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# Response of Spleen and Jejunum of Mice Infected with *Schistosoma mansoni* to Mulberry Treatment

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**Abstract.-** Schistosomiasis is the second most predominant tropical disease in Africa after malaria. In the developing world, it has a great public health and socio-economic importance. Here, we aimed to assess the antioxidant and anti-schistosomal activities of *Morus alba* leaves (MLE) methanolic extract (200, 400 and 800 mg/kg) on the noticed tissue damage caused by *Schistosoma mansoni* infection in mice. The infection resulted in marked histopathological abnormalities in the spleen and jejunum. Moreover, infection induced splenomegally and the spleen appeared with disorganized red and white pulps while the jejunum of the infected mice appeared with some inflammation, vacuolation of the epithelium, and destruction of some villi. Also, the number of goblet cells within the infected villi was significantly increased. In addition, schistosomiasis caused oxidative damage where the level of glutathione (GSH) was reduced significantly while the levels of malondialdehyde (MDA) and nitrite/nitrate were elevated significantly. On the other hand, oral gavage of MLE extract ameliorated the tissues damage and oxidative stress induced by Schistosomasis. The present study indicates that MLE extract possess a highly promising ameliorative effect against histopathological damages and oxidative stress induced by Schistosomasis.

**Keywords**: *Schistosoma mansoni*, *Morus alba*, oxidative stress, glutathione level.

## INTRODUCTION

Helminth parasites of the genus *Schistosoma* are the causative agents of schistosomiasis, which is a neglected disease, so it remains a significant public health problem in tropical and subtropical regions (Quack *et al.*, 2006; Steinmann *et al.*, 2006). In Egypt, the disease is well established and it is estimated that up to 70% of the rural population in endemic areas is affected (Al Sherbiny *et al.*, 2003).

The massive egg production of schistosomes is leading to granuloma formation in the gut, intestine, bladder, spleen, liver and lungs (Ross *et al.*, 2002; Araújo *et al.*, 2010), and a substantial number of eggs are trapped in the liver and intestine (Helmy *et al.*, 2009). The intestine or urinary system bleeding, liver and spleen enlargement are the most common pathological changes found in chronic schistosomiasis (Burke *et al.*, 2009).

In recent decades, there has been a growing interest to search for extracts and pure compounds, especially those derived from plants that exhibit potential schistosomicidal properties. This is as one alternative method to the conventional chemical control, particularly in the absence of a vaccine and the probability of drug resistance (Ndamba *et al.*, 1994; McManus and Loukas, 2008).

*Morus alba* (Moraceae) is a white mulberry, and it has many medicinal properties so it has been used since ancient times in folk medicine (Nade *et al.*, 2009). *M. alba* has been used for ailments of respiratory system, as well as, edema, wound healing and diabetes. It has been reported that *M. alba* have antibacterial, neuroprotective, hypolipidemic and hypotensive activities (Chai *et al.*, 2005; Kang, 2006; Yadav *et al.*, 2008).

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A number of earlier investigations indicated that *M. alba* exhibits an antioxidant effect due to the presence of phenolic compounds and some vitamins which act as a good source of natural antioxidants (Fukai *et al.*, 2003). The current study was aimed at investigating the antioxidant potential role of *M. alba* in reducing oxidative stress in the spleen and jejunum of mice infected with *S. mansoni*.

## MATERIALS AND METHODS

*Animals*

Fifty 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 at an age of 9 to 11 weeks and were approved by state authorities and followed the Egyptian rules for animal protection.

*Preparation of* Morus alba *leaves extract (MLE)*

Leaves of *Morus alba* plant were collected from mulberry trees which cultivated in El-Maadi, Cairo governorate, identified by the Department of Botany, Faculty of Science, Helwan University, dried at a temperature not exceeding 40°C and powdered. The investigated dried powdered leaves were separately extracted with 70% methanol. The methanolic plant extract was filtered and evaporated to dryness *in vacuo* at a temperature not exceeding 50°C. The dried plant extract was kept in dark bottle for investigation. According to Kalantari *et al.* (2009) three doses 200, 400 and 800 mg/kg body weight were prepared by dissolving in distilled water (Alam *et al.,* 2002).

Schistosoma mansoni *infection*

*S. mansoni* cercariae were obtained from Schistosome Biological Supply Center at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. Mice were exposed to 80±10 *S. mansoni* cercariae per mouse by the subcutaneous injection method, modified by Oliver and Stirewalt (1952).

*Experimental design*

Five groups, each of ten mice, were used in this study. First group (-MLE) was non-infected and served as a vehicle control (uninfected) group. It received 100 µl water/mouse orally for 10 days. After 46 days post infection (*p.i.*) with 80±10 *S. mansoni* cercariae, infected animals were divided into the remaining four groups, the second group is infected (-MLE) group. The 3rd, 4th & 5th groups were infected with *S. mansoni*. Thereafter, the infected animals of these groups, received orally 200, 400 and 800 mg/kg body weight of MLE, respectively, once daily for 10 days.

*Preparation of spleen and jejunum tissues*

On day 56 *p.i.* with *S. mansoni* and MLE administration, the animals of all groups were decapitated. Spleen and jejunum were removed, weighed and rapidly cut into smaller pieces. A few pieces were fixed in 10% neutral buffered formalin for histopathological investigations, while others were homogenized in ice-cold medium containing 50 mM Tris–HCl and 300 mM sucrose, pH 7.4 (Tsakiris *et al.*, 2004) and finally stored at -80°C until use in the various biochemical determinations.

*Spleen index*

At the end of the experimental period, each mouse was weighed; the spleen was removed and weighed. Finally, the spleen index was calculated (ratio of spleen weight in mg/mouse to body weight in g/mouse).

*Histopathology*

Formalin-fixed spleen and jejunum were embedded in paraffin, and 5 µm sections were stained with hematoxylin and eosin. Mice jejuna were stained with Alcian blue for the identification of the goblet cells. For each animal, the number of goblet cells in the jejunum was counted on at least ten well-orientated villous crypt units (VCU). Results were expressed as the mean number of goblet cells per ten VCU (Allen *et al.*, 1986).

*Estimation of the reduced glutathione (GSH) level*

GSH level in spleen and jejunum was determined by the methods of Ellman (1959). The method is based on the reduction of Ellman's reagent (5, 5'dithiobis (2-nitrobenzoic acid) "DTNB") with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 412 nm.

*Determination of nitrite/nitrate and malondialdehyde (MDA) levels*

The nitrite/nitrate and MDA levels were determined according to Green *et al.* (1982) and Ohkawa *et al.* (1979), respectively.

*Statistical analysis*

The obtained data were presented as means ± standard error. 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). $P \leq 0.001$ was considered as significant for all statistical analysis in this study.

## RESULTS

*S. mansoni* resulted in a highly significant increase in splenic index (ratio of spleen weight in mg/mouse to body weight in g/mouse) (Fig. 1). Oral gavage of methanolic extract of *M. alba* leaves (MLE) to infected mice reduced the index significantly. The maximum effect of MLE extract was at a dose of 200 mg/kg that showed a complete recovery in the splenic index indicating its ameliorative effect on schistosomiasis.

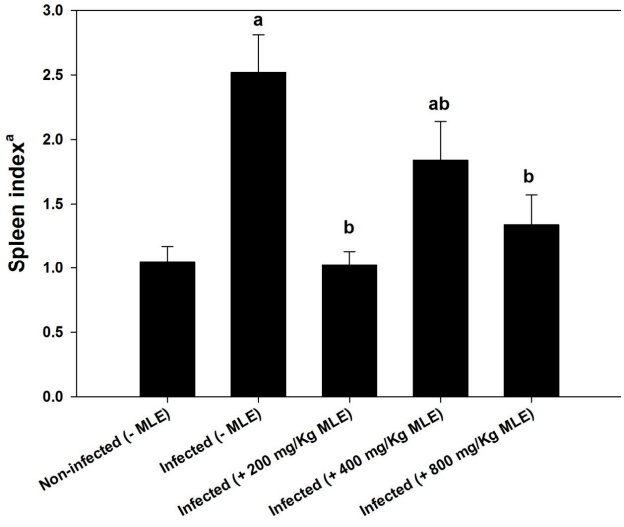


Fig. 1. *Morus alba* induces changes in spleen index of mice infected with *S. mansoni*. Values are Means ± SE.
[a]Ratio of spleen weight in mg/mouse to body weight in g/mouse.

The normal spleen was composed of white and red pulps surrounded by a capsule of dense connective tissue (Fig. 2). The white pulp was composed of a central, T-cell rich zone, and a peri-arterial lymphoid sheath surrounded by B-cell-rich primary follicles. The white pulp was separated from the red pulp by the marginal sinus embedded in a layer of marginal zone lymphocytes. On day 55 post-infection with *S. mansoni*, the white pulp enlarged due to cellular proliferation. The limit between white and red pulp started to disappear (Fig. 2), and the spleen increased in size. Vacuolation of some splenic cells was detected. Most of the cells were darkly stained and the sinusoidal spaces were large. The histological lesion is still present after treatment of mice with *M. alba* but the tissue is much improved after treatment with the dose of 800 mg/kg.

Histopathological examination of the intestine of the infected non-treated mice revealed also chronic inflammation and numerous large granulomas in the mucosa, submucosa and in some instances penetrating the muscular layer. In most instances, a plenty of granulomas with central egg trapped could be seen (Fig. 3). In *Morus alba* treated groups, the intestine showed apparent amelioration where there were fewer and non-developed granulomas. In most cases, there were few eggs without concentric fibrosis (Fig. 3). Moreover, infection of mice with *S. mansoni* was able to significantly increase the number of goblet cells (Fig. S1, Fig. 4). This number was increased more than 2 fold compared to the non-infected control mice. *M. alba* could reduce the increased number of goblet cells specially when mice were treated with a dose of 400 mg/kg (Fig. 4).

Data in Table I showed that the infected mice with *S. mansoni* decreased the level of GSH in selected organs under investigation (spleen and jejunum). Gavage of the three doses of MLE to *S. mansoni* infected mice was able to increase the GSH level in both organs when compared to the infected mice.

The nitrite/nitrate level was raised significantly ($P \leq 0.001$) as a result of schistosomiasis in spleen and jejunum versus non-infected group (Table I). The ameliorative effect of MLE was noticed in infected mice at the different doses.

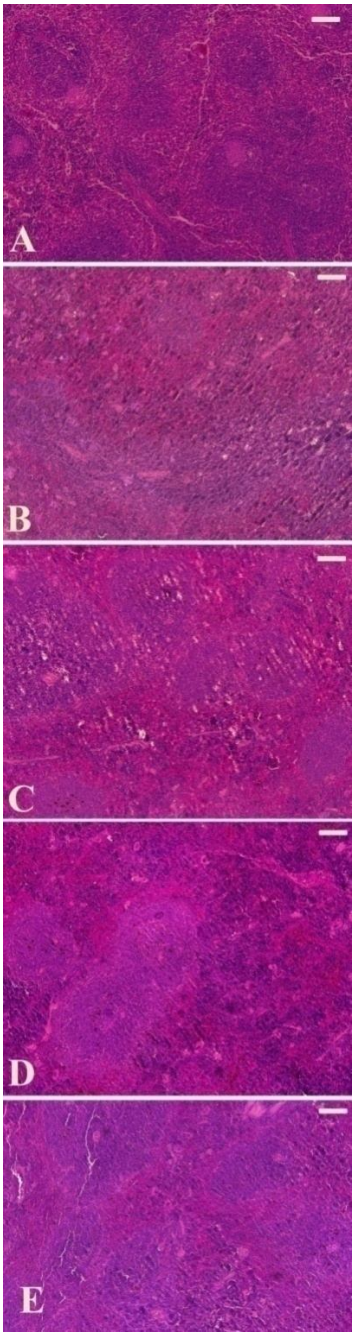


Fig. 2. Histological structure of mouse spleen infected with *S. mansoni* on day 46 *p.i.* (A) Non-infected spleen with normal architecture. (B) Infected spleen with disorganized pulps. (C, D and E) Infected-treated mice with a dose of 200, 400 and 800 mg MLE/kg, respectively. Sections appeared with improved tissue damage. Spleen appeared with less lesion and improved tissue damage. Sections are stained with hematoxylin and eosin. Bar = 50 µm.

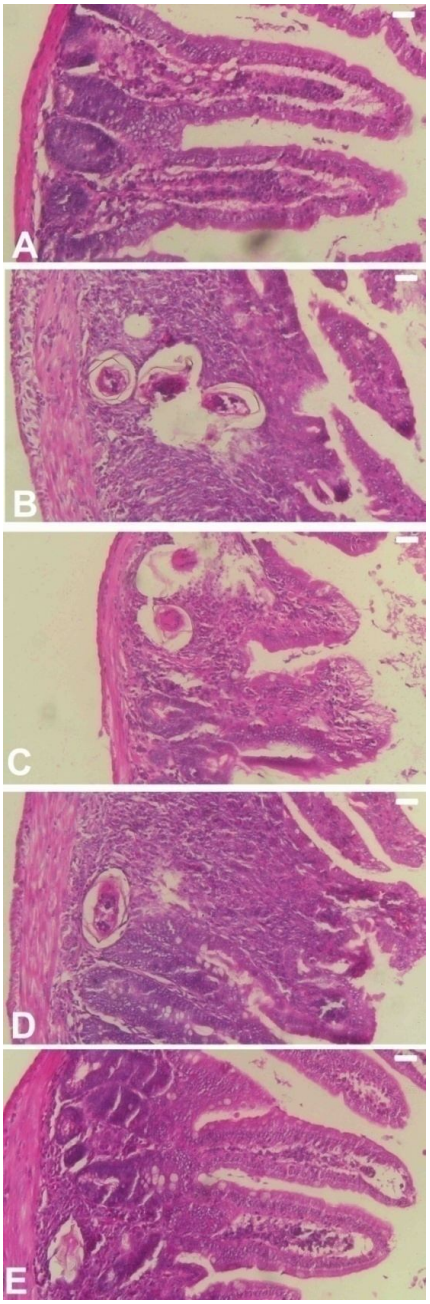


Fig. 3. Histological structure of mouse jejunum infected with *S. mansoni* on day 46 *p.i.* (A) Non-infected jejunum with normal architecture. (B) Infected jejunum with granuloma with large accumulation of inflammatory cells (C, D and E) Infected-treated mice with a dose of 200, 400 and 800 mg MLE/kg, respectively. Sections appeared with improved tissue damage. Sections are stained with hematoxylin and eosin. Bar = 50 µm.

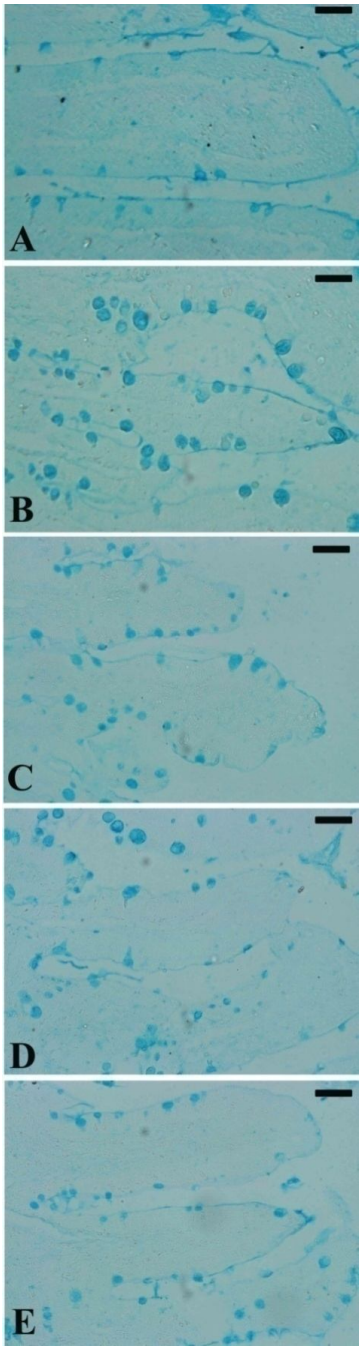


Fig. 4. Changes in goblet cell numbers in mouse jejunum infected with *S. mansoni* and treated with *Morus alba* leaves extract. (A) Non-infected jejunum (B) Infected mouse jejunum with more goblet cells. (C, D and E) Infected jejunum with decreased number of goblet cells. Infected-treated mice with a dose of 200, 400 and 800 mg/kg MLE, respectively. Sections are stained with Alcian blue. Bar = 50 µm.

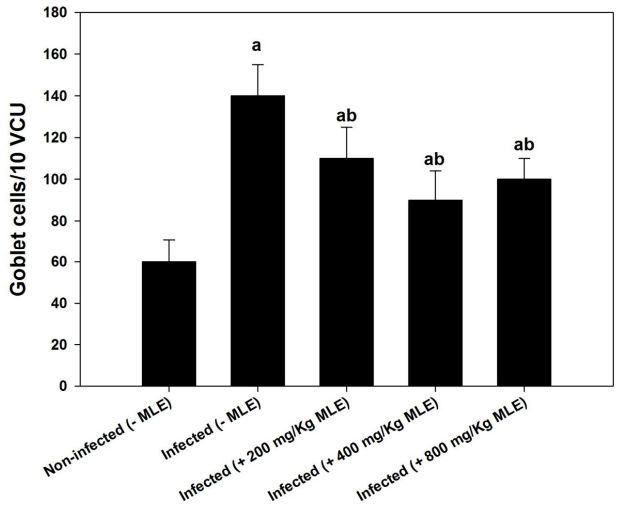


Fig. 5. *Morus alba* induced changes in goblet cell number in jejunum of mice infected with *S. mansoni* on day 55 *p.i.*. Values are means ± SD. a: Significant against non-infected control group at $P \leq 0.001$, b: Significant against infected non-treated group at $P \leq 0.001$.

In spleen the most effective dose was 200 mg/kg, while, 400 and 800 mg/kg induced recovery in jejunum.

Similarly, *S. mansoni* infection to mice resulted in a highly significant increment of MDA level in spleen and jejunum at P≤0.001 as compared to the control group as shown in Table I. Treatment of the schistosome infected mice with MLE induced a highly significant reduction of MDA level in spleen, the maximum effect was recorded at a dose of 400 mg/kg. Moreover, the most effective dose of *M. alba* extract in jejunum was 200 mg/kg.

## DISCUSSION

The spleen index in the present work showed a highly significant increment as a result of schistosomal infected mice. Our findings were in agreement with Soomro *et al.* (2001), Silva-Souza and Vasconcelos (2005), da Silva *et al.* (2012) and Corrêa *et al.* (2013). Also, Soomro *et al.* (2001) and Corrêa *et al.* (2013) noticed that splenomegaly is prominent in *S. mansoni* where, the schistosome infection resulted in a highly significant increment in the total spleen weights of infected mice (da Silva *et al.,* 2012). In addition, Silva-Souza and Vasconcelos (2005) indicated that *S. mansoni*

**Table I.- Effect of *M. alba* leave extracton glutathione (GSH), nitrate/nitrite and lipid peroxidation level as expressed by malondialdehyde (MDA) equivalents in splenic and intestinal homogenates of *S. mansoni* infected mice.**

| | Non-infected | Infected | Infected + Treated with *M. alba* extract | | |
|---|---|---|---|---|---|
| | | | 200 mg/Kg | 400 mg/Kg | 800 mg/Kg |
| GSH (mg/g) | | | | | |
| Spleen | 49.16±2.69 | 26.14±1.78 $^{a**}$ | 27.24±1.25 $^{a**}$ | 29.19±2.36 $^{a**}$ | 27.77±0.72 $^{a**}$ |
| Intestinal | 06.06±0.29 | 05.05±0.98 | 07.45±0.39 | 6.48±0.40 | 10.94±1.48 $^{a**b**}$ |
| Nitrite/nitrate (µmol/g) | | | | | |
| Spleen | 51.86±1.99 | 81.67±2.61 $^{a**}$ | 57.92±2.24 $^{b**}$ | 55.02±1.67 $^{b**}$ | 50.11±1.40 $^{b**}$ |
| Intestinal | 118.9±1.70 | 280.0±2.66 $^{a**}$ | 102.1±2.27 $^{a**b**}$ | 130.0±2.33 $^{a*b**}$ | 116.3±2.43 $^{b**}$ |
| MDA (nmol/g) | | | | | |
| Spleen | 99.31±1.98 | 125.6±2.02 $^{a**}$ | 84.51±0.99 $^{a**b**}$ | 80.42±0.80 $^{a**b**}$ | 113.3±2.19 $^{a**b**}$ |
| Intestinal | 05.79±0.98 | 20.63±1.77 $^{a**}$ | 08.94±1.58 $^{b**}$ | 19.93±1.60 $^{a**}$ | 13.89±1.62 $^{a**b*}$ |

Values are means ± SE. a: Significant against vehicle (non-infected) control group at $P \leq 0.05$, b: Significant against infected control group at $P \leq 0.05$,* Significant at $P \leq 0.01$ and ** Significant at $P \leq 0.001$, n = 6.

histopathology is characterized by the presence of granuloma with parasitic eggs, with an increase in the size of the spleen. In addition, our results cleared that the treatment with MLE reduced the spleen index in *S. mansoni* infected mice, where the most effective dose is 200 mg/kg. Schistosomiasis mostly; affecting the intestine causing granuloma formation (El Banhawey *et al.,* 2007; Riad *et al.,* 2008). Mature *S. mansoni* are depositing eggs in the intestinal wall that either pass to the gut lumen and are expelled in the faeces or travel to the liver (Gryseels *et al.,* 2006). Eggs release antigens that produce varying degrees of granulomatous response in the intestines of the definitive host (Hirata *et al.,* 1993).

Riad *et al.* (2008) reported that, granulomas were evident in the subserosa, muscularis and submucosa. Besides, an apparent increase of goblet cells number, per villus was recorded. All these results were cleared in the infected non-treated mice of our investigation. Otherwise, the infected mice treated with MLE showed fewer and non-developed granulomas in the intestinal tissue and in most cases, there were few eggs without concentric fibrosis. In addition, it reduced the increased number of goblet cells; indicating to the antifibrotic and anti-inflammatory roles of *M. alba* leaves (Choi and Hwang, 2005; Amer *et al.,* 2013).The extent of granuloma formation and egg deposition in the tissues determine the severity of the disease. Moreover, an imbalance between pro-and anti-oxidant processes has been demonstrated both *in vitro* (Feldman *et al.,* 1990) and *in vivo* (Gharib *et al.,* 1999).

*S. mansoni* induced a highly significant reduction in GSH level in spleen and jejunum, which indicates that schistosomiasis causes more liberation of free radicals. On the other hand, MLE gavage to *S. mansoni* infected mice resulted in highly significant increment in GSH level of spleen and jejunum. Our results are in agreement with the observation of (El Sokkary *et al.,* 2002; Amer *et al.*, 2013; Diab *et al.*, 2013**;** de Oliveira *et al.*, 2013).

de Oliveira *et al.* (2013) reported that the non-enzymatic antioxidant capacity in spleen were decreased as a result of schistosome infection. El-Sokkary *et al.* (2002) concluded that there were reductions in glutathione, superoxide dismutase, and vitamin E in the spleen of *S. mansoni* infected mice. In addition, the level of GSH was increased as a result of treatment of infected mice with an antioxidant. Likewise, Amer *et al*. (2013) and Diab *et al.* (2013) speculated that *Morus alba* leaves extract gavage to infected mice resulted in a highly significant increment of GSH level.

In the current investigation, evidence of increased nitrite/nitrate and MDA levels in spleen and jejunum of *S. mansoni* infected mice was seen. On the contrary, the treated *S. mansoni* infected mice with MLE caused a highly significant decrease

in nitrite/nitrate and MDA levels of the studied organs which are in agreement with El Sokkary *et al.* (2002), Amer *et al.* (2013) and Diab *et al.* (2013).

Raso *et al.* (2001) and El Sokkary *et al*. (2002) deduced that the spleen oxidative stress was prompted by *S. mansoni* infection. Our data revealed that the nitrite/nitrate and MDA levels of spleen were increased significantly in schistosoma-infected mice were dead by 56 day post-infection. In addition, the main organ affected during the course of the pathology, the spleen, was shifted to a pro-oxidant state (La Flamme *et al.,* 2001; de Oliveira *et al.*, 2013). The imbalance of oxidative parameters may be due to the egg deposition, changes in vascular tone and soluble immune mediators (Wynn *et al.*, 2004; Pearce, 2005).

In the acute and chronic stages of *S. mansoni* infection, both structural and functional changes occur in the intestine of infected mice (Bogers *et al.*, 2000). The chronic infection of mice with *S. mansoni* severely disturbs the gastrointestinal motility. This is characterized by a hypercontractile activity of the small intestine in the chronic stages of infection and by a disturbed transit time of a semi-liquid meal through the small intestine (Bogers *et al.*, 2000; Moreels *et al.*, 2001). Granulomatous tissue causes the loss of elasticity in the intestinal wall and as the disease progresses the tissue can become calcified (Warren, 1982).

El Sokkary *et al.* (2002) speculated that the oxidative stress that generated in the spleen due to schistosomiasis was reduced by an antioxidant. Amer *et al.* (2013) and Diab *et al.* (2013) observed that *M. alba* treatment prevented the increase in nitrite/nitrate and MDA, probably in part by scavenging the very reactive components. The MLE contain 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).

Collectively, all the mentioned changes in the spleen and jejunum pathology induced a state of oxidative stress in infected mice with *S. mansoni* and this stress was significantly reduced by MLE treatment indicating to its antioxidant properties and antischistosomal activity.

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