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RESEARCH ARTICLE

Nephroprotective Effects of *Morus Alba* Linn against Isoniazid-Induced Toxicity in Albino Rabbits

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A B S T R A C T

Isoniazid (INH) is the first line drug for the treatment of tuberculosis and can cause nephrotoxicity in human beings and animals. Nephroprotective effects of *Morus alba L* was studied in healthy albino rabbits (1.25-1.75 kg) of either sex. Rabbits were divided randomly into five groups (n=8). Group 1 was control group. In group II, INH was administered to induce nephrotoxicity at the dose rate of 100mg/kg/day. In group III, INH (100 mg/kg) was administered in combination with silymarin (100 mg/kg). The combined effects of INH (100 mg/kg) and hydroalcoholic extracts of *Morus alba* at the dose rates of 400 mg/kg and 800 mg/kg were observed in group IV and group V respectively. Biochemical analysis (blood urea nitrogen and serum creatinine) showed nephrotoxicity in rabbits receiving only INH while group III and IV showed significant ($P<0.05$) nephroprotective activity as compared to control group. Histopathological analysis also revealed the nephroprotective effects of *Morus alba*. HPLC analysis of serum showed the reduced concentration of INH in hydroalcoholic extract treated animals. It can be concluded that INH can induce nephrotoxicity as observed by biochemical, histopathological and HPLC analysis. These changes can be reduced by the concomitant administration of silymarin and hydroalcoholic extract of *Morus alba* L.

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INTRODUCTION

Varieties of natural products, medicinal plants and dietary components have been evaluated as nephro-protective agents. Currently, increasing health concerns necessitate reliance on alternate remedies for sustaining good health. Medicinal plants are extensively used for the management and treatment of various diseases. Plant drugs are considered nontoxic and devoid of side effects (Bhawna and Kumar, 2009; Jahan *et al*., 2012; Iqbal *et al*., 2012). Mulberry is native to china; it is an important plant that belongs to the family Moraceae. Worldwide mulberry contains more than 150 species, *Morus alba* L. (white mulberry) is dominant specie among them (Srivastava *et al*., 2006). *Morus alba* L. (*M. alba* L) is monoecious, deciduous, medium sized tree and with a height of about 30 meters and width of about 1.8 meters, it is native to Asia, Africa, Europe, South and North America. In China, *M. alba* L is used widely in folk medicines specially the stem, root bark, leaves and fruits (Kumar and Chauhan, 2008). The leaves of this plant are widely used as fodder for domestic animals and silkworms. It is also used as vegetable and fruit in European countries and in Japan the dry leaves of *M. alba* are used as tea (Ercisli and Orhan, 2007; Katsube *et al*., 2009). In Italy, the white berries of *M. alba* are used as fresh fruit or added in to various food products which includes pulp, juices, wine, ice cream, jam, desserts and marmalade (Pawlowska *et al*., 2008).

M. alba L has a wide range of therapeutic spectrum and is used in various ailments of liver and pancreas. It's important constituent mulberroside A that has shown uricosuric and nephroprotective effects (Wang *et al*., 2011). It is also an important source of nutrition and contains significant amount of carbohydrates, proteins, fats, vitamins, fibers and minerals. Recent studies has shown that this plant has hypolipidemic, free radical scavenging potential, antiviral (Lee *et al*., 2014), antibacterial, antioxidant (Hajizadish *et al*., 2014), anti-inflammatory (Lim *et al*., 2014), astringent, emollient, antihyperglycemic (Wang *et al*., 2014), neuroprotective, skin tonic, anticonvulsant (Gupta *et al*., 2014) and antihypertensive activities (Butt *et al*., 2008). Many of

these biological and pharmacological activities are due to the presence of naturally occurring flavonoids. These flavonoids are polyphenolic compounds and present in leaves, fruits, roots and seed of plants that play an important role in plant color and also good for human health (Ross and Kasum, 2002; Zafar *et al*., 2013). *M. alba* is the richest source of polyphenolic compounds especially antho-cyanins and flavonoids, because of these bioactive compounds this plant shows tremendous therapeutic activities.

Nephrotoxicity is mostly caused by the overdose or toxic effects of some drugs and harmful substances (Galley, 2000). Various chemicals and drugs have the capability of damaging the glomerulus, causing increased permeability to large size molecules. Glomerulopathies are mostly caused by drugs, of which glomerulo-nephritis is most common form of lesions produced. Moreover, numerous cases of various glomerular changes such as focal segmental glomerulosclerosis and crescentic glomerulonephritis have also been reported (Izzedine *et al*., 2006). Administration of isoniazid chronically can induce hepatotoxicity (Vanhoof *et al*., 2003). Similarly, when isoniazid and hepamerz were given in combination there was marked increase in the level of creatinine and blood urea nitrogen that caused nephrotoxicity leading to increased mortality in rabbits (Maryam *et al*., 2010). We have already reported the protective effects of some plants extracts against chemical induced skin irritation (Waheed *et al*., 2013) and INH + Rifampicin induced nephro-toxicity (Hashmi *et al.*, 2013) in albino rabbits. The present study is to investigate whether oral administration of hydroalcoholic extract of *Morus alba* L. has any protective effects against isoniazid induced nephrotoxicity in albino rabbits.

MATERIALS AND METHODS

Plant material: *M. alba* L. leaves were collected from the vicinity of University of Agriculture Faisalabad, Pakistan and identified by a botanist at the Department of Botany, University of Agriculture, Faisalabad. The leaves were dried under shade for 2 weeks then were powdered with mechanical grinder. Dry powder (400 g) was obtained by grinding and passing through mesh sieve No 40 then stored in airtight container at 4ºC. *Morus alba* L latex was extracted using water and ethanol in the ratio of 70:30 as a solvent. The extract was lyophilized by freeze drying apparatus (Christ, Germany model# Alpha 1-4 LSC).

Animals: Adult albino rabbits were purchased from the local market of Faisalabad. Rabbits were of the same breed having weight around 1500-1800 g. Rabbits were kept in the animal room at the Department of Physiology and Pharmacology, University of Agriculture, Faisalabad at room temperature (22±2ºC) with proper ventilation facility. They were acclimatized for 1 week. Rabbits were fed with seasonal fodder and water ad-libitum. The experiments were carried out in accordance to the guidelines of Directorate of Graduate Studies and Institutional Animal Ethical Committee.

Experimental protocol: The rabbits were divided into five groups containing eight animals in each group. Group1 served as control and received normal diet throughout the experiment, Group II received isoniazid (Pacific Pharmaceuticals Pvt. Ltd., Lahore, Pakistan, 100 mg/kg BW/day orally) for 28 days, Group III received silymarin (Abbott laboratories, Karachi, Pakistan, 100 mg/kg BW/day orally+INH (100 mg/kg BW/day) for 28 days. Group IV and V received hydro-alcoholic extract of *M. alba* L at the dose rate of 400 mg/kg BW/day and 800 mg/kg BW/day along INH 100 mg/kg BW/day, respectively for 28 days.

Biochemical assays: After the treatment period, blood was collected from jugular vein of rabbits and centrifuged using the table top centrifuge (REMI) at 3000 rpm to get serum. Level of blood urea nitrogen and creatinine was estimated using Span diagnostic kit on chemical analyzer (microlab3000) for assessment of renal toxicity.

Histopathological analysis: Formalin fixed kidney biopsies were processed in graded ethanolic concentrations and fixed in paraffin blocks. Kidney fragments were arranged perpendicular to the plane of the section in the block and 6 micrometer thick transverse fragments were cut and mounted on glass slides and stained with hematoxylin and eosin (H and E stain). Microscopy was completed on Olympus PM-10ADS automatic light microscope (Olympus optical Co., Tokyo, Japan) with a 40X objective.

HPLC analysis: The drug was analyzed in serum following the HPLC method described in Hashmi *et al*. (2013).

Statistical analysis: Results were expressed as two way analysis of variance (ANOVA) and statistical differences among different treatment groups was determined by Duncan's Multiple Range test and $P<0.05$ was considered as significant.

RESULTS

Table-1indicates that the Mean±SEM values of BUN is significantly ($P<0.01$) increased in group treated with isoniazid alone (43.54±5.17 mg/dl) as compared to group treated with silymarin + isoniazid (29.67±1.45 mg/dl), the group treated with *M. alba* 400 mg/kg hydroalcoholic extract (30.17±1.86 mg/dl) and control group (26.88±1.96 mg/dl). Mean values of serum creatinine (mg/dl) significantly ($P<0.01$) increased in group treated with isoniazid alone (1.002±0.03 mg/dl) as compared with group treated with silymarin + isoniazid (0.884±0.02 mg/dl), the group treated with *M. alba* 400 mg/kg hydroalcoholic extract (0.866±0.02 mg/dl), the group treated with *M. alba* 800 mg/kg hydroalcoholic extract (0.910±0.03 mg/dl) and is comparable with control group (0.850±0.02 mg/dl) Table 1.

Kidney biopsies of control group showed normal architecture. In group II, nuclei of the tubular epithelial cells are pyknotic and condensed in appearance, edema, mild to moderate congestion present in renal parenchyma and focal tubular necrosis was observed on histological examination (Fig. 1a). While, in group III, the nuclei of the tubular epithelial cells were normal in appearance with

Table 1: Mean ± SEM values of Blood Urea Nitrogen (mg/dl) and Serum Creatinine (mg/dl) with per oral drugs and *Morus alba* extracts daily administration for 28 days in rabbits (n=8)

Groups	Biochemical Examination	
	Blood Urea Nitrogen (mg/dl)	Serum Creatinine (mg/dl)
Group I (Untreated)	26.88±1.96 B	0.85±0.02 B
Group II (INH)	43.54±5.17 A	1.002±0.03 A
Group III (INH + Silymarin)	29.67±1.45 B	0.884±0.02 B
Group IV(INH + 400mg Hydroalcoholic Extract)	30.17±1.86 B	0.866±0.02 B
Group V(INH + 800mg Hydroalcoholic Extract)	33.80±1.93 AB	0.91±0.03 B

Means sharing similar letter in a column are statistically non-significant (P>0.05).

Table 2: Mean Values of Isoniazid concentration at 2nd and 4th week of treatments as determined by HPLC

Groups	Concentration (µg/ml) at 2nd Week	Concentration (µg/ml) at 4th Week
Group II (INH)	8.18±1.70	75.32±4.85
Group III (INH + Silymarin)	13.06±2.24	44.26±4.16
Group IV (INH + 400mg Hydroalcoholic Extract)	56.07±6.12	21.67±3.07
Group V (INH + 800mg Hydroalcoholic Extract)	69.84±4.80	31.3±4.00

mild congestion in renal parenchyma (Fig. 1b). The histological observations of group IV and V revealed that the renal parenchyma is normal in appearance, glomerulus is bounded by a clear space, the lumen of the capsule of Bowman, in proximal convoluted tubules nuclei are normal in appearance and mild degree of vascular congestion observed at some places (Fig. 1c).

HPLC analysis:

In group II, the mean INH concentration in rabbit's serum was 8.18µg/ml and 75.32 µg/ml at 2nd and 4th week respectively. In group III, the mean INH concentration in rabbit's serum was 13.06µg/ml and 44.26µg/ml at 2nd and 4th week respectively (Table 2). In group IV, at 2nd week, the mean INH concentration in rabbit's serum was 56.07 µg/mL. While at 4th week the mean isoniazid concentration decreased to 21.67 µg/mL. Similar trend was observed in group V (Table 2).

DISCUSSION

The rational use of drugs is required to prevent untoward side effects especially on vital organs such as kidney. It is more important to identify the high risk patients and quick identification of the drug which produces injury in the body (Singh *et al*., 2003). Isoniazid therapy is usually considered a safe and effective against TB (Carmona *et al*., 2005). Any how isoniazid can cause severe untoward reactions such as hepatotoxicity (Vanhoof *et al*., 2003).

In the present study, BUN and creatinine level was increased significantly (P<0.01) after administration of INH as compared to control group. In kidney architecture there was increased incidence of necrosis in tubular epithelial cells as compared with other groups. INH alone showed nephrotoxic activity which may be responsible for mortality and other complications in rabbits. HPLC analysis also showed a marked increase in INH concentration in serum. The INH concentration at 4th week was markedly higher as compared with INH concentration at 2nd week. These analyses suggested that

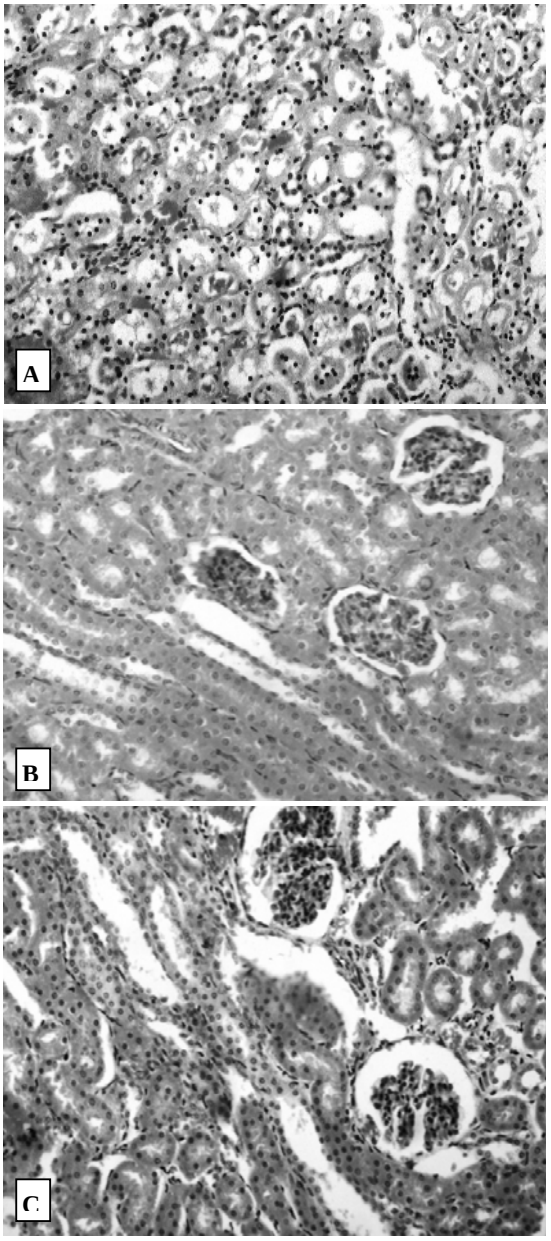

Fig. 1: Kidneys of rabbits treated with INH 100mg/kg body weight (A), INH 100mg/kg body weight + Silymarin 100mg/kg body weight (B), INH 100mg/kg body weight + Hydroalcoholic extract of M. alba 400mg/kg body weight (C) with daily oral administration for 28 days (H & E, 400x)

rabbits receiving INH have high concentration of INH in serum which may be responsible for causing kidney damage and other serious complications. Because unique physiological characteristics of kidney make it potential targets for drug toxicity (Bayomi *et al*., 2013), these unique characteristics of kidney includes a greater supply of blood to kidney, filtration of toxic substances and drugs by glomerular filtration and active tubular secretion. Other risk factor which may affect the kidney includes metabolic enzymes present in kidney (Gąsiorowski *et al*., 2013).

Concomitant administration of Silymarin along with INH decreased BUN, and creatinine level significantly (P<0.01) as compared to group receiving nephrotoxic

drug. Histopathological studies showed normal kidney architecture but at few places condensed nuclei are present while at other places nuclei have normal structure indicating the ameliorated effect of silymarin against nephrotoxicity induced by Isoniazid (Fig 1b). HPLC analysis showed a marked increase in INH concentration in serum at 2nd week and 4th week of serum analysis. INH concentration at 4th week was much higher than INH concentration at 2nd week. In comparison with nephro-toxic group the INH concentration in silymarin treated group at 4th week was lower. This reduced concentration of INH in serum may be due to interaction of silymarin with INH (Choudhary *et al*., 2014), due to this interaction the isoniazid absorption decreased from stomach of rabbits or silymarin reduced the INH concentration in blood and less toxic effects were observed by INH. The exact mechanism is still unknown and further study helps to reveal this interaction.

Silymarin has anti-nephrotoxic activity against cisplatin induced nephrotoxicity in albino rats (Karimi *et al*., 2005). It was also observed that glomeruli and renal tubules showed normal cellular architecture but at some places mild necrosis and glomerular atrophy were observed (Abdelmeguid *et al*., 2010). Concomitant administration of silymarin with non-steroidal anti-inflammatory drugs reduced the hepatic and renal toxicities in osteoarthritic patients (Hussain *et al*., 2007). Similarly, silymarin showed appreciable renal protective potential by normalizing the concentration of urine and serum markers such as creatinine and BUN. Antioxidant enzymes level and concentration of glutathione was enhanced, while decreased in lipid peroxidation were observed dose dependently with silymarin (Khan and Siddique, 2012). These reported results are in accordance with our findings.

Hydroalcoholic extract of *M. alba* (400 mg/kg/day) along with INH normalized the level of BUN and creatinine significantly ($P<0.01$) with respect to toxic control group. However, hydroalcoholic extract of *M. alba* (800 mg/kg/day) showed non-significant ($P>0.05$) reduction in the level of BUN as compared with toxic control group. Histopathological findings also supported the biochemical studies and showed normal architecture of kidney in rabbit receiving 400 mg/kg/day *M. alba* extract. However, at few places mild congestion observed in renal parenchyma with some condensed nuclei in tubular epithelial cells while at other places normal nuclei was observed, the glomerular structure was normal and intact with no pathological lesions. On the other hand the rabbits receiving *M. alba* extract at the dose rate of 800 mg/kg/day showed mild to moderate degree of congestion in parenchyma and similarly the nuclei are condensed at few places while normal in structure at other places. Mild degree of necrosis was also observed at some places. These results showed that animals receiving 400 mg/kg/day of *M. alba* along with INH showed better protective activity may be due to antioxidant and anti-inflammatory activities (Hajizadish *et al*., 2014; Lim *et al*., 2014) as compared with animals receiving 800 mg/kg/day of *M. alba* as evidenced by both biochemical analysis of serum and histopathological studies.

At 2nd week higher concentration of INH was observed in serum in both groups receiving different doses of hydroalcoholic extract of *M. alba*. This concentration of INH is comparable with INH concentration present in serum of nephrotoxic group. However at 4th week the drastic decrease in the level of INH was seen in both groups receiving 400 mg/kg/day and 800 mg/kg/day of *M. alba* extract. The percentage reduction of INH concentration in both hydroalcoholic treated group was comparable and is markedly decreased in comparison with other treatment groups. This reduction of INH in serum of rabbits with administration of hydroalcoholic extract of *M. alba* may be responsible for nephroprotective activity of *M. alba* due to its pharmacokinetic interactions (Choudhary *et al*., 2014).

In previous studies it was reported that in Chinese medicines *M. alba* L showed analgesic, improved eye sight, protect liver from hepatotoxins and lowers the blood pressure by facilitating discharge of urine or used as urine enhancer (Chen and Li, 2007; Jia *et al*., 1999). Similarly, *M. alba* bark is used in treatment of edema, wheezing, cough, headache, fever, red dry and sore eyes and to enhance urination (Taylor *et al*., 2006). The frequency of urination increased markedly in *M. alba* receiving animals in comparison with rabbits receiving other treatment. Due to enhanced urination less concentration of nephrotoxic drug accumulates in kidney and more clearance of INH takes place. As less concentration of nephrotoxic drug is available in kidney so the functional units of kidney remain intact, as evidenced by histopathological studies. HPLC analysis also proved the enhanced clearance of INH by kidney as 4th week serum analysis showed less concentration of INH in hydroalcoholic treated group as compared with 4th week serum concentration of INH in other treated groups. Similarly, Mulberroside A an important stilbene glycoside separated from *M. alba* is generally used in many conventional Chinese medicines for the treatment of arthritis, rheumatism, gout, and edema through purging diuresis. Activity of mulberroside A had been evaluated in hyperuricemic mice, enhanced BUN level and serum creatinine confirmed impairment of renal function. The results of the study suggested that mulberroside A regulates the renal organic ion transporters which results in decreased level of serum uric acid and normalizing the renal function in hyperuricemia. However, the mechanism of regulating renal organic ion transporters was still unknown. Histopathological studies also revealed that mulberroside A reduced histological changes in kidney. This was the first study suggesting the nephroprotective and uricosuric activity of *M. alba* (Wang *et al*., 2011). In our study mulberroside A might be assumed to show nephroprotective activity by regulating the renal organic ion transporters which induced diuresis and excrete a greater concentration of INH and showed nephroprotective activity. This study provides a scientific base for the use of hydroalcoholic extract of *M. alba* as an alternate drug candidate for the management of isoniazid-induced nephrotoxicity.

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