseases and herbivores. "Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defenses against predation or infection" (Lakshmi and Bai, 2015).

Dodonaea viscosa is belonged to order Sapindales and family Sapindaceae (USDA National Plant Database, 2006). *Dodonaea viscosa* has a pantropical distribution occurring in temperate regions of Australia, Africa, Mexico, New Zealand, India, Samoa, Guam, Northern Mariana Islands, Virgin Islands, Puerto Rico, Florida, Arizona, South America and Hawaii (West *et al.*, 1984).

Dodonaea viscose is a dioecious or monoecious multi-stemmed shrub or single-stemmed small tree up to 7 m tall. Recent phytochemical studies have confirmed that *Dodonaea viscosa* contains all the major secondary plant metabolites like alkaloids, flavonoids, saponins, tannins, steroids and gum mucilage. A number of chemical constituents have been isolated from *Dodonaea viscosa* (Ramamurthy *et al.*, 2013).

Hossain (2018) reported that *Dodonaea viscosa* considered as a laxative, spasmolytic,antiviral, anti-inflammatory, antimicrobial and hypotensive agents. Also, Venkatesh *et al.* (2008) stated that *Dodonaea viscosa* possessed antidiabetic, antimicrobial, insecticidal, antioxidant, cytotoxic, antifertility, wound, anti-inflammatory, analgesic, anti-ulcer, antispasmodic, anti-diarrheal and detoxification effects.

The present work aimed to investigate the potential anti-Schistosomal activity of *Dodonaea viscosa* against experimental infection in mice through the determination of the number of live eggs in hepatic tissue; histopathological investigation of liver tissue as well as measuring granuloma size and assessing liver enzymes.

MATERIALS AND METHODS

Animals:

24 male albino mice were purchased from Animal House of Theodor Bilharz Researches Institute. They were housed in polystyrene cages in which the floor was covered with sawdust to minimize the possibility of painful contact with a hard

surface. Mice were kept in a 12 h light-dark cycle with full access to food and water. The room temperature was 25±3 ºC. The experiment was approved by the Committee of Laboratory Animal.

Plant Material:

Leaves of *D. viscosa* were collected from Suez Canal University in the month of February and March 2015. *D. viscosa* leaves were identified and authenticated by the botanist in Botany Department, Faculty of Science, Suez Canal University, Egypt. (Voucher herbarium specimen number: MEDP 26), The leaves were completely dried under the shade and coarsely powdered.

Preparation of Crude Extracts:

One hundred grams of the powder was subjected to continuous hot extraction in soxlet apparatus with water; The concentrated extract was poured into glass Petri plates and brought to dryness at 60 °C in the oven until a paste-like mass was obtained. Then paste form extract was sealed in Petri plates and stored at -20° C. The crude extract was prepared by diluting the paste in saline and storing in air-tight bottles at -4 °C for mice treatment. The therapeutic dose has been calculated and prepared as mentioned by (Khalil *et al.*, 2006).

Infection of Mice:

S. mansoni cercariae were supplied from Schistosome Biological Supply Center at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Mice were exposed to 50±10 *S. mansoni* cercariae per mouse by the tail immersion method, modified by Oliver and Stirewalt (1952).

Experimental Design:

Twenty-four mice were divided into four equal (n = 6) groups as follows.

Group 1: Control mice (negative control).

Group 2: mice treated with *D. viscosa* crud extract (300 mg/kg b.wt/ day)

Group 3: infected mice (positive control).

Group 4: infected mice treated with *D. viscosa* crud extract (300 mg/kg b.wt/ day).

Blood Sampling:

Blood samples were collected at the end of the experimental period (14 consecutive days). Blood samples were collected from heart puncture; blood samples were taken in a test tube without anticoagulant. The samples were put in an inclined position for 20minutes at room temperature, and then put in the refrigerator and then the samples were centrifuged at 3000 rpm for 10 minute and the clear serum was separated carefully, collected and stored in epindorf tubes at -20 C until estimation of serum chemistry.

Parasitologic Procedures:

Mice were dissected to open abdominal and thoracic cavities. After carefully severing the ribs near the spinal column on the left side of the thoracic cavity of a mouse, a small slit in the hepatic portal vein was made. A 20-gauge needle was inserted into the descending aorta. The perfusate (citrated saline: 8.5g sodium chloride and 15g sodium citrate per litre) was collected in a beaker by pumping perfusion fluid through the needle. Using phosphate-buffered saline, worms were collected and any adhering to mesenteric veins were removed using forceps. The recovered worms were then counted and sexed.

