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The Histological and Biochemical Effects of Saffron on *Schistosoma mansoni* Infected Mice

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ABSTRACT

The present study was designed to evaluate the antischistosomal activity of the herbal medicine; saffron on mice infected with *Schistosoma mansoni* species. Mice were divided into three groups; each group contains seven mice. The first group was served as normal control and mice of the other two groups was individually infected with 60±5 *Schistosoma mansoni* cercaria. One of them was the infected group and the other group mice were orally treated with 0.2 ml of saffron extract (100mg/kg) six weeks' post infection three times weekly for four weeks. After the last dose; all mice were sacrificed. Mice liver infected with *Schistosoma mansoni* cercaria displayed an extremely elevation ($P \leq 0.001$) in liver weight, ova count, ALT and AST levels. Otherwise, it induced an extremely reduction ($P \leq 0.001$) in both albumin and total protein levels. Saffron extract modulated all the previous mentioned parameters to be near of normal values. Histologically, mice liver infected with *Schistosoma mansoni* cercaria showed portal fibrosis, granulomatous nodules and focal cell infiltration. Saffron extract ameliorated the histological structure of liver by reducing the different histopathological effects induced with *Schistosoma mansoni* cercaria. Finally, saffron has a powerful antischistosomal activity in mice infected with *Schistosoma mansoni* cercaria.

1. INTRODUCTION

Schistosomiasis remains to be a tropical disease that ranks with malaria as a major source of morbidity affecting approximately 210 million people in 76 countries of the world (Steinmann et al., 2006). Schistosomiasis is still one of the most prevalent epidemic diseases in Egypt. Egyptians have a long history of symptoms caused by schistosomiasis, notably hematuria (Salem *et al.*, 2010). Mature *S. mansoni* female worms produce hundreds of eggs a day, many which are trapped in the liver and intestine. These induce a local inflammatory response (Kaplan et al., 1998). Schistosomal eggs induced liver fibrosis which is a common pathological process leading to the development of irreversible cirrhosis (Xiong et al., 2003) and inability of the liver to perform its biochemical functions (Rehermann and Nascimbeni, 2005).

El-Agamy et al. (2011), reported that the mice infected with *S. mansoni* exhibited an elevation values of both ALT and AST. They also mentioned that the liver histopathological signs after infection with *S. mansoni* showed fibrotic changes, as well as inflammatory changes as portal infiltration, interface hepatitis and focal necrosis.

Many medicinal plants were studied to investigate their antischistosomal potency and found to be effective, like: garlic *Allium sativum* (Riad et al., 2008), wild carrots *Daucus carota* (Shalaby et al., 1999), myrrh *Commiphora molmol* (Massoud, 1999) and ginger *Zingiber officinale* (Sanderson et al., 2002).

Saffron, *Crocus sativus* (Family Iridaceae) commonly known as Zaa'fran is used as a flavoring agent and an

important ingredient of Arabic coffee. Saffron is known to contain various chemical constituents including crocin-1, picrocrocin, startry, vitamins, Bl and B2, fixed oils, carotenoids, colichicine, quercitin, proteins, wax and mucilage (Tarantilis et al., 1995). The saffron total extract, aqueous and ethyl acetate fractions significantly resulted in suppression of parasitemia in mice infected with different parasites including Malaria (Nader et al., 2015). The saffron and its constituents can easily reach the different contaminant bacteria because of their volatility and/or water-solubility and contribute to bacterial killing (Concepcion et al., 2011). The aqueous and ethanolic extracts of saffron exhibit hepatoprotective effects against liver damages induced by carbon tetrachloride (CCl4) in mice and significantly decreased the elevated levels of AST and ALT in plasma (Milad et al., 2011). Numerous studies indicated the health promoting properties of saffron are attributed primarily to crocin, a unique carotenoid with powerful antioxidant capacity (Giaccio, 2004). It was reported that saffron has an anti-inflammatory (Nam et al., 2010) and antioxidant properties (Trujillo et al., 2013).

