

## The Antioxidant Effect of *Morus alba* Leaves Extract on Kidney, Testes, Spleen and Intestine of Mice

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**Abstract.-** The current study aims at determining the antioxidant properties of *Morus alba* leaves extract (MLE) on various organs of mice. Forty male Swiss albino mice were divided into control group and three different groups which received three different doses of MLE orally for 10 days. The glutathione, nitrite/nitrate, malondialdehyde levels were estimated in kidney, testis, spleen and jejunum of adult male albino mice. Also, the levels of urea, creatinine and uric acid were determined in blood plasma of mice. The oral gavage of *M. alba* leaves extract induced a highly significant increment in glutathione level while it caused a highly significant reduction of nitrite/nitrate and malondialdehyde in all studied organs. In addition, blood urea nitrogen, creatinine and uric acid were improved. We speculated that, MLE scavenged the free radicals in different mice organs under investigation due to its antioxidant activity.

**Keywords:** *Morus alba* leaves, kidney, testis, spleen, jejunum, mice.

### INTRODUCTION

**T**raditional medicines show reliance on phytochemicals rich plants extracts to cure various maladies. Genus *Morus* (Mulberry) is one of such example that consists of over 150 species, among these *Morus alba* is dominant. *M. alba* an deciduous tree belonging to the family of *Moraceae*, is widely cultivated in tropical, subtropical and temperate areas (Agarwal and Kanwar, 2007). It contains an appreciable amount of flavones, triterpenes, proteins, amino acids, carbohydrates, fats, fibers, mineral contents and some vitamins such as vitamin C or their precursors (Chen *et al.*, 1995; Butt *et al.*, 2008). So, Hussein *et al.* (2010) stated that *M. alba* leaves used as a feed for ruminants and other animals due to its high contents of crude protein (15-25%). All parts of *M. alba* are of great therapeutic worth and their main mechanism of action involves their antioxidant activities. *M. alba* is useful for healing of various diseases (*i.e.*, treat fever, protect the liver, improve eyesight and lower

blood pressure) for thousands of years as well as for snakebites and as an antidote to action poisoning. Moreover, in recent years, *M. alba* tea made from leaves is getting attentions in various Asian countries as it is claimed to be an antidiabetic drink (Zhishen *et al.*, 1999; Butt *et al.*, 2008; Volpato *et al.*, 2011).

The consumption of *M. alba* leaf tea has increased over the past decades because of its hypotensive, antidepressant, anti-inflammatory and kidney protective effects. Recent studies have suggested that *M. alba* possesses antistress, diuretic, anticough, antidiarrheal properties and acts as an analgesic agent (Lee *et al.*, 2013; Zafar *et al.*, 2013). Besides, it shows antiviral and antimicrobial effects and has a neuroprotective role (El-Beshbishy *et al.*, 2006).

The antioxidant defenses enable the body system to restore the prevailing reducing environment and repair the tissue damage (Halliwell and Gutteridge, 1999). In addition, consumption of fresh fruits and vegetables as a dietary intake of antioxidants to improve human health has been attributed mainly to their high contents of beneficial phytochemicals and other micronutrients. These phytochemicals can inhibit or delay the oxidation of susceptible cellular substrates so prevent oxidative stress and protect against cellular damage, many

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chronic and degenerative disorders (Boyer and Liu, 2004; Opara and Al-Ani, 2010; Erukainure *et al.*, 2012).

So, this study was aimed at evaluating the antioxidant activity of *M. alba* leaf methanolic extract on kidney, testis, spleen and jejunum of male albino mice.

## MATERIALS AND METHODS

### *Animals*

Forty male 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 Egyptian rules for animal protection.

### *M. alba leaves extract (MLE) preparation*

Leaves of *M. 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, Egypt and dried at a temperature not exceeding 40°C and powdered (2mm mesh size). The investigated dried powdered leaves were separately extracted with 70% methanol. The methanolic plant extract was filtered and evaporated to dryness in vacuum at a temperature not exceeding 40°C. The dried plant extract was kept in dark bottle for investigation. Three doses 200, 400 and 800 mg/kg mice body weight (b.wt.) were prepared by dissolving in distilled water.

### *Experimental design*

Animals were allocated to four groups of ten mice per group. Group I served as a vehicle control and received distilled water (100 µl water/mouse) by oral gavage for 10 days. Animals of groups II, III, IV were gavaged with 200, 400 and 800 mg/kg body weight of *M. alba* methanolic extract (MLE), respectively, once daily for 10 days. The animals of all groups were cervically dislocated on day 10 post-treatment with MLE. Kidney, testis, spleen and jejunum were weighed and 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 the oxidative stress determination. Also, blood was collected using EDTA as an anticoagulant to separate the plasma. Blood plasma was stored at -70°C until used for estimation of reduced glutathione (GSH), nitrite/nitrate and malondialdehyde (MDA) and kidney function tests.

### *Biochemical studies*

GSH level was estimated in kidney, testis, spleen and jejunum homogenates 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 directly proportional to GSH concentration and its absorbance was measured at 405 nm (Ellman, 1959).

The nitrite/nitrate and MDA were assayed colorimetrically in homogenates of kidney, testis, spleen and jejunum according to the methods of Green *et al.* (1982) and Ohkawa *et al.* (1979).

Creatinine, blood urea nitrogen and uric acid were assayed in plasma, using kits of Roche Diagnostics. (Mannheim, Germany), and measured by 901 Hitachi automated system (Tokyo, Japan).

### *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 8.0).  $P \leq 0.001$  was considered as a significant for all statistical analysis in this study.

## RESULTS

Table I shows the effect of MLE on GSH, nitrite/nitrate and MDA in kidney, testis, spleen and intestine of mice shows (Table I). that *M. alba* has induced a significant increase in the level of renal GSH (at a dose of 800 mg/kg), splenic GSH (at both doses of 200 and 400 mg/kg) and intestinal GSH (at both doses of 400 and 800 mg/kg) on the contrary, the level of testicular GSH was significantly decreased at both doses of 200 and 400 mg/kg.

**Table I.-** Effect of *M. alba* leaves extract (MLE) on glutathione (GSH) level (mg/g) nitrite/nitrate level ( $\mu\text{mol/g}$ ) and MDA level (nmol/g) in kidney tests, spleen and intestine of mice.

Group	Kidney (n=10)	Testes (n=10)	Spleen (n=10)	Intestine (n=10)
<b>GSH</b>				
Control	23.80 $\pm$ 1.75	221.6 $\pm$ 0.99	49.16 $\pm$ 2.69	06.06 $\pm$ 0.29
200 mg/Kg MLE	28.54 $\pm$ 1.94	122.5 $\pm$ 1.72 <sup>a*</sup>	60.64 $\pm$ 2.64 <sup>a*</sup>	07.85 $\pm$ 0.71
400 mg/Kg MLE	24.56 $\pm$ 1.69	157.7 $\pm$ 2.27 <sup>a*</sup>	73.39 $\pm$ 3.03 <sup>a*</sup>	15.58 $\pm$ 1.44 <sup>a*</sup>
800 mg/Kg MLE	76.50 $\pm$ 1.80 <sup>a*</sup>	220.4 $\pm$ 1.40	47.88 $\pm$ 2.21	11.90 $\pm$ 0.39 <sup>a*</sup>
<b>Nitrite/nitrate</b>				
200 mg/Kg MLE	70.19 $\pm$ 1.61	07.90 $\pm$ 0.68	51.86 $\pm$ 1.99	118.9 $\pm$ 1.70
400 mg/Kg MLE	35.93 $\pm$ 1.95 <sup>a*</sup>	05.98 $\pm$ 0.46 <sup>a*</sup>	52.25 $\pm$ 1.56	65.26 $\pm$ 2.10 <sup>a*</sup>
800 mg/Kg MLE	24.93 $\pm$ 1.86 <sup>a*</sup>	06.11 $\pm$ 1.07	46.10 $\pm$ 2.28 <sup>a*</sup>	101.7 $\pm$ 2.55 <sup>a*</sup>
	39.70 $\pm$ 2.54 <sup>a*</sup>	07.72 $\pm$ 1.43	31.92 $\pm$ 2.47 <sup>a*</sup>	72.79 $\pm$ 1.97 <sup>a*</sup>
<b>MDA</b>				
Control	37.39 $\pm$ 2.09	129.2 $\pm$ 1.13	99.31 $\pm$ 1.98	05.79 $\pm$ 0.98
200 mg/Kg MLE	35.92 $\pm$ 1.44	117.8 $\pm$ 1.16 <sup>a*</sup>	54.14 $\pm$ 2.36 <sup>a*</sup>	05.26 $\pm$ 0.87
400 mg/Kg MLE	33.89 $\pm$ 1.88 <sup>a*</sup>	120.7 $\pm$ 1.32 <sup>a*</sup>	42.06 $\pm$ 3.51 <sup>a*</sup>	05.50 $\pm$ 0.50

Values are means  $\pm$  SE. \* Significance against control group at  $P \leq 0.001$ .

Nitrite/nitrate level showed a highly significant reduction after MLE administration. The maximum effect was at a dose level of 400 mg/kg b.wt. in kidney, at 800 mg/kg in spleen and at 200 mg/kg in jejunum while nitrite/nitrate showed a non-significant reduction in testis. MLE (200, 400 & 800) mg/kg b. wt. induced a non-significant decrease in MDA level in jejunum, while the MLE at a dose of 800 mg/kg, caused a highly significant reduction in kidney, testis and spleen tissue homogenates.

All investigated doses of MLE caused a highly significant reduction in plasma creatinine level as compared to the control group. In addition, MLE induced a highly significant reduction in blood urea nitrogen level at doses 200, 400 mg/kg and in plasma uric acid level at a dose 400 mg/kg (Table II).

## DISCUSSION

Evidence indicates that *M. alba* can exert several health-beneficial effects such as hepatoprotective, gastroprotective and reproductive effects (Zafar *et al.*, 2013). Antioxidant properties of mulberry on renal and intestinal tissues could enhance the immune system and enable the reproductive organs to perform the function in a good manner.

**Table II.-** Effect of *M. alba* leaves extract (MLE) on plasma urea, creatinine, uric acid of mice.

Group (mg/Kg MLE)	Urea (mg /dl)	Creatinine (mg /dl)	Uric acid (mg /dl)
Control	49.33 $\pm$ 2.24	0.14 $\pm$ 0.007	0.87 $\pm$ 0.06
200	22.66 $\pm$ 1.58 <sup>a*</sup>	0.06 $\pm$ 0.009 <sup>a*</sup>	0.78 $\pm$ 0.06
400	27.33 $\pm$ 1.58 <sup>a*</sup>	0.06 $\pm$ 0.01 <sup>a*</sup>	0.47 $\pm$ 0.01 <sup>a*</sup>
800	49.33 $\pm$ 1.70	0.05 $\pm$ 0.007 <sup>a*</sup>	0.84 $\pm$ 0.07

Values are means  $\pm$  SE. \* Significance against control group at  $P \leq 0.001$ , n=10.

*M. alba* leaves could be a natural antioxidant sources and the 70% alcohol extract of the Egyptian *M. alba* has a powerful antioxidant properties (El-Beshbishy *et al.*, 2006; Sadighara *et al.*, 2013). So, in the present study, *M. alba* methanolic extract administration to mice induced a highly significant elevation in glutathione level and a highly significant reduction in both levels of nitrite/nitrate and MDA at most ingested doses (200, 400, 800) mg/kg b.wt. in all organs under investigation. Our results are in agreement with Butt *et al.* (2008); Hussein *et al.* (2010); Hamdy (2012) and Nade *et al.* (2013).

It was reported that *M. alba* significantly improved the change in glutathione level (Butt *et al.*, 2008; Hussein *et al.*, 2010), where, the

mechanism of such protection of *M. alba* leaves methanolic extract may be due to the augmentation of glutathione which is one of the cellular antioxidants (Madhumitha and Indhuleka, 2012; Nade *et al.*, 2013). Moreover, the *M. alba* reduced lipid peroxidation (Hussein *et al.*, 2010; Nade *et al.*, 2013).

Reactive oxygen species can initiate lipid peroxidation and DNA damage leading to carcinogenesis and cell death if the antioxidant system is impaired. The antioxidant potency of some phenolic compounds from *M. alba* has been reported in different experimental models (El-Beshbishy *et al.*, 2006; Nade *et al.*, 2013). Flavonoids may act *in vivo* to decrease oxidative damage to DNA, protein and lipids (through donation of hydrogen atom to free radical) leading to a reduced risk of some diseases. 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; Zafar *et al.*, 2013). Also, Quercetin 3-(6-malonylglucoside) which among flavonoids; is most significant for antioxidant potential of mulberry leaves (Butt *et al.*, 2008; Iqbal *et al.*, 2012). In addition, the presence of the phenolic groups in the phytochemicals especially naringenin and quercetin in *M. alba* could be responsible for  $\cdot\text{OH}$  radical scavenging activity. Thus, it is quite clear that changes observed in GSH could be attributed to the enhancement in antioxidant status in blood and tissues of normal rats (Madhumitha and Indhuleka, 2012).

It was reported that *M. alba* has stilbene glycoside Mulberroside A and is successfully used for the management of gout and hyperuricemia in folk Chinese medicine. The mulberroside A shows uricosuric and nephroprotective effects. In hyperuricemia mice it decreases the serum level of urea nitrogen, creatinine, albumin,  $\beta_2$ -microglobulin and enhanced the creatinine clearance (Wang *et al.*, 2011). In addition, Zafar *et al.* (2013) reported that concomitant administration of hydro alcoholic extract of *M. alba* along with isoniazid significantly reduced the nephrotoxicity as evidenced by marked reduction in blood urea nitrogen and creatinine.

Collectively, the findings of the present investigations emphasize the antioxidant activities of

the *M. alba* methanolic extract in different mice organs where the *M. alba* gavage improved the oxidative stress in intestine, kidney, testis and spleen, consequently; renal functions was modulated, likewise; the immune system since the spleen combines the innate and adaptive immune system.

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