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Role of *Morus alba* in Ameliorating *Schistosoma mansoni*-Induced Renal and Testicular Injuries in Mice

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Abstract. - Schistosomiasis is the second most significant parasitic disease in the world after malaria. Herbal medicine is the most widely used form of medicine in the world today where the medicinal plants contain curative bioactive ingredients. The study aimed to investigate the role of *Morus alba* leaves extract in ameliorating *Schistosoma mansoni*-induced renal and testicular injuries in mice. Experimental mice infected with *S. mansoni* cercariae and treated with the crude methanolic extract after 46 days postinfection. Histopathological effects were determined in 5 µm thick sections. Oxidative stress was assessed by determining glutathione (GSH) level, nitrite/nitrate and lipid peroxidation as indicated malondialdehyde (MDA) production in the kidney and testis, besides estimation of plasma uric acid and testosterone levels. The ameliorating effect of *M. alba* was extending to improve the histopathology of kidney and testis of infected mice. Infection with *S. mansoni* caused progressive depletion of GSH level and significant enhancement of MDA and nitrite/nitrate levels. Treatment of mice with *M. alba* extract ameliorated the tissues damage and oxidative stress induced by schistosomasis, as indicated by significant improvement of GSH level and significant decrease in MDA and nitrite/nitrate levels formation as compared to Schistosome infected group. The present study indicates that *M. alba* extract possess a highly promising ameliorative effects against histopathological damages and oxidative stress induced by *S. mansoni* infection.

Keywords: *Schistosoma mansoni*, *Morus alba*, oxidative stress, mice, glutathione, peroxidation, malondialdehyde, schistosomiasis.

INTRODUCTION

Schistosomiasis is one of the most common parasitic diseases affecting liver and intestine and causing granuloma formation and hepatic fibrosis. Schistosomiasis also causes certain necrotic changes in the liver tissue (El-Aasar *et al*., 1989). *Schistosoma mansoni* is a digenetic trematode responsible for high social and economic impact for human (Waisberg *et al*., 2008). In Egypt, *S. mansoni* is a continuing health problem despite attempts to control this parasitic infection over many years (Helmy *et al*., 2009). Schistosomiasis has been estimated to afflict as many as 207 million people in 76 countries, with 779 million more being at risk of infection (Steinmann *et al*., 2006), causing more than 208,000 deaths per year (Gryseels *et al*., 2006; de Oliveira *et al*., 2013).

S. mansoni infection may imbalance oxidative parameters by different causes or mechanisms such as egg deposition, changes in vascular tone and soluble immune mediators. However, schistosomiasis is a complex syndrome affecting several organs and functions to different degrees (Carod-Artal, 2010; Wilson *et al*., 2011).

Abdel Aziz *et al*. (1991) and Josiah and Manko (2003) reported pathological changes in kidney and testis. Nephropathies associated with *S. mansoni* infections have been described both in experimental animals and in humans. Moreover, schistosome infected mice showed distortion of the testicular cytoarchitecture and disruption of spermatogenesis (Houba *et al*., 1977; Josiah and Manko, 2003).

Praziquantel is the drug of choice for the treatment of schistosomiasis, but the development of resistant strains (Pica-Mattoccia and Cioli, 2004), and reduction in curing rates, has reinforced the need to develop new safe and effective methods against schistosomiasis (Botros *et al*., 2003).

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Therefore, Yang *et al*. (2012) and Enyara *et al*. (2013) have emphasized upon the development of new drugs from plants which contain a rich source of bioactive components with fewer side-effects.

Mulberry trees especially *Morus alba is* a widely available plant found throughout the year in several countries. *M. alba* leaves are used in flavored mulberry tea and also make fodder for ruminants and other animals due to its high crude protein content (15-25%) (Sanchez, 2000). The consumption of *M. alba* trees has increased over the past decades because of its hypotensive, hypoglycemic, antidepressant, antioxidant, antiphlogistic, anti-parasitic, anti-diarrheal and kidney protective effects (Zou *et al*., 2012; Amer *et al*., 2013; Lee *et al*., 2013; Zafar *et al*., 2013).

This study is aimed at investigating the effecting of methanolic extract of *M. alba* on tissues damages and oxidative stress induced by *S. mansoni* in the kidney and testis of mice.