2. MATERIALS AND METHODS

2.1. Experimental animals

Twenty-one male Swiss albino mice weighing 20±2 g, were purchased from Schistosoma Biological Supply Program (SBSP) unit at Theodor Bilharz Research Institute (TBRI), Giza Egypt. Mice were housed in plastic cages and maintained under laboratory conditions at (TBRI). They could feed on a standard pelleted diet containing 24% protein, 4% fat and about 4-5% fiber and water. The mice were kept for about one week before experimentation for adaptation under laboratory condition. The experiments were approved by the state authorities and it followed the Egyptian rules on animal protection, as well as specific local institutional laws for protection of animals under the supervision of authorized investigators.

2.2. Experimental design

Animals were divided into three groups with seven mice in each group, as the following; the 1st group is a healthy control group received a normal diet (non-infected; non-treated); the 2nd group is an infected one and received a normal diet (infected; non-treated). Mice were infected with *S. mansoni* and served as infected mice group and the 3rd group is an infected group that treated with a therapeutic dose of saffron; received a normal diet (infected; treated). Mice were infected with *S. mansoni* and treated orally with saffron extract in a dose of 100 mg/kg for 4 weeks' post infection.

2.3.Infection of mice

S. mansoni cercariae were supplied by the Schistosoma Biological Supply Program (SBSP) at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Male albino mice were infected with 60±5 *S. mansoni* cercariae via subcutaneous route (Oliver and Stirewalt, 1952). Cercariae shedding out of *Biomphalaria alexanderina* snails were used to infect the mice. The experiments were carried out at Animal Biotechnology Department, Genetic Engineering and Biotechnology Institute, Sadat City University, Sadat City, Egypt.

2.4.Saffron Extraction

Saffron (*Crocus sativus*) was purchased from a local market, Cairo City, Egypt. A dried powder (10 g) from saffron separately was mixed with 100 ml organic solvent (ethanol, hexane and ethyl acetate). The mixture was placed at room temperature for 24 h on shaker with 150 rpm. Solution was filtered through muslin cloth and then re-filtered by passing through Whatman Filter No. 1. The filtrate thus obtained was concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solution of crude extract for saffron of organic solvent were prepared by mixing well the appropriate amount of dried extract with respective solvent to obtain a final concentration of 100 mg/ml. The solution was stored at 4°C after collecting in sterilized bottles until further use (Purshotam and Pankaj, 2011).

2.5. Liver weights

At the end of experimentation, the liver weight of each mouse was taken in different mice groups.

2.6. Liver and intestine Ova count

Cheever and Anderson, (1971) mentioned that after the perfusion of each mouse; a piece of liver or intestine was taken to find out the number of eggs. Each piece of liver or intestine was dried in filter paper weighted and placed separately in 5ml of 5 % potassium hydroxide (KOH) solution. They were incubated under 37°c for 24 hours, until the complete digestion of liver and intestine. The digested liver or intestine was shaken well on a magnetic mixer for one minute and 0.1 ml was pipetted by a micropipette,

placed on a slide to be examined under the light microscope.

2.7. Biochemical analysis

The activity of Serum Alanine aminotransferase (ALT) was measured colorimetrically in accordant with the method described by (Bardley et al., 1972). Aspartate aminotransferase (AST) activity was estimated by enzymatic rate method (Henry et al., 1960). Total proteins were estimated in accordant with (Burtis et al., 1999); thus, the intensity of the color formed is proportional to the total protein concentration in the sample. The serum albumin concentration was estimated by bromocresol green colorimetric reaction, in accordant with the method as described by (Doumas et al., 1971).

2.8. Histological examination

Specimens of liver were taken from mice in all groups. Liver tissue was fixed in 10% neutral buffered formalin solution for 24h at room temperature at 37°C, following dehydration in ascending series of ethanol (70, 80, 90 and 100%). Tissue sample were processed with paraffin wax. Sections (5-microns) were stained with hematoxylin and eosin (H & E) and were examined under light microscope. The sections were viewed and photographed (Banchroft et al., 1996).

2.9. Statistical analysis

The results were expressed as mean ± SD of different groups. The differences between the mean values were evaluated by one-way analysis of variance ANOVA followed by Tukey-Kramer multiple comparison test (Armitage and Berry, 1987) using Graph Pad Prism software. *P* values < 0.05 were statistically significant.

3. RESULTS

3.1. Liver weight

Data tabulated in table (1) explained that the values of liver weights (Mean ± SD) in *S. mansoni* infected mice recorded an extremely elevation compared to control ($P \leq 0.001$). Saffron extract induced restoration of the normal weights of liver in *S. mansoni* infected mice.

Table (1): Effect of saffron extract on the liver weights in *S. mansoni* infected mice.

Liver weights (gm)	Groups
2.119 ± 0.2148	**Control**
3.88 ± 0.1002***	**Infected**
2.209 ± 0.0701^{ns}	**Infected +Saffron**

All data are expressed as mean ± SD; (***) extremely significant ($P \leq 0.001$), (**) very significant ($P \leq 0.01$), (*) significant ($P \leq 0.05$) and (ns) not-significant ($P > 0.05$).

3.2. Ova count

Data recorded in table (2) clarified that the recorded egg load in intestine is more than found in liver and saffron extract induced extremely reduction in ova count ($P \leq 0.001$); compared to *S. mansoni* infected mice. The ability of saffron in reduction of ova count in liver is more than in intestine.

Table (2): Effect of saffron extract on the ova count in liver and intestine in *S. mansoni* infected mice.

Intestine Egg load	Liver egg load	Groups
5853±138.5	3153±61.8	**Infected**
2943±97.54***	646.6±32.07***	**Infected +Saffron**

3.3. Biochemical Findings

The Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) values was extremely significant elevated ($P \leq 0.001$) in *S. mansoni* infected mice in relation to normal values recorded in control group (table 3). The saffron extract induced restoration of the normal values of ALT and AST by inducing an extremely significant reduction in their values in relation to the infected group.

Table (3): Effect of saffron extract on the levels of alanine aminotransferase (ALT) and the Aspartate aminotransferase (AST) in different mice groups in relation to normal control.

Groups	ALT (U/L)	AST (U/L)
Control	38.00± 6.387	88.33±11.17
Infected	150.2±8.256***	236.5±13.58***
Infected +Saffron	34.33±5.046^{ns}	96.00±22.36^{ns}

*** Significant difference from control and ns: no significant difference from control

3.4. Data recorded in table (4) explained that the levels of albumin and total protein was extremely significant reduced ($P \leq 0.001$) in *S. mansoni* infected mice in relation to normal values recorded in control group. On the other hand, saffron extract induced an

elevation in the level of albumin and total protein to be near of normal values. The effect of saffron extract is more potent in elevation in total protein more than albumin that induced very significant reduction ($P \leq 0.01$) in albumin level in relation to control whether; induced a non-significant change in total protein level in relation to normal values.

Table (4): Effect of saffron extract on the levels of albumin and the total protein in different mice groups in relation to normal control.

Groups	Albumin(g/dl)	Total protein(g/dl)
Control	4.083±0.2483	6.083± 0.3488
Infected	2.700±0.2366***	3.550± 0.3391***
Infected +Saffron	3.500±0.3742**	6.167± 0.3266ns

*** Significant difference from control and ns: no significant difference from control

3.5. Histological findings

Hematoxylin and eosin-stained liver sections for control mice showed normal lobular pattern of a central vein (CV). From this vein the hepatic cords (HC) separated by hepatic sinusoids (HS) are radiated. Portal tracts (PT) were seen bordering the hepatic lobule. Each portal tract was consisted of three consistent structures, namely branches of portal vein (PV), hepatic artery and bile ductules (BD). Hepatocytes were large and polyhedral in shape with slightly eosinophilic granular cytoplasm. They varied in size and have large rounded vesicular single or double nuclei and prominent nucleoli (Fig. 1A).

