

## Antischistosomal and Hepatoprotective Activity of *Morus alba* Leaves Extract

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**Abstract.-** Schistosomiasis is an important disease in the tropics with huge impact on the socio-economic development of affected regions. The study aimed to investigate the antischistosomal and hepatoprotective activity of *Morus alba* leaves extract. Mice were allocated to eight groups. Group I served as vehicle control. Groups II, III and IV were gavage with 100 µl of 200, 400 and 800 mg/kg mulberry leaves extract, respectively for 10 days. Groups V, VI, VII and VIII were infected with 100±10 *Schistosoma mansoni* cercariae. On day 46 p.i. with *S. mansoni*, the animals of group VI, VII and VIII received 100 µl mulberry extract by gavage once daily for 10 days at a dose of 200, 400 and 800 mg/kg body weight, respectively. All mice were sacrificed at day 55 post-infection. Worm recovery and egg counting in the hepatic tissues were determined. *S. mansoni* was able to induce inflammation and injury of the liver. This was evidenced (i) as increases in granuloma size, (ii) as increased production of nitric oxide and malondialdehyde, and (iii) as lowered glutathione levels. All these infection-induced parameters were significantly altered during mulberry treatment. Based on these results, it is concluded that mulberry could ameliorate preexisting liver damage and oxidative stress conditions due to schistosomiasis.

**Key words:** *Schistosoma mansoni*, *Morus alba*, liver, schistosomiasis, hepatoprotective activity.

### INTRODUCTION

Schistosomiasis remains one of the most prevalent parasitic infections in the world and is endemic in more than 75 countries (World Health Organization, 2010). More than 10% of the world's population – are at risk of being infected with schistosomiasis and approximately 207 million people in tropical and subtropical zones are infected with this disease (Steinmann *et al.*, 2006; World Health Organization, 2010). Laboratory animals have been frequently used as a model for the analysis of pathological studies in human infection (Cheever *et al.*, 2002). The reference drug for treatment of schistosomiasis is praziquantel (PZQ), however, in Egypt, some patients received three doses of PZQ failed to be completely cured (Ismail *et al.*, 1999).

Medicinal plants have been useful in the development of new drugs and continue to play an invaluable role in the drug discovery processes

(Farnsworth, 1994). Some medically important plant species as *Zingiber officinale*, *Nigella sativa* and *Asparagus officinalis* show an effect against schistosomiasis (Hoareau and Dasilva, 1996; Tanwer and Vijayvergia, 2010).

*M. alba* L. of Moraceae family (mulberry) has long history of use in Chinese oriental medicine. It is claimed that almost all parts of this plant is useful in cardiovascular, liver and spleen disorders (Fukai *et al.*, 2003). Recent research has shown that this herb has free radical scavenging activities, hypolipidemic, antioxidant, antibacterial, antiviral, astringent, emollient and antiinflammatory properties (El-Beshbishy *et al.*, 2006; Du *et al.*, 2003; Chung *et al.*, 2003). Mulberry leaf contains triterpenes (lupeol), sterols (β-sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids and organic acids (Doi *et al.*, 2001).

Since the liver plays a vital role in maintaining health and in the same time is highly susceptible to disease and injury, we designed the current study to investigate the hepatoprotective effect of mulberry leaves against *Schistosoma mansoni* infection in mice.

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## MATERIALS AND METHODS

### Animals

Swiss albino mice were bred under specified pathogen-free conditions and fed a standard diet and water *ad libitum*. The experiments were performed only with male mice (25-30 g) at an age of 9-11 weeks and were approved by state authorities and followed Saudi Arabian rules for animal protection.

### Infection of mice

*S. mansoni* cercariae were from Schistosome Biological Supply Center at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Mice were exposed to  $100 \pm 10$  *S. mansoni* cercariae per mouse by the tail immersion method, modified by Oliver and Stirewalt (1952).

### Preparation of the mulberry leaf extract

Fresh matured leaves of *Morus alba* tree were collected from Riyadh, Saudi Arabia. The samples were authenticated by Dr. Jacob Thomas (Botany Department, College of Science, King Saud University, Saudi Arabia) on the basis of taxonomic characters and by direct comparison with the herbarium specimens with a voucher number KSU-11272 available at the herbarium of Botany (King Saud University, Saudi Arabia). Mulberry leaf extract was prepared according to the method described by Mohammadi *et al.* (2012) with some modification. Air-dried powder (100 g) of mulberry leaves were extracted by percolation at room temperature with 70% methanol and kept at 4 °C for 24 h. The obtained extract was concentrated under reduced pressure (bath temperature 50°C) and dried in a vacuum evaporator. The residue was dissolved in distilled water, filtered and used in our experiment.

### Experimental design

Animals were allocated to 8 groups of eight mice each and treated as mentioned in Table I.

Mice were infected with  $100 \pm 10$  *S. mansoni* cercariae. On day 45 p.i. with *S. mansoni*, the animals of the infected groups received 100 µl of *M. alba* extract by gavage once daily for 10 days. On day 55 p.i. with *S. mansoni*, the animals of all groups were sacrificed.

**Table I.- Experimental design.**

| Group                       | Treatment regime   |
|-----------------------------|--|
| Non-infected control        | Group I received 100 µl water<br>Group II received 200 mg/Kg Mulberry<br>Group III received 400 mg/Kg Mulberry<br>Group IV received 800 mg/Kg Mulberry   |
| <i>S. mansoni</i> -infected | Group V received 100 µl water<br>Group VI received 200 mg/Kg Mulberry<br>Group VII received 400 mg/Kg Mulberry<br>Group VIII received 800 mg/Kg Mulberry |

Livers from infected and non-infected control animals were aseptically removed from mice, cut up in small pieces, washed in sterile physiological saline, and rapidly frozen and stored at -80 °C, if not otherwise stated. Part of the liver was weighed and homogenized in a PRO Scientific D-Series Benchtop homogenizer in order to prepare a 50% (w/v) homogenate in an ice-cold medium containing 50 mM Tris-HCl and 300 mM sucrose (Tris-sucrose buffer). The initial homogenate was centrifuged at  $500 \times g$  for 10 min at 4°C. The supernatant was diluted 1:10 with the Tris-sucrose buffer to give 10% and was then used for the various biochemical determinations.

### Perfusion and worm recovery

Mice were sacrificed nearly at the end of the eighth week post exposure (day 55 p.i.); Schistosome worms were recovered from the hepatic portal and mesenteric veins by perfusion technique described by Smithers and Terry (1965).

### Egg count in the liver

The eggs in the liver of infected mice were counted according to Pelligrino *et al.* (1962). In brief, the number of eggs per gram of liver tissue was determined by weighing a piece of liver (0.1 g) and divided it into four fragments, each fragment crashed between a slide and cover slip. The fragments were examined by light microscope.

### Granuloma size

Tissue samples of the liver of all groups were immediately fixed after animal dissection in 10% neutral buffered formalin, dehydrated and processed for paraffin sectioning. Sections were then deparaffinized, stained with hematoxylin and eosin

stains. To assess the size of tissue granuloma, the mean diameter ( $\mu\text{m}$ ) was measured. For each group, 30 granulomas were chosen from different sections and different mice.

#### Biochemical analysis

##### Glutathione

Glutathione (GSH) was determined chemically in liver homogenate using Ellman's reagent (Ellman 1959). The method is based on the reduction of Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid) with GSH to produce a yellow compound. The chromogen is directly proportional to GSH concentration, and its absorbance was measured at 405 nm.

##### Lipid peroxidation

Lipid peroxidation in liver homogenate were determined according to the method of Ohkawa *et al.* (1979) by using 1 ml of trichloroacetic acid 10% and 1 ml of thiobarbituric acid 0.67%, followed by heating in a boiling water bath for 30 min. Thiobarbituric acid reactive substances were determined by the absorbance at 535 nm and expressed as malondialdehyde (MDA) equivalents formed.

##### Nitric oxide

The assay of nitrite in the liver homogenate was performed according to the method of Berkels *et al.* (2004). The method was based on the reaction of liver homogenate with the Griess reagent. The Griess reagent is based on the two-step diazotization reaction in which acidified nitrite produces a nitrosating agent, which reacts with sulphanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine dihydrochloride to form the chromophoric azo-derivative red at 540 nm.

##### Statistical analysis

One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's test using a statistical package program (SPSS version 17.0). All p values are two-tailed and  $p < 0.05$  was considered as significant for all statistical analysis in this study.

## RESULTS

Analysis of the parasite at day 55 p.i. showed differences in the total number of the recovered worms in all the infected-treated mice compared to the infected-untreated one (Table II). Mulberry treatment with 200, 400 and 800 mg/kg significantly affect the worm burden with a reduction rate of 40%, 26% and 46%, respectively (Table II). Moreover, mice treated with mulberry (200 mg/kg) displayed a highly significant increase in egg reduction, estimated by  $2180 \pm 101$  egg/ g liver (Table II).

**Table II.-** Worm recovery and egg density in the hepatic tissues of mice infected with *S. mansoni*, treated or non-treated with *M. alba* leaves extract.

| Group                  | Worm recovery |             |              | Egg / g liver   |
|------------------------|---------------|-------------|--------------|-----------------|
|                        | Male          | Female      | Total        |                 |
| Group V (Water)        | 7 $\pm$ 1.5   | 8 $\pm$ 2   | 15 $\pm$ 1.1 | 2453 $\pm$ 94   |
| Group VI (200 mg/kg)   | 5 $\pm$ 2     | 4 $\pm$ 1.5 | 9 $\pm$ 1.5* | 2180 $\pm$ 101* |
| Group VII (400 mg/kg)  | 6 $\pm$ 1     | 5 $\pm$ 1.4 | 11 $\pm$ 1*  | 2365 $\pm$ 89   |
| Group VIII (800 mg/kg) | 4 $\pm$ 1.4   | 4 $\pm$ 1   | 8 $\pm$ 1.3* | 2350 $\pm$ 91   |

Values are means $\pm$ SD (n=8). \*: Significant change at  $P \leq 0.05$

Granulomas were marked by concentric fibrosis with many fibroblasts encircled the trapped eggs. Mulberry administration induced a significant reduction in the size of granuloma compared to infected nontreated one (Figs. 1, 2). The granuloma diameter reached approximately  $405 \pm 26$   $\mu\text{m}$  in infected livers. Mulberry treatment with 200, 400 and 800 mg/kg could significantly reduce the granuloma diameter to  $250 \pm 19$ ,  $230 \pm 19$  and  $260 \pm 22$   $\mu\text{m}$ , respectively (Fig. 2).

*S. mansoni* also induced a highly significant increase in hepatic NO and MDA (Table III) by approximately 1.5 fold. However, mulberry induced no significant reduction in the *S. mansoni*-induced increase in both MDA and NO, respectively (Table III).

*S. mansoni* induced a significant ( $P \leq 0.05$ ) decrease in the level of glutathione in the liver of mice (Fig. 3). Treatment with 200 and 800 mg/kg of

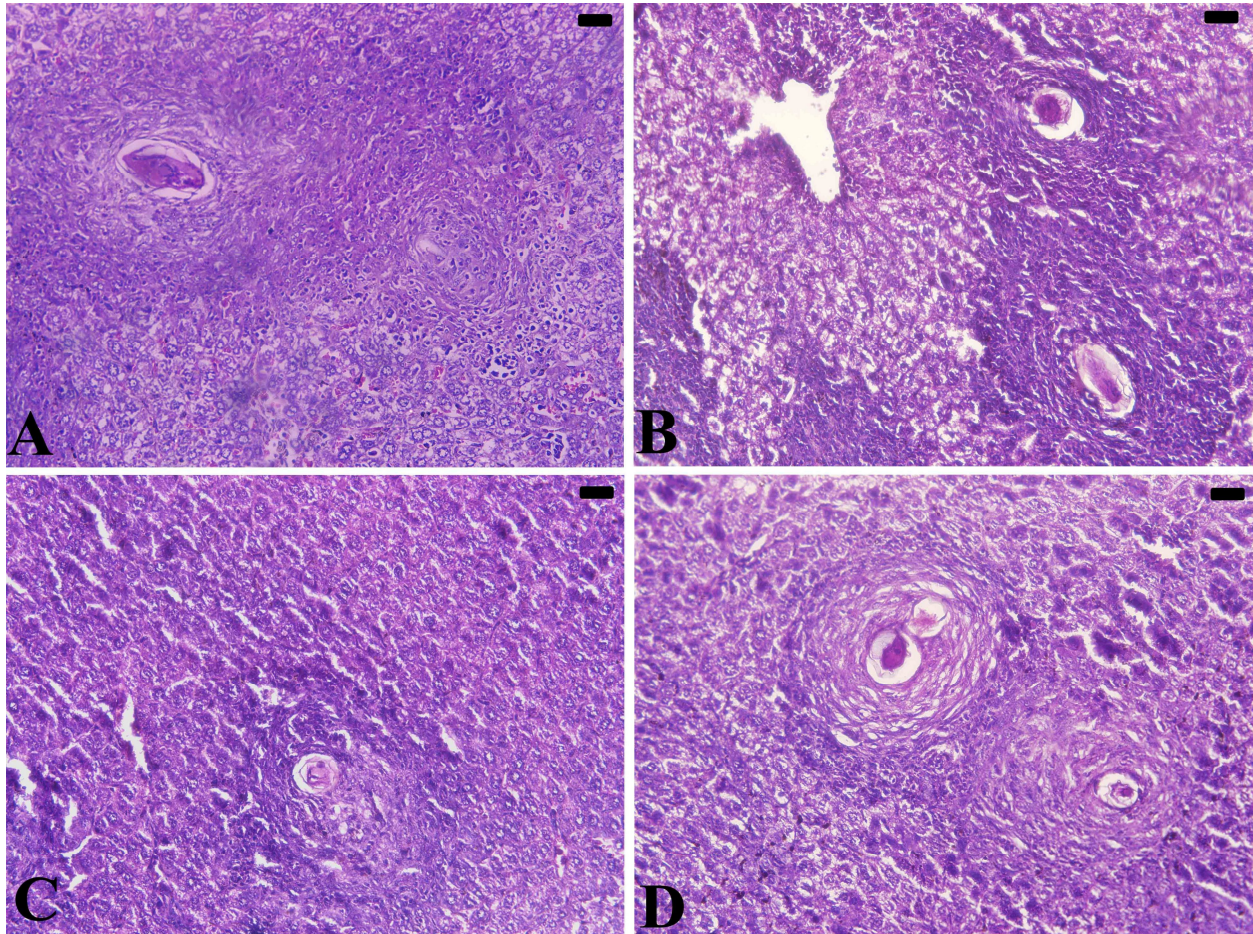


Fig. 1. Sections of mouse liver infected with *S. mansoni* on day 55 p.i. A, Non-infected liver with normal architecture. B, C, D, Infected treated liver with 200, 400 and 800 mg/kg mulberry, respectively. Sections appeared with prominent inflammation around granuloma. Sections are stained with hematoxylin and eosin. Bar=50 µm.

**Table III.- Malonaldehyde (MDA) and nitric oxide (NO) level in hepatic tissues of mice infected with *S. mansoni*, treated or non-treated with *M. alba* leaf extract.**

| Group                       |                  | MDA<br>(nmol/g)        | NO<br>(µmol/g)          |
|-----------------------------|------------------|------------------------|-------------------------|
| Non-infected control        | I (Water)        | 46±1.6                 | 384±17                  |
|                             | II (200 mg/kg)   | 45±3                   | 214±27 <sup>a</sup>     |
|                             | III (400 mg/kg)  | 38±2 <sup>a</sup>      | 358±20                  |
|                             | IV (800 mg/kg)   | 44±1.5                 | 332±35 <sup>a</sup>     |
| <i>S. mansoni</i> -infected | V (Water)        | 70±8 <sup>a</sup>      | 596±66 <sup>a</sup>     |
|                             | VI (200 mg/kg)   | 60±12 <sup>a,b,c</sup> | 524±44 <sup>a,b,c</sup> |
|                             | VII (400 mg/kg)  | 72±6 <sup>a,b</sup>    | 562±37 <sup>a,b,c</sup> |
|                             | VIII (800 mg/kg) | 61±10 <sup>a,b,c</sup> | 603±36 <sup>a,b</sup>   |

<sup>a</sup>Significant difference as compared with normal control group ( $p \leq 0.05$ ). <sup>b</sup>Significant difference as compared with corresponding control group ( $p \leq 0.05$ ). <sup>c</sup>Significant difference as compared with infected group ( $p \leq 0.05$ ).

Mulberry rescued the reduced level of glutathione caused by *S. mansoni* (Fig. 3).

## DISCUSSION

Liver is the largest organ in the vertebrate body and the site for intense metabolism. It plays an astonishing array of vital functions in the maintenance and performance of the body. Granulomatous inflammations in the liver serve a protective role by walling off and eliminating invaders and irritants, cavitation and fibrosis often aggravate the pathology of the disease (Boros, 1978). Administration of mulberry for 10 days has a pronounced antischistosomal activity where it diminished granuloma sizes extensively and



decreased the number of involutive granulomas as compared with the infected control mice. These findings might suggest a possible antifibrotic role of mulberry. Also, the histological examination revealed a notable suppression in the granulomatous diameter.

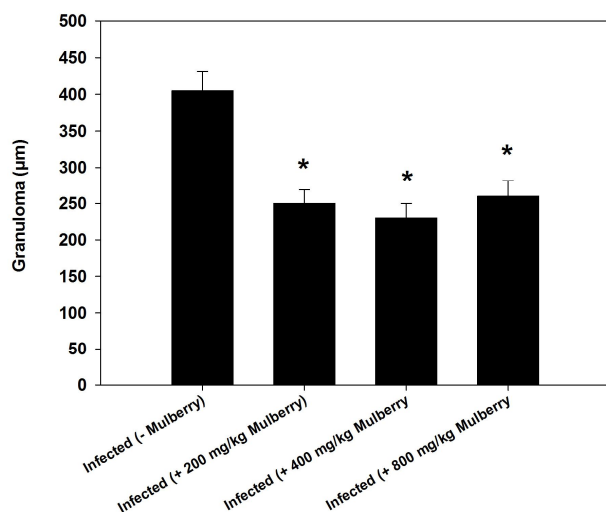


Fig. 2. Mulberry reduced granuloma size in liver of mice infected with *S. mansoni*. Values are means±SD. \*: Significant change at  $P \leq 0.05$ .

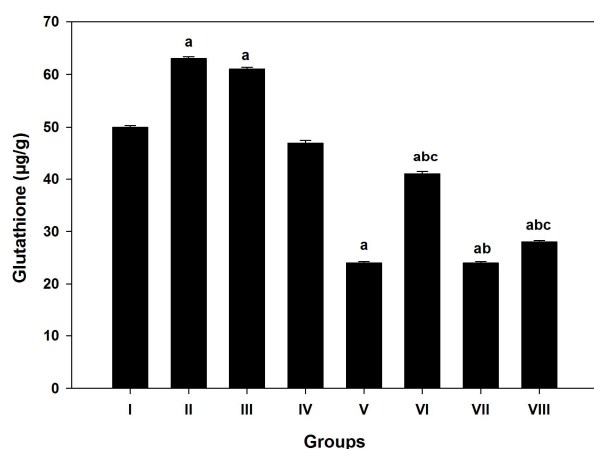


Fig. 3. Effect of mulberry on the level of glutathione in liver homogenate of infected mice with *S. mansoni*. Values are means±SD (n=8). <sup>a</sup> Significant difference as compared with normal control group ( $p \leq 0.05$ ). <sup>b</sup> Significant difference as compared with corresponding control group ( $p \leq 0.05$ ). <sup>c</sup> Significant difference as compared with infected group ( $p \leq 0.05$ ). For details of groups, see Table III.

Schistosomiasis is associated with liberation of free radicals and disturbance in the cellular antioxidant system. It has been revealed that there is an important role of antioxidant processes in mediating liver injury in schistosomiasis due to an increased production of reactive oxygen intermediates (La Flamme *et al.*, 2001). Hence, the suppressive effect of mulberry on granuloma formation is probably due, in part, to the fact that mulberry has antioxidants effect (Du *et al.*, 2003; El-Beshbishy *et al.*, 2006). The leaf of mulberry contains triterpenes (lupeol) Sterols ( $\beta$ -Sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids and organic acids (Doi *et al.*, 2001).

It has been reported that schistosomiasis caused an impairment of liver GSH content of mice (Cunha *et al.*, 2012). Decreasing the antioxidant capacity of the liver, leading to the generation of lipid peroxides that may play a major role in the pathology associated with schistosomiasis (Cunha *et al.*, 2012). In the present study, hepatic GSH decreased significantly in infected treated mice as compared with the normal control group, which indicates that schistosomiasis causes more liberation of free radicals. Here, mulberry is seen to be the most effective antioxidant as shown in the present study. Mulberry decreased hepatic lipid peroxidation and GSH depletion. GSH plays an important role in antioxidant defense directly through scavenging of reactive oxygen species and indirectly through functions as a cofactor of antioxidant enzymes (Franco *et al.*, 2007).

Indeed, infections cause an inflammatory response in the liver of mice. This response manifests itself as a perturbed liver structure and as a significant production of NO (Chang and Chen, 2005). Raso *et al* (2001) have viewed that the oxidative stress is mainly due to NO produced by the stimulated iNOS.

In the present study, it was observed that the infection caused significant increases in the hepatic MDA levels. Mulberry treatment prevented the increase in MDA, probably in part by scavenging the very reactive components. Moreover, high rate of oxidative processes, formation of hepatic MDA due to the peroxidative damage to the liver

microsomal membrane lipid and impairment of the antioxidant defense characterize schistosomiasis (El Shenawy *et al.*, 2008). Collectively, our data indicate that mulberry exhibits a significant antioxidant and antischistosomal activities, protecting host tissue from injuries induced by parasites.

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