MATERIALS AND METHODS

Animals

Fifty male Swiss albino mice, 9-11 weeks of age, were maintained under specified pathogen-free conditions and fed on standard diet and provided with water *ad libitum*.

Preparation of M. alba leaves extract (MLE)

Leaves of *M. alba* cultivated in El-Maadi, Cairo governorate were dried at 40°C, powdered (2mm mesh size), and were extracted with 70% methanol, which was filtered and evaporated to dryness *in vacuo* at 50°C. The dried plant extract was kept in dark bottle for investigation. Three doses *viz.*, 200, 400 and 800 mg/kg body weight were prepared by dissolving in distilled water.

Infection of animals

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

Experimental design

Animals were allocated to five groups each of ten mice. One group was non-infected and received water (100 µl water/mouse) by oral gavage for 10 days. The remaining mice were infected with 80±10 *S. mansoni* cercariae. The infected animals were divided into four groups 46 days post-infection (*p.i.*). One of these was infected (untreated), while the remaining three infected mice received MLE orally at 200, 400 and 800 mg/kg mice body weight, once daily for 10 days.

Kidney index

At the end of the experimental period, each mouse was weighed. Its left kidney was then removed and weighed. The kidney index was calculated as ratio of kidney weight (mg) to mice body weight (g).

Histological studies

On day 55 *p.i.* and MLE administration, the animals were killed by cervical dislocation. Kidney and testis were immediately removed, weighed and each divided into two parts. One part was fixed in 10% formalin, dehydrated, embedded in paraffin, sectioned and stained with haematoxylin and eosin for histopathological investigations. The second part was homogenized immediately to give 50% (w/v) homogenate in ice-cold medium containing 50 mM Tris–HCl and 300 mM sucrose, pH 7.4 (Tsakiris *et al*., 2004). The homogenate was centrifuged at 500×g for 10 min. The supernatant (10%) was used for estimation of various biochemical parameters.

Biochemical studies

The glutathione (GSH) level in kidney and testis was determined by the method of Ellman (1959), whereas the nitrite/nitrate and malondialdehyde (MDA) levels were assayed according to the method of Green *et al*. (1982) and Ohkawa *et al*. (1979), respectively.

Uric acid was assayed in plasma using kits of Roche Diagnostics Co. (Mannheim, Germany), while plasma testosterone level was determined through ELISA using kit of Roche Diagnostics Co. (Mannheim, Germany).

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). $P \leq 0.05$, $p \leq 0.01$ and $P \leq 0.001$ were considered as low, moderate and highly significant for all statistical analysis in this study.

RESULTS

The reduction in kidney weight due to *S. mansoni* infection was expressed as kidney index. On day 55 *p.i.*, the index in the infected mice was decreased about 43% when compared to the non-infected mice (Fig. 1). Treatment of the infected mice with *M. alba* leaves extract (400 mg/kg mice BW) was able to improve the loss in kidney index (Fig. 1).

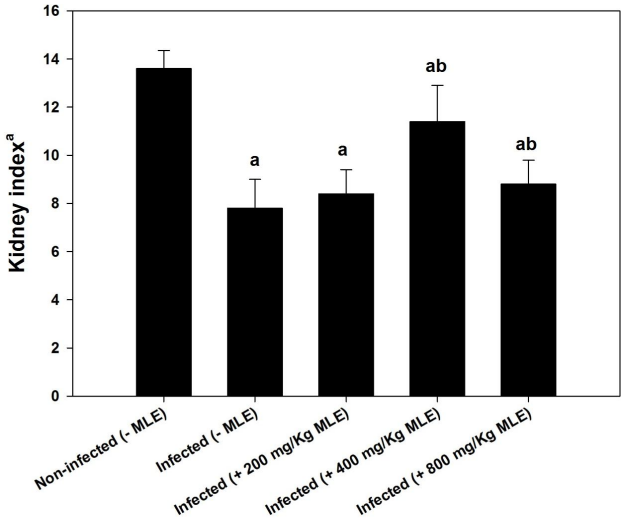


Fig.1. *M. alba* induces changes in kidney index of mice infected with *S. mansoni*. Values are means ± SE.

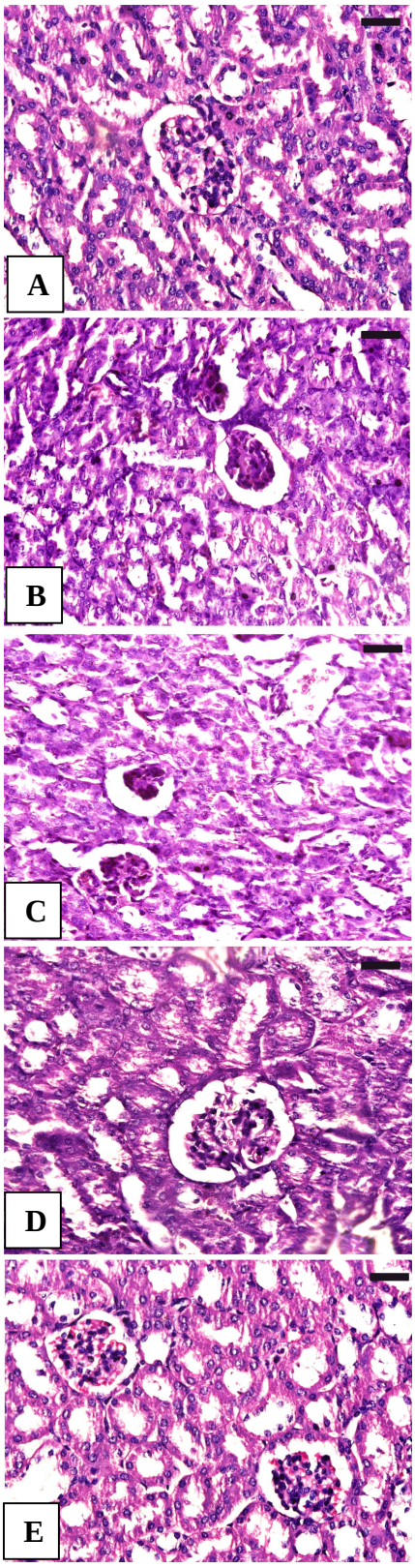
At the histological level, the architecture of both renal (Fig. 2) and testicular (Fig. 3) tissues showed great alterations due to the infection with *S. mansoni*. The glomeruli of the infected kidney appeared shrunken, while most of the kidney tubules were vacuolated (Fig. 2). *M. alba* was able to improve the kidney injury especially when the infected mice treated with a dose of 800 mg/kg. Testicular histopathological examination demonstrated that schistosomiasis caused seminiferous tubule injury as manifested by tubular degeneration and vacuolization after schistosomiasis induction (Fig. 3), which were alleviated by *M. alba* post-treatment, especially with a dose of 800 mg/kg mice BW (Fig. 3).

The induced injury by *S. mansoni* in renal and testicular tissues was responsible for the highly significant increase in blood plasma uric acid (Fig. 4) and highly significant decrease in blood plasma testosterone level (Fig. 5) in infected control group compared to non infected control group. MLE ameliorated the induced changes *p.i.* especially at the dose of 800 mg/kg when various abnormal biochemical values also returned to normal values again.

Table (I) summarizes the changes of GSH, nitrite/nitrate and MDA levels in both renal and testicular tissues. GSH level showed a highly significant reduction as a result of *S. mansoni* infection. On the other hand, *M. alba* methanolic extract gavage induced an increment of GSH level at different doses (200, 400 and 800 mg/kg mice BW) as compared to infected control group indicating the ameliorative effect of MLE *p.i*. Also, the nitrite/nitrate level was raised significantly as a result of *S. mansoni* infection in kidney and testis. Treatment with methanolic MLE to infected mice at 200, 400 and 800 mg/kg BW induced a highly significant decrease of nitrite/nitrate level in the selected organs (Table I). Likewise, *S. mansoni* infection induced a highly significant increment in MDA level in the studied organs (kidney and testis) as compared to non infected control group. Oral gavage of the three doses of methanolic extract of *M. alba* in *S. mansoni* infected animals showed a highly significant reduction ($P \leq 0.001$) in MDA level in the organs under investigation versus control infected group (Table I).

DISCUSSION

Kidney pathology can occur in *S. mansoni* infections and can even lead to renal failure (Wang *et al*. 2004). Attention was drawn to the glomerular lesions associated with schistosomiasis. In this study, the histological picture of the infected kidney with schistosomiasis showed great alterations, besides the reduction in the plasma uric acid level. El-Sawaf and Abo- Elgoud (2010) indicated that the



glomeruli of mice infected with *S. mansoni* showed an increase in cellularity and mesangial matrix deposition leading to expansion of the glomerular tuft and obliteration of Bowman's space and the appearance of some shrunken glomeruli. Moreover, Frank *et al*. (1999) mentioned that in cases of schistosomal- glomerulopathy, the kidney became shrunken due to interstitial renal fibrosis. The induced renal injuries leads to a disturbance in the uric acid level (Mohammed *et al*., 2006).

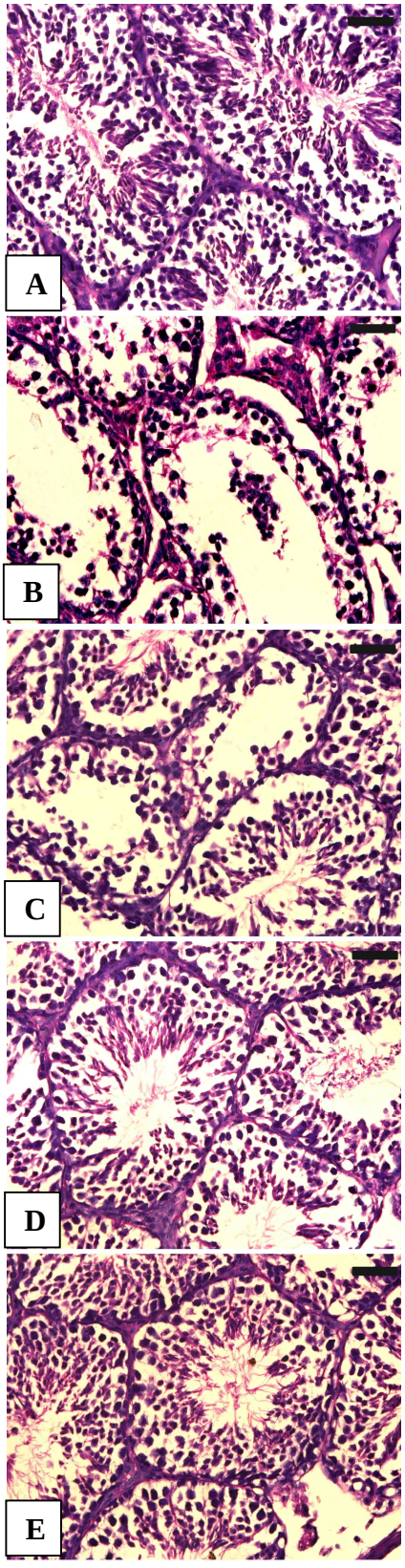
Not only the renal tissue, but also the testicular architecture of the infected mice revealed some alteration. Several reports indicated that *S. mansoni* infection induced a testicular damage that leads to a disturbance in spermatogenesis and the testosterone level and then affect fertility (Lansoud-Soukate *et al*., 1991; Mostafa and Soliman, 2010).

Results of the present work showed a highly significant decrease in GSH level as a result of schistosomiasis in the selected organs, on the other hand, nitrite/nitrate and MDA (end products of lipid peroxidation) levels were increased significantly. These data are in agreement with those of El-Shenawy and Soliman, (2003), Kouriba *et al.* (2005) and Halliwell and Gutteridge (2007).

Schistosomiasis causes a reduction in the levels of protective endogenous antioxidants and increases generation of free radicals (El-Sokkary *et al*., 2002; El-Shenawy and Soliman, 2003; El-Shenawy, 2008). This case of imbalance in the antioxidant-oxidants system was creating an oxidative stress (Michiels *et al*., 1994).

The infection with *S. mansoni* induced a drastic reduction of GSH stores in the kidney (Halliwell and Gutteridge, 2007). Likewise, El-Sokkary *et al.* (2002) indicated that the activities of antioxidant enzymes are markedly decreased in mice infected with *S. mansoni*, while nitrite/nitrate

Fig.2. Histological structure of mouse kidney infected with *S. mansoni* on day 46 *p.i.* (A) Non-infected kidney with normal architecture, (B) Infected kidney with shrunken glomeruli and vacuolated tubules, (C, D and E) Infected-treated mice with a dose of 200, 400 and 800 mg/kg MLE, respectively. Kidney appeared with fewer lesions and tissue damage appears to be minimized. Sections are stained with hematoxylin and eosin. Bar=50 µm.



level was increased in the kidney of schistosome infected mice. In addition, Kouriba *et al*. (2005) stated that the oxidative damage to lipids and proteins in kidneys of infected mice was increased. Under conditions of inflammation and oxidant stress, nitrite/nitrate is often produced; its cytotoxicity is primarily due to the production of peroxynitrite ($ONOO^-$), a toxic oxidant generated when nitrite/nitrate couples with O_2 (Szabo, 1996).