Hematoxylin and eosin-stained liver sections from Schistosoma-infected mice showed portal fibrosis due to massive and concentrated deposition of eggs in portal areas, whereas the liver parenchyma maintains its normal architecture (Fig. 1B). Eggs are found in tissue sections with an intense fibrotic reaction and extensive mononuclear cells infiltration around them (granulomatous nodules). In addition to the diffuse portal cell infiltration, there was intra-lobular focal and diffuse cell infiltration. On the other hand, liver sections from saffron-treated infected mice displayed a noticeable reduction in the size of the granulomatous nodules, and the mononuclear cell infiltration was greatly reduced (Fig. 1C).

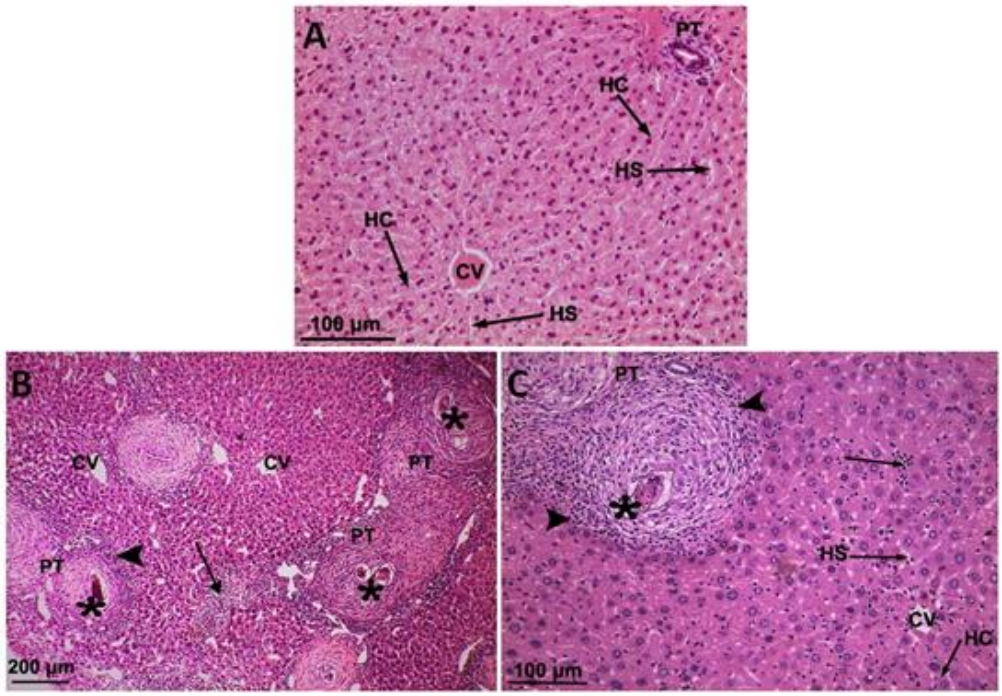

Figure 1: Hematoxylin and eosin-stained liver sections from control **(A)**, infected **(B)** and saffron-treated mice **(C)** showing central vein **(CV)**, portal tract **(PT)**, hepatic cords **(HC)**, hepatic sinusoids **(HS)**. Asterisks **(*)** point to the periovular granulomas enclosed by diffuse mononuclear cell infiltration (arrowheads), arrows point to intralobular focal cell infiltration, which is lager in section from infected mice **(B)** than in section from treated ones **(C)**.

The Masson Trichrome-stained liver sections from infected (Fig. 2A) and saffron-treated infected mice (Fig.2 B) showed periovular granulomas, resulting in chronic portal fibrosis due to extensive collagen deposition around parasitic eggs within the branches of portal vein and bile ductules within the portal areas. The number and size of these granulomas exhibit noticeable reduction in sections from saffron - treated mice compared to the non-treated ones.

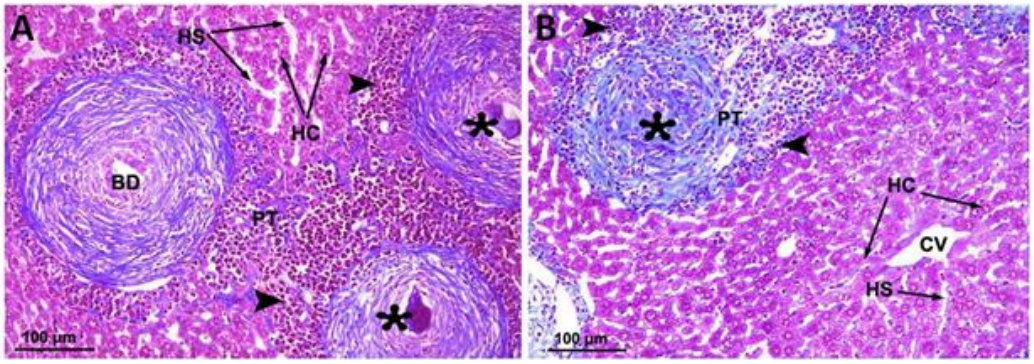

Figure 2: Masson trichrome-stained liver sections from infected **(A)** and saffron-treated mice **(B)** showing central vein **(CV),** portal tract **(PT),** hepatic cords **(HC),** hepatic sinusoids **(HS).** Concentric lamellae of collagenous depositions seen surrounding a hyperplastic bile ductule **(BD)** and schistosomal eggs within intra-hepatic branches of portal vein forming periovular granulomas **(*)** enclosed by mononuclear cell infiltration (arrowheads)**,** which is more intense in section from infected mice **(A)** than in section from treated ones **(B).**

4. DISCUSSION

In schistosomiasis, granulomas develop around the ova (periovular granulomas) within different tissues of the host including liver. The egg shell protects the ova from destruction by the host defense mechanisms. The ova release a variety of substances which are toxic to host tissues and antigenic, leading to antigen-specific humoral and cell-mediated immune responses. The formation of granulomas in the liver is a manifestation of delayed type hypersensitivity to soluble egg antigens, which are released by the trapped ova (Elbaz and Esmat, 2013).

Although granuloma formation is beneficial for the host because it blocks the hepatotoxic effects of antigens released from parasitic eggs, this process may lead to fibrosis with excessive accumulation of collagen and extracellular matrix proteins in the periportal areas (Morais et al., 2008). Granuloma formation is a protective phenomenon for the host as it sequesters toxic and antigenic substances and eventually it destroys the egg and removes residual debris. Deletorious effects of granulomas include focal tissue injury and induction of noticeable fibrosis. Granulomas contain macrophages, epithelioid cells, giant cells, eosinophils, lymphocytes and a few mast cells. The inflammatory cells rest on collagenous matrix produced by fibroblasts. This matrix displaces normal organ parenchyma (Weinstock, 1992).

Hepatic fibrosis can be defined as an increase in the amount of fibrous connective tissue in relation to the parenchyma of the liver (Pauly and Ruebner, 1987). Fibrosis results when the rate of collagen synthesis is higher than that of collagen degradation (Chen et al., 2002). It is a result of egg-induced chronic peri-sinusoidal inflammation leading to deposition of fibrous material and vascular destruction. These fibrotic changes produce a portal fibrosis throughout the intrahepatic branches of the portal vein displaying the so-called clay 'pipe stem' in (Lambertucci, 1993). Although the term cirrhosis has been misused, the liver parenchymal cells essentially are unharmed and thus the terms periportal or portal fibrosis have been preferred (Baddamwar, 2004).

Our histopathological findings revealed a remarkable reduction in the number and size of granulomatous inflammatory infiltrations in the liver sections from saffron-treated mice compared to non-treated ones. These go in line with previous work on ginger-treated infected mice (Mostafa et al., 2011; Hassan et al., 2016). It is worth noting that, oral supplementation of ginger extract to infected animals was effective in reducing worm burden and the egg load in the liver coincided with the reduction in granuloma diameters. Ginger extract had also the effect to counteract liver fibrosis in response to *S. mansoni* infection via reduction of inflammatory mediators that play a

crucial role in schistosomal liver fibrosis and its complications (Aly and Mantawy, 2013).

Oxidative stress represents a crucial factor in the development of diseases such as hepatic inflammation and hepatic cirrhosis (Dalle-Donne et al., 2006; Bandegi et al., 2014). Schistosomiasis causes a progressive reduction in the levels of protective endogenous antioxidants (Diab et al., 2013) and increases generation of free radicals (Muema et al., 2015). This leads to a case imbalance in parasite-oxidant/ host-antioxidant system inducing oxidative stress (Michiels et al., 1994). The oxidative processes associated with *Schistosoma mansoni* infection suppresses the host enzymatic detoxification activities, playing a role in pathogenesis of schistosomiasis (El Sokkary et al., 2002). Oxidative free radicals induce host tissue damage (Fromenty et al., 1997). It is known that oxidative stress due to schistosomiasis at the site of granulomatous inflammation leads to the generation of lipid peroxidation (LPO) products, which may play a central role in the pathology of schistosomiasis (Aly et al., 2010). LPO products cause cell injury and necrosis due to losing the fluidity and integrity of cell membrane (Dkhil et al., 2014). It is worth mentioning that saffron can prevent chronic-stress induced oxidative damage of liver (Bandegi et al., 2014). Thus, these substances should be useful as new pharmacological tools for ameliorating chronic stress-induced oxidative damages, including schistiosomal hepatopathy.

In the present study, all infected mice showed a significant increase in serum ALT, AST which are measures of liver integrity. This seems consequent with hepatic cell damage and impaired cell membrane permeability (Ghanem et al., 1970) or due to heavy schistosomal egg deposition (Giboda et al., 1994). Hepatoprotective activity of saffron was evaluated on *Schistosoma mansoni* infected mice by estimation of serum hepatic enzymes. Hepatic cells appear to participate in a variety of enzymatic metabolic activities. Infection of *Schistosoma mansoni* damages the hepatic cells leading to a significant increase in serum levels of ALT and AST (Table 3). The Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) values was extremely significant elevated ($P \leq 0.001$) in *S. mansoni* infected mice in relation to normal values recorded in control group. These findings agree with previously reported findings by (El-Gowhary et al., 1993). The infected mice treated with curcumin restore the hepatic ALT and AST activities that were increased by *S. mansoni* infection. This amelioration in the activities of liver enzymes could be attributed to the reduction in hepatic granuloma size and fibrosis as well as absence of necrotic hepatic tissue in infected treated mice (Mahmoud and Elbessoumy, 2014). This is the same finding induced with saffron in *S. mansoni* infected mice.

Serum levels of total protein and albumin were reduced significantly as compared to control group (Table 4). However, Supplementation of curcumin to infected mice resulted in an elevation of total protein and albumin levels compared with infected non-treated mice. Similar observations were noticed by (El-Ansary et al., 2007 and El-Emam et al., 2011). Saffron extract performed the same curative effect against *S. mansoni* infected mice.

Due to the schistosomal infection, the liver weight was greatly increased in a significant manner due to egg deposition in liver; this finding agreed with (Soliman et al., 2000) who mentioned the same finding.

In the present investigation, the alcoholic extract of saffron exhibited an anti-schistosomal activity in mice as showed by significantly reduction of egg count in the hepatic and intestinal tissues of treated mice. This finding was considered by several authors as a strong evidence of the efficiency of antischistosomal drugs (Suleiman et al., 2004 and Mati et al., 2010).

5. Conclusion

The results of this study indicate on the powerful efficiency of saffron extract in *S. mansoni* infected mice by modulation the levels of ALT, AST, albumin, total proteins, egg count and liver weights towards the normal values. It also ameliorated the histological structure of liver. Finally, alcoholic extract of saffron has an anti-Schistosomal effects against *S. mansoni* infected mice.

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