Tissue Egg Count in the Liver:

The number of *S. mansoni* eggs in the liver samples isolated from each mouse was estimated after alkali digestion (Cheever, 1968). Briefly, 0.5 g of each liver was placed in dedicated glass bottles each containing 2 ml of 4% KOH and incubated at 37°C overnight. The following day, each sample was placed in a 60°C incubator for 1 h. The bottles were then vigorously shaken and 0.1 ml aliquots were removed and placed on glass slides. The slides were then cover-slipped and examined under a microscope for *S. mansoni* ova. The average number of ova/0.1 ml aliquot was determined and the number of ova/gram tissue then calculated

Assessment of Hepatic Function Markers:

The activities of alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST) were determined in serum using commercial kits (Human Company, Germany, Cat. No. EC 2.6.1.2),

based on the kinetic method of Schumann *et al.* (2002).

Histopathological Studies:

Control and treated groups were anaesthetized and dissected immediately. Tissue samples of mice liver were excised and cut into small pieces. Hepatic tissue was fixed rapidly in 10% formalin for 24 hr, washed under running tap water for 24 hr., dehydrated in ascending grades of ethyl alcohol until it reaches absolute ethyl alcohol, cleared in terpineol for two days, then washed in benzene for 10 minutes and embedded in three changes of pure paraffin wax. serial transverse sections of all selected organs, 5 microns thick were cut and mounted on clean glass slides stained in haematoxylin (Harris HX) and eosin, cleared in xylene and mounted in Canada balsam (Drury and Wallington, 1980). After that sections of selected organs were carefully examined and photographs were taken as requested.

Hepatic Inflammation Measurements:

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:

Statistics were calculated with SPSS for windows version 17.0, the means value obtained in the different groups were compared by one way ANOVA followed by Duncan's were used to investigate the effect of *Dodonaea viscosa* extracts on the treatment of experimentally infected animals. All results were expressed as mean values ± SE and significance were defined as $p< 0.05$ (Field, 2000).

RESULTS

Egg count

The results in table (1) represent the effects of *D. viscosa* on egg counts of *S. mansoni* infected mice. The results were analyzed to determine if there is a difference in egg count means of S. *mansoni* infected mice from the controls by using a one-way analysis of variance test. The results showed a significant increase in egg count of S. *mansoni* infected mice as compared negative control group. It also showed that egg count was significantly decreased ($p < 0.05$) in *S. mansoni* infected mice treated with *D. viscose* as compared with the *S. mansoni* infected group.

Table (1): Effect of *Dodonaea viscosa*, on egg count in male mice infected with S. mansoni

Groups	No. of eggs
Control	0.00±0.00
D. viscosa	0.00±0.00
S. mansoni infected	48.00±6.072 [a]
D. viscosa + infected	19.67±4.624 [b]

Values are expressed as mean ± S.E. for six mice in each group.

[a] $P < 0.05$ denotes value significantly different from control using one way ANOVA followed by Duncan. [b] $P<0.05$ infected mice treated with *D. viscosa* compared to *S. mansoni* infected. using one way ANOVA followed by Duncan. Other values proved no significance.

AST and ALT levels:

The results in table (2) represent the effects of *D. viscosa* on serum aspartate transaminase (AST) and alanine transaminase (ALT) activity on *S. mansoni* infected mice. The results were analyzed to determine if there is a difference in aspartate transaminase and alanine transaminase means of *S. mansoni* infected mice from the controls by using a one-way analysis of variance test. The present results showed a significant increase in the activity of AST as well as ALT in *S. mansoni* infected mice as compared negative control group. It also showed that the levels of ALT and AST were significantly decreased ($P < 0.05$) in *S. mansoni* infected mice treated with *D. viscose* as compared with the *S. mansoni* infected group.

Table (2): Effect of *D. viscosa*, on liver function enzymes in male mice infected with *S. mansoni*

Groups	ALT	AST
Control	0.213±.035	0.173±.036
D. viscosa	0.275±.018	0.229±.010
S. mansoni infected	0.570±.133 [a]	0.338± .041[a]
D. viscosa + infected	0.276±031 [b]	0.250±.072

Values are expressed as mean ± S.E. for six mice in each group.
[a] $P < 0.05$ denotes value significantly different from control using one way ANOVA followed by Duncan. [b] $P<0.05$ infected mice treated with *D. viscosa* compared to *S. mansoni* infected. using one way ANOVA followed by Duncan. Other values proved no significance.

Histopathological Findings:

Control and *Dodonaea viscosa* Groups:

The liver of control and *D. viscosa* treated groups showed normal hepatic tissue. The section of normal rat liver showed a large number of hepatic lobules. The hepatic lobule contains numerous cords of hepatocytes radiating from the central vein to the periphery of the lobule. The hepatocytes are separated by narrow sinusoids as seen in the liver sections. Hepatic cells are arranged concentrically around the central vein. The large hepatocytes have more or less centrally situated one large round nucleus and homogenous cytoplasm.

***S. mansoni* Infected Mice:**

The sections of the liver of the *S. mansoni* infected group showed histopathological abnormalities such as; loss of normal architecture with oval or irregularly shaped neoplastic hepatocytes. Sever lymphatic infiltration and the formation of the *S. mansoni* egg-induced liver granuloma (Fig.1).

S. mansoni* Infected Mice Treated with *Dodonaea viscosa

In comparison with the *S. mansoni* infected group, the histopathological changes were less severe in *D. viscose* treated groups and this was evident by minimal diameter in granuloma as shown in figure 2.

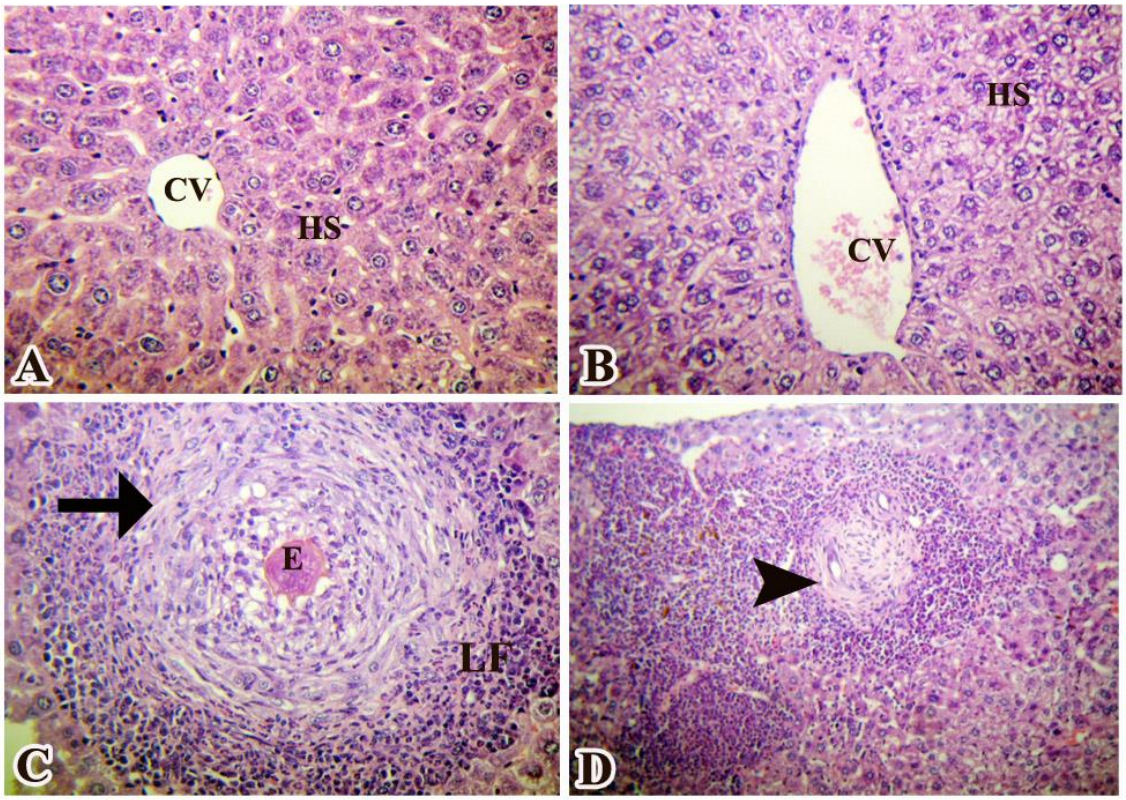

Fig 1: (A) Liver section of control mice displayed hepatic strands (HS) and prominent central vein (CV). **(B)** Liver section of uninfected *D.viscose* treated mice **(C)** Liver section of *S. mansoni* infected group showed Granuloma (arrow) associated with lymphatic infiltration (LF). The egg (E) is at the center of the granuloma. **(D)** The liver section of S. mansoni infected group treated with *D. viscose* extract displayed a reduction in granuloma diameter (arrowhead).