Besides tissue damage, the reactants generated during *S. mansoni* infection impair mitochondrial function and increase lipid peroxidation (Fromenty *et al*., 1997). The oxidative processes that occur upon infection with *S. mansoni* seem to go uncontrolled, since the enzymatic activities involved in O_2 and H_2O_2 detoxification are depressed. Such events may be, at least in part, responsible for the pathology associated with schistosomiasis (El-Sokkary *et al*., 2002).

Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species leading to carcinogenesis and cell death if the antioxidant system is impaired (Farber *et al*., 1990; El-Beshbishy *et al*., 2006). As a consequence, the infected host develops a rigorous condition of health impairment which may result in irreversible tissue damage (Halliwell and Gutteridge, 2007). The main organs affected during the course of the pathology *viz.*, the liver, kidneys and spleen, are shifted to a pro-oxidant state (El-Sokkary *et al*., 2002; Facundo *et al*., 2004; de Oliveira *et al*., 2013). The host reaction presumably involves reactive oxygen intermediates production which is associated with inflammation (Halliwell and Gutteridge, 2007).

In the present investigation, the methanolic extract of *M. alba* at doses of 200, 400 and 800 mg/kg mice BW exhibited antischistosomal activity in mice as showed by elevation in GSH level and a

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

Table I. - Effect of *M. alba* leaves extract (MLE) on glutathione (GSH), nitrate/nitrate and malondialdehyde (MDA) levels in renal and testicular homogenates of *S. mansoni* infected mice.

Group	GSH		Nitrate/nitrate		MDA	
	Renal	Testicular	Renal	Testicular	Renal	Testicular
Non-infected (- MLE)	23.81±1.75	221.63±0.99	70.19±1.61	7.91±0.68	37.39±2.09	129.25±1.14
Infected (- MLE)	8.54±1.79 ^{a*}	114.32±1.73 ^{a*}	107.13±2.21 ^{a*}	22.90±0.89 ^{a**}	126.75±2.11 ^{a*}	220.22±1.5 ^{a*}
Infected (+ 200 mg/Kg MLE)	21.12±1.69 ^{b*}	119.98±2.43 ^{a*b}	52.31±1.61 ^{a**b**}	12.10±0.90 ^{ab**}	110.67±2.38 ^{a*b*}	213.83±1.64 ^{a*b}
Infected (+ 400 mg/Kg MLE)	22.44±1.29 ^{b*}	146.42±2.14 ^{a*b*}	42.16±2.23 ^{a**b**}	11.29±1.42 ^{ab**}	98.97±2.29 ^{a*b*}	217.97±1.60 ^{a*}
Infected (+ 800 mg/Kg MLE)	23.58±0.94 ^{b*}	197.69±2.45 ^{a*b*}	57.33±2.122 ^{a**b**}	12.55±1.39 ^{a*b**}	91.78±3.34 ^{a*b*}	205.61±1.96 ^{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.

reduction in both of nitrite/nitrate and MDA levels in the renal and testicular tissues of treated mice. Besides that plasma uric acid level is significantly reduced and plasma testosterone level is elevated. These results are in agreement with those of Butt *et al*. (2008), Hamdy (2012) and Hamzaa *et al*. (2012).

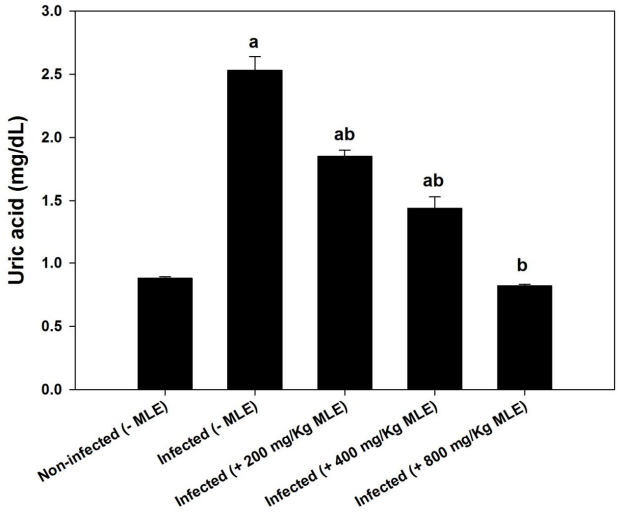


Fig.4. Effect of *M. alba* leaves extract (MLE) on plasma uric acid level on *S. mansoni*-infected mice. Values are means ± SE.

