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Research Article

EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF *MENTHA ARVENSIS* IN CISPLATIN-INDUCED NEPHROTOXICITY

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ABSTRACT

Present study was performed to evaluate the effect of *Mentha arvensis* on cisplatin-induced nephrotoxicity in Sprague-Dawley rats. The *M. arvensis* hydroalcoholic extract (MAHE) was administered orally at two dose levels (200 mg/kg and 400 mg/kg). The kidney function test (estimation of serum creatinine, total protein, blood urea nitrogen [BUN], and urea), oxidative stress study (estimation of superoxide dismutase [SOD] activity, glutathione content, and lipid peroxides [LPO]), and histological studies were also conducted. MAHE was found effective at both doses, although high dose (400 mg/kg) was found more effective, which was evidenced by decrease in serum creatinine, total protein, BUN, urea, and LPO and increased in SOD activity. Histopathological studies were also confirmed the nephroprotective action of MAHE. Present investigation revealed that *M. arvensis* showed nephroprotective effect on cisplatin nephrotoxicity in Sprague-Dawley rats which may be due to the presence of flavonoids and related compounds.

Keywords: *Mentha arvensis*, Pudina, Nephroprotective activity, Nephrotoxicity.

INTRODUCTION

The rate of drug-induced nephrotoxicity has been increasing with the ever increasing number of drugs and easy availability of over-the-counter medication *viz*. nonsteroidal anti-inflammatory drugs (NSAIDs). Angiotensin converting enzyme inhibitors, antibiotics, NSAIDs, and contrast agents are the major culprit drugs contributory to damage of kidney [1]. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various phytocontituents. Earlier literatures have prescribed various herbs for the cure of renal disorders. Co-administration of several medicinal plants possessing the nephroprotective activity along with different nephrotoxic agents which may attenuate its toxicity [2].

Mentha arvensis (Mint), is an aromatic perennial herb commercially cultivated for its oil popularly known as pudina in India, is an important medicinal plant of widespread utility both in crude and extracted form. Many studies have established that *M. arvensis* leaves extracts have potent anti-inflammatory, anti-microbial, neuroprotective, ulcer protective, and hepatoprotective properties [3-7]. Traditionally, *M. arvensis* leaves are used in kidney disorders [8]. The present study was aimed to evaluate the nephroprotective activity of hydroalcoholic extract of leaves of *M. arvesis* hydroalcoholic extract (MAHE) in the cisplatin-induced nephrotoxicity.

MATERIALS AND METHODS

Animals

All studies were approved by the Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute, Bhopal. (Registration no.1283/c/09/CPCSEA; Reference no.: PBRI/13/IAEC/PN-349). Healthy albino Sprague-Dawley rats of either sex weighing 150-180 g were maintained on standard rodent feed and water ad libitum. Rats were closely observed for any infection, and those showing sign of infection were excluded from the study and replaced. The rats were randomly distributed in the group of 6 each for study.

Plant material

The plant *M. arvensis* has been procured from the local market of Bhopal, Madhya Pradesh and authenticated by Dr. Zia Ul Hasan, Professor and Head, Department of Botany, Saifia Science College, Bhopal, Madhya Pradesh (Voucher specimen no. 440/Bot./Saifia/13). The plant leaves washed, shade-dried, and powdered mechanically. The powdered quantity then weighed and stored in airtight poly bags. The stored drug was first deffatted with petroleum ether and extracted with 70% methanol using continuous hot percolation by the soxhlet apparatus.

Experimental design

All the experiments were performed in compliance with the guideline for the care and use of laboratory rats. Rats were divided into four groups:

Group I: (Normal control) rats were fed on a standard diet and were treated with (10 mL/kg) vehicle for 10 days and on 5th day normal saline by oral administration, was served as a control group.

Group 2: (Cisplatin control) rats were given vehicle for 10 days and on 5th day cisplatin (7.5 mg/kg b w) was injected by intraparitoneal route followed by 1 hr of vehicle oral administration.

Group 3: (MAHE 200 mg/kg) Rats of this group were administrated with oral MAHE (200 mg/kg) for 10 days and on 5th day cisplatin (7.5 mg/kg b w) was injected by intra-paritoneal route followed by 1 hr of MAHE (200 mg/kg)(1/10th of LD_{50}) oral administration.

Group 4: Rats of this group were administrated with oral MAHE (400 mg/kg) extract for 10 days and on 5th day cisplatin (7.5 mg/kg b w) was injected by i.p. followed by 1 hr of MAHE (400 mg/kg) (1/5th of LD_{50}) oral administration.

Biochemical assays

For biochemical assays, blood samples were collected from rats after 10th day of the treatment. Sera were obtained by centrifugation of the blood sample and stored at −20°C, and creatinine, total protein, blood urea nitrogen (BUN), and urea were estimated using a fully automated Bio-chemical auto analyzer (Erba Manheim Lica Scan II™). The commercial diagnostic kits (Erba Manheim, Span Diagnostics Ltd.) were used in these analyses.

Superoxide dismutase (SOD) activity was measured using the methods of Paoletti *et al.*, 1986 [9]. Glutathione (GSH) activity was determined from the method of Ellman, 1959 [10]. Lipid peroxidation (LPO) was estimated in the renal tissue as the concentration of thiobarbituric acid reactive product according to Ohkawa *et al.,* 1979 [11].

Histological examination

Rats were euthanized under light anesthesia with diethyl ether, and kidney was dissected out. The kidney was perfused with buffer saline to remove access blood and then kidney was isolated and stored at −20°C. The kidney was fixed with 10% neutralized buffered formalin. Fixed materials were embedded in paraffin wax and sections of 5 μm thickness were cut. Slides were stained with hematoxylin and eosin for histological examination.

Statistical analysis

All the results were expressed as mean ± standard deviation. Statistical comparison was made between drugs treated groups and control group. All data were analyzed by one-way ANOVA followed by Benferroni's test. $p<0.05$ were considered as level of significance.

RESULTS

Biochemical results

Effect of MAHE on serum creatinine

Serum creatinine level was significantly ($p<0.001$) elevated in cisplatin control group compared to normal control group. MAHE (200 mg/kg) significantly ($p<0.05$) decreased the serum creatinine level as compared to cisplatin control group. MAHE (400 mg/kg) significantly ($p<0.001$) decreased the serum creatinine level as compared to cisplatin control group and serum creatinine level significantly ($p<0.05$) decreased as compared to MAHE (200 mg/kg) treated group (Table 1).

Effect of MAHE on total protein level

Serum total protein level was significantly ($p<0.001$) elevated in cisplatin control group compared to normal control group. MAHE (200 mg/kg) significantly ($p<0.001$) decreased the Serum total protein level as compared to cisplatin control group. MAHE (400 mg/kg) significantly ($p<0.001$) decreased the serum total protein level as compared to cisplatin control group (Table 1).

Effect of MAHE on BUN level

Serum BUN level was significantly ($p<0.001$) elevated in cisplatin control group compared to normal control group. MAHE (200 mg/kg) significantly ($p<0.001$) decreased the serum BUN level as compared to cisplatin control group. MAHE (400 mg/kg) significantly ($p<0.05$) decreased the serum BUN level as compared to cisplatin control group (Table 1).

Effect of MAHE on urea level

Serum urea level was significantly ($p<0.001$) elevated in cisplatin control group compared to normal control group. MAHE (200 mg/kg) significantly ($p<0.001$) decreased the serum urea level as compared to cisplatin control group. MAHE (400 mg/kg) significantly ($p<0.05$) decreased the serum urea level as compared to cisplatin control group (Table 1).

Effect of MAHE on SOD activity

Cisplatin treated rats showed significant ($p<0.001$) decrease in activity of SOD as compared to normal rats. Treatment of MAHE (400 mg/kg) along with cisplatin significantly ($p<0.05$) increased the activity of SOD compared to cisplatin control group (Table 2).

Effect of MAHE on GSH

GSH level was significantly ($p<0.001$) decreased in cisplatin control group as compared to normal control. MAHE (400 mg/kg) treatment significantly ($p<0.05$) improved the GSH level while it could not show any significant change at MAHE (200 mg/kg) group (Table 2).

Effect of MAHE on LPO

Cisplatin treatment significantly ($p<0.001$) increased the LPO levels in kidney tissues of cisplatin control group. MAHE (400 mg/kg) significantly ($p<0.001$) decrease the increased LPO at MAHE (400 mg/kg)+ cisplatin treated group as compared to cisplatin control group (Table 2).

Histological study

The sections of kidney of normal control rats showed normal renal tubules (RT) and renal corpuscles. The Bowman's capsule and the glomeruli appeared to be prominent and normal (Fig. 1a). In the cisplatin control group, the kidney showed acute tubular necrosis, dilated proximal convoluted tubules (PCT), slogging of epithelium due to desquamation and atrophic glomeruli, cellular debris in the tubular lumen and increased tissue in interstitium, were an indication of cisplatin-induced nacrosis of renal, which were signs of nephrotoxicity (Fig. 1b). Histological section of the kidney of the rat treated with cisplatin and MAHE (200 mg/kg) showed marked improvement in comparison with cisplatin control group, and it reverted the histological appearance seen in the latter group to normal in examined tissue fields. There were areas of tubular injury with tubular atrophy, tubular necrosis, and interstitial fibrosis, but these were of less intensity than in cisplatin control group (Fig. 1c). Kidney histological sections of rats treated with cisplatin + MAHE (400 mg/kg) showed marked improvement, showing normal kidney histology and architecture, with interstitial fibrosis. There were some foci showing tubular injury with, tubular atrophy, tubular necrosis and interstitial fibrosis which were less intense than those seen in cisplatin control group and cisplatin + MAHE (200 mg/kg) group (Fig. 1d).

DISCUSSION

In the present investigation, nephroprotective potential of hydroalcoholic leaf extract of *M. arvensis*, at two dose levels (200 and 400 mg/kg) was evaluated by estimation of kidney function test, i.e. by estimating the amount of serum creatinine, total proteins BUN and urea, study of oxidative stress by means of estimation of LPO, % inhibition of SOD activity and GSH in kidney homogenate of cisplatin-treated rats and by histological examination of kidney of cisplatin-treated rats.

Cisplatin was used to induce nephrotoxicity in Sprague-Dawley rats and to cause acute renal failure. Cisplatin is a potent antitumor drug. Cisplatin-based combination chemotherapy regimens are extensively used as front-line therapy in the treatment of ovarian germ cell tumors, testicular cancer, epithelial ovarian cancer, lung cancer, head and neck cancer, advanced cervical cancer, and malignant melanoma. Although cisplatin has been a mainstay for therapy of cancer, its use is mainly limited by two factors: Acquired resistance to cisplatin and severe side effects in normal tissues which include neurotoxicity, ototoxicity, nausea and vomiting, and nephrotoxicity [12].

Table 1: Effect of MAHE on serum creatinine, total protein, BUN, and urea

Treatment	Creatinine (mg/dl)	Total protein (mg/dl)	BUN (mg/dl)	Urea (mg/dl)
Normal control	0.50±0.13	7.10±0.93	15.72±2.63	33.63±5.62
Cisplatin control	4.57±0.87[a]	15.01±2.64[a]	31.93±5.09[a]	68.34±10.89[a]
MAHE (200 mg/kg)+cisplatin (7.5 mg/kg)	3.20±0.65[c]	10.16±1.65[b]	25.06±3.26[b]	53.62±6.98[b]
MAHE (400 mg/kg)+cisplatin (7.5 mg/kg)	1.80±0.51[b,d]	10.06±1.38[b]	21.32±3.36[c]	45.62±7.20[c]

All data are presented in Mean±SD (n=6), [a]$p<0.001$ as compared to normal control group, [b]$p<0.001$ as compared to cisplatin control group, [c]$p<0.05$ as compared to cisplatin control group, [d]$p<0.05$ as compared to MAHE (200 mg/kg) treated group, MAHE: *Mentha arvensis* hydroalcoholic extract, SD: Standard deviation, BUN: Blood urea nitrogen

Table 2: Effects of MAHE on SOD, GSH and LPO

Treatment	SOD (% inhibition)	GSH (mmol/g tissue)	LPO (nmol/g tissue)
Normal control	106.82±20.64	15.84±4.48	33.55±4.78
Cisplatin control	50.15±10.23[a]	6.70±1.26[a]	77.36±7.28[a]
MAHE (200 mg/kg)+ cisplatin (7.5 mg/kg)	75.54±17.24	9.61±0.92	61.14±16.34
MAHE (400 mg/kg)+ cisplatin (7.5 mg/kg)	89.68±7.59[b]	12.60±1.91[b]	55.15±6.83[b]

All data are presented in Mean±SD (n=6), LPO: Lipid peroxides, SOD: Superoxide dismutase, GSH: Glutathione, [a]p<0.001 as compared to normal control group, [b]p<0.05 as compared to cisplatin control group, MAHE: *Mentha arvensis* hydroalcoholic extract, SD: Standard deviation

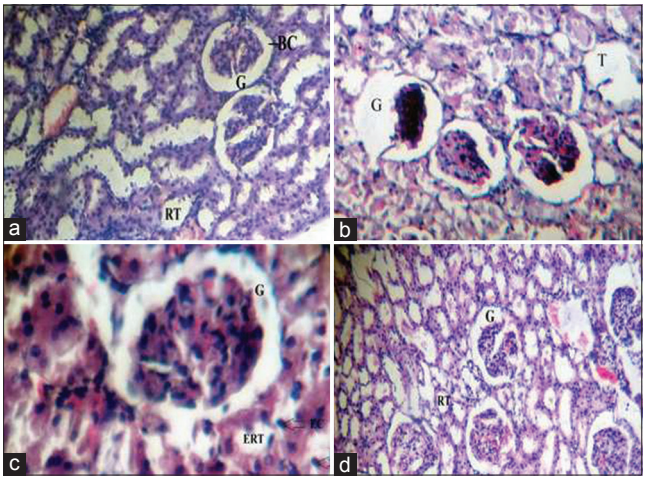

Fig. 1: Sections in the kidney cortex of (a) Normal control rat showing a Bowman's capsule (BC), glomerulus (G) and renal tubules (RT); (b) cisplatin control rat showing enlarged and congested renal tubules with wide lumens (T), leucocytic infiltrations (arrow) and atrophied glomerulus (G); (c) section in kidney of a rat treated with cisplatin and *Mentha arvensis* hydroalcoholic extract (MAHE) (200 mg/kg) and showing improved glomeruli (G) and enlarged renal tubules (ERT) with somewhat wide lumen, and epithilial cell; (d) section in kidney of a rat treated with cisplatin and MAHE (400 mg/kg) and showing normal glomeruli (G) and RT with somewhat wide lumen

Creatinine is a spontaneously generated cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys through glomerular filtration and proximal tubular secretion. There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels increase. This suggests diminished ability of the kidneys to filter these waste products from the blood [13]. In the present investigation, serum creatinine level was significantly decreased by MAHE at both doses showed its nephroprotective action on cisplatin-induced nephrotoxicity.

The total protein is the amount of protein present in investigating blood sample. It was reported that cisplatin-induced renal toxicity was evidenced by the elevated biochemical markers such as serum urea, serum creatinine, and total protein [14].

Urea is a by-product of metabolism of protein. This waste product is generated in the liver, then filtered from the blood and excreted in the urine by the kidneys. The BUN test measures the nitrogen amount contained in the urea in the blood sample. High BUN levels indicate kidney dysfunction [15]. It was reported that serum concentration of creatinine, urea, BUN, and total proteins depends largely on the glomerular infiltration [16]. In the present study, serum urea, BUN, and total protein level were significantly decreased by MAHE at both doses showed its nephroprotective action on cisplatin-induced nephrotoxicity.

SOD is the first line of defense against free radical induced oxidative stress. It is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radical to hydrogen peroxide [16]. Cisplatin caused a significant decrease in SOD activity. The present investigation showed SOD activity increased by the administration of MAHE (400 mg/kg) implicates its antioxidant and nephroprotective activity.

GSH is an intracellular reductant and plays a pivotal role in catalysis, metabolism, and transport. It protects cells against peroxides, free radicals, and other toxic compounds [17]. Reduced GSH neutralizes the hydroxyl radical and plays an important role against oxidative stress and inflammatory responses [18]. The nephrotoxicity induced by cisplatin in rats is due to decrease of GSH -S-transferase-γ activity [19]. Cisplatin decreases the GSH content in kidney, indicating oxidative stress. Cisplatin inhibits the activity of antioxidant enzymes **(**GSH and LPO) in rat kidneys suggests that cisplatin nephrotoxicity results from generation of reactive oxygen species [15]. The present study showed GSH content increased by the administration of MAHE (400 mg/kg) implicates its antioxidant and nephroprotective activity.

LPO is an autocatalytic process, which is a common reason of cell death. Malondialdehyde (MDA) is one of the end products in the LPO procedure. MDA is generated during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of LPO [17]. LPO are the products of chemical damage produced by oxygen free radicals to the polyunsaturated fatty acids of cell membranes. The results show a measure of total serum LPO, an indicator of whole body free radical activity. High levels of LPO are associated with cancer, stroke, heart disease, and aging [20]. In the present investigation, administration of cisplatin caused increase in renal concentration of MDA indicated increased LPO which showed the renal oxidative stress and LPO. The elevated level was decreased by the administration of MAHE (400 mg/kg) showed its antioxidant and nephroprotective activity.

It was reported that, histological changes of rat kidney after cisplatin treatment revealed acute tubular necrosis which confirmed irreversible injury to the kidney. Cisplatin intoxication also showed severe atrophy of glomerulus, which was evident due to a reduction in its size. Marked dilation of PCT with slogging of almost entire epithelium due to desquamation of tubular epithelium was evident. Cellular debris in the tubular lumen and increased tissue in the interstium is also an indication of cisplatin-induced renal necrosis [21]. In the present study, we found that kidney of cisplatin treated rats showed tubular degeneration, atrophy of glomeruli, dilatation of RT and renal blood vessels. Administration of MAHE at both doses improved the histological changes induced in the kidney by cisplatin.

The leaves of *M. arvensis* reported to contain monoterpenes such as (menthone, menthofuran, methyl acetate and limonine), flavonoids (caffeic, chlorogenic and rosmarinic), sesquiterpenes (viridiflorol), triterpenes (squalene, ursolic acid and sitosterol), phytol, cardiac glycosides, tocopherols, tannin, and minerals [22,23]. Phytochemical screening of MAHE showed the presence of glycosides, flavanoids, triterpene, steroids, tannin, and phenolic compounds.

Anti-oxidant activity of *M. arvensis* has been already reported [24]. Flavanoids are well known potent antioxidant and free radical scavengers. The leaves of *M. arvensis* are a rich source of flavanoids which have been shown to possess several biological properties related to antioxidant mechanism. In the present investigation, the nephroprotective effect showed by MAHE may be due to the presence of flavonoids and related compounds.

CONCLUSION

In the present study, results suggested that MAHE (200 and 400 mg/kg) significantly reduced cisplatin-induced elevated serum levels of creatinine, total protein, BUN, and urea while increase in SOD activity, GSH content, and LPO in oxidative stress. The histological findings also supported the nephroprotective action of MAHE. It can be concluded from the present study that nephroprotective effect of *M. arvensis* was showed due to the presence of one or more of flavanoids.

The present study is proved the traditional use of *M. arvensis* in nephrotoxicity. Further, the isolation of active phytocompound is underway to know the responsible compound for the nephroprotective action of *M. arvensis*.

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