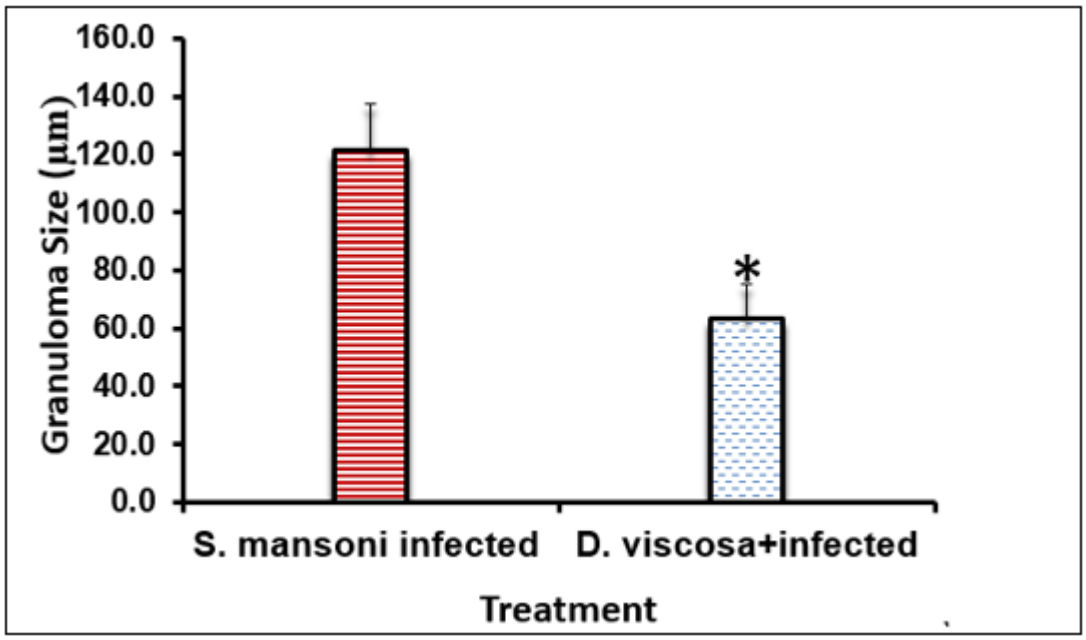

Fig 2: Quantitative analysis of hepatic granuloma in untreated S. *mansoni* infected and *D. vicosa* treated infected mice. * P<0.05 infected mice treated with *D. viscosa* compared to *S. mansoni* infected, using independent sample t-test.

DISCUSSION

For centuries, medicinal plants have been used to combat parasitism, and in many parts of the world are still used for this purpose. In ethnoveterinary medicine, which draws inspiration from traditional practice, there seems to be a range of plant/s or plant extract suitable for treating almost every parasitic disease of livestock (Mirzaei-Aghsaghali, 2012).

Medicinal chemists have synthesized a number of drugs that can be used against many but by far not all endoparasites. A major problem is that many of these drugs were developed many years ago and some parasitic strains have become resistant to them. The development of new antiparasitic drugs has not been much of a priority for the pharmaceutical industry because many of the parasitic diseases occur in poor countries where the populations cannot afford to pay a high price for the drugs. Thus, investment in drug development against parasitic diseases is a risky affair (Trouiller *et al.*, 2002). *D. viscosa* extract showed appreciable anti-schistosomal activity in infected mice after administration at 300 mg/kg for 14 consecutive days. Accordingly, the significant reduction of live ova might be due to the effect of *D. viscosa* administration on worm fecundity. Some drugs seem to act initially on the reproductive organs of the worms (Standen, 1955; Abdulla *et al.,* 2009). However, the level of treatment may have a partial activity during treatment. The drug may cause cessation of egg-laying in most of the females or may affect in some way the function of oviposition. Despite this variability, the mean number of eggs present in the tissues could be used as an indicator of schistosome fecundity and that a direct comparison of this variable could be made between treated and untreated host mice (Lamberton, *et al.,* 2017).

S. mansoni infection results in a hepatocellular injury, which in turn, leads to the release of the enzymes from the injured hepatic cells into the blood circulation (Dkhil, 2014). In the present study, the significant higher AST and ALT activity in the infected groups may due to the existence of the inflammatory hepatic granuloma reported to be present as a result of egg deposition and the presence of worms as well as its toxins. In this respect, a significant elevation of ALT activities in liver tissues of infection with *S. mansoni* is reported by El-Sayed, et *al.* (2011). Other investigators found increases in serum transaminases in *S. mansoni* infected animals (Awadalla *et al.,* 1975; Mansour et al., 1982; Hamed *et al.,* 2005). The current study indicated that treatment infected mice with *D. viscosa* extract displayed a positive impact on the liver function were ALT and AST showed a significant decrease as compared with infected mice. Our results were in accordance with Shalaby *et al.* (2012) who stated that *D. viscosa* ethanolic extract exhibited potent antioxidant activity. Where it attenuates the increments of MDA, AST, ALT, ALP, GGT, total protein, and increased GSH and SOD levels against induced liver fibrosis.

In conclusion, *D. viscosa* has moderate anti-schistosomal activity in mice. It significantly reduced the production of eggs out up in *S. mansoni* infected mice. In addition, this study showed that *D. viscosa* clearly reduces the liver granuloma size and this modulation of the liver granulomas was reflected on some degree of improvement in the status of the liver. This will have an obvious economic advantage for the local populations, as the developed medicinal plant products will be the cheapest therapeutic alternative for *S. mansoni*.

Competing Interests:

The authors declare that there are no competing interests

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