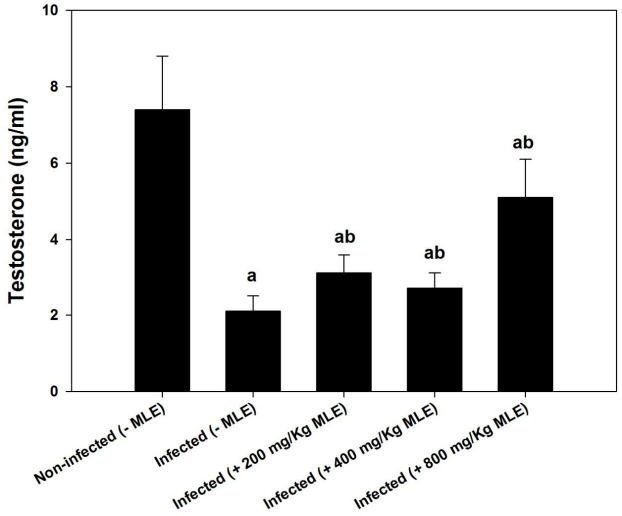


Fig.5. Effect of *M. alba* leaves extract (MLE) on plasma testosterone level on *S. mansoni*-infected mice. Values are means ± SE.

The *M. alba* leaves counteract by improving the GSH concentrations in kidney and other organs

of rats. Moreover, *M. alba* treatment significantly lessened the increase level of MDA (Butt *et al*., 2008; Hamdy, 2012). Lee *et al*. (2007) have also shown that *M. alba* extract showed an antioxidant activity in induced inflammation in rats in which the levels of nitrate/nitrite, MDA have also shown decreased significantly in kidney. The extract, therefore, protects the cells against inflammation and peroxidation. Hamzaa *et al*. (2012) reported that *M. alba* obviously raised the serum testosterone level. The authors attributed this increase to the phenolic contents of *M. alba.*

Jeong *et al.* (1999) proposed the mechanism of phenolic compounds, whose supplementation enhances lipid and protein metabolism, owing to hormonal regulation by the stimulation of noradrenalin secretion, thereby affecting the levels of steroid hormones, including testosterone, corticosterone, and other hormones in rats. Also, the effect of *M. alba* could be linked to the abundance of flavonoids which is an effective aromatase inhibitor. The cytochrome P-450 aromatase is required for the conversion of androgens to estrogens, and hence, aromatase inhibitors would decrease the concentration of estrogens and maintain a higher level of testosterone.

Presence of phenolic compounds especially the presence of different flavonoids and among them quercetin 3- (6-malonylglucoside) is most important for antioxidant potential of *M. alba* plant through donation of hydrogen atom to free radical. This power may be attributed to their ability to decompose free radicals by quenching active singlet oxygen and by trapping and quenching radicals before they reach a cellular target (El-Beshbishy *et al*., 2006; Aditya Rao *et al*., 2012).

Chungo *et al.* (2003) reported that, *M. alba* leaves extract reduce *in vitro* and *in vivo* oxidation process, which was more pronounced in the reduction of lipid peroxidation and this effect returned to presence of mulberroside A and oxyresveratrol which obtained from *M. alba* plant extract. Also these compounds showed an inhibitory effect against $FeSo_4/H_2O_2$-induced lipid peroxidation in rat microsomes and a scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl radical (Chungo *et al*., 2003; Butt *et al*., 2008). Moreover, Wang *et al*. (2011) concluded that mulberroside caused a decrease in serum uric acid levels.

In conclusion, the administration of methanolic extract of *M. alba* with different doses to schistosome infected mice resulted in an increase in GSH level and reduction in nitrite/nitrate and lipid peroxidation levels in kidney and testis indicating the antischistosomal activity of methanolic extract of *M. alba* leaves.

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