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Nephroprotective effect of *Murraya koenigii* on cyclophosphamide induced nephrotoxicity in rats

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ABSTRACT

Objective: To evaluate the nephroprotective effect of defatted methanolic extract and aqueous extract of *Murraya koenigii* (*M. koenigii*) against cyclophosphamide drug.
Methods: Nephrotoxicity was induced by cyclophosphamide in 7 days at 150 mg/kg body weight through intraperitoneal route in rat model. Nephroprotective activity of *M. koenigii* extract (100 mg/kg and 200 mg/kg in intraperitoneal route) was measured, including nephrological source, oxidative stress parameters like superoxide dismutase, glutathione, the lipid peroxide and *in vivo* assay like blood urea nitrogen, creatinine were determined and analyzed by One way analysis of variance followed by Tukey's test.
Results: The study result showed that important phytochemicals such as carbohydrates, flavonoids, tannin, alkaloids, glycosides, protein and steroids were found to be present in the extract of *M. koenigii*. The renal function markers like blood urea nitrogen and creatinine level were found to be decreased significantly by *M. koenigii* extract treatment. A significant difference was found to be at $P < 0.01$.
Conclusions: The present study reveals the protective role of *M. koenigii* extract against cyclophosphamide induced nephrotoxicity.

1. Introduction

The kidneys are reddish brown, paired structures that lie on either side of the vertebral column in the lumbar region of the body. The kidney is concerned with many homeostatic mechanisms. It maintains the overall chemical composition of the intracellular environment by regulating the quantity of water, sodium chloride, potassium, phosphate and numerous other substances in the body [1].

Since most of the anticancer drugs and their metabolites are nephrotoxic and cleared from the body mainly through renal pathway, which cause major damage to the kidney. Impaired renal function can result in delayed drug biotransformation and excretion of chemotherapeutic agents, resulting in systemic toxicity. The 5-year prevalence was 43% for renal impairment and 71% for chronic kidney disease among renal impairment patients [2].

Cyclophosphamide is a widely used anticancer drug acts by alkylating mechanism. Cyclophosphamide undergoes metabolic activation by hepatic enzymes and forms 4-hydroxy cyclophosphamide, which convert into two cytotoxic metabolites acrolein and phosphoramide mustard. These cytotoxic metabolites on enzymatic activation form covalent bonds with DNA and proteins, causing cell death. Angiogenesis largely supports tumor growth. Only a small fraction of cyclophosphamide is eliminated by kidney as the tubular reabsorption of the drug is very high [3].

Plants have been used as medicines for thousands of years all over the world. According to World Health Organization bulletin, an approximate 80% of the population from developing countries mostly are still dependent on plant-based medicines for their primary healthcare issues [4].

Murraya koenigii (*M. koenigii*) family Rutaceae, commonly known as Curry leaf plant is a highly valued plant for its medicinal value and characteristic aroma [5]. The plant grows in forests of 500–1600 m height. *M. koenigii* is an unarmed,

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semi-deciduous aromatic shrub [6]. Since ancient times, traditional healthcare system has relied on medicinal plants or their bioactive compounds for primary health needs of most population in the world [7]. The pharmacological activities of the crude extracts and various parts leaf, bark, roots and seeds of *M. koenigii* have been reported. It has been found to possess biological activities as an antioxidant and free radical-scavenging activity, hypoglycemic activity, hepatoprotective activity, anticancer activity *etc.* [8–11]. It has been reported to contain rich amounts of flavonoids and phenolic acids such as tannic acid, caffeic acid [12]. The extract of the leaves of *M. koenigii* was found to be useful in the treatment of kidney disorders [13]. The present study is aimed to evaluate the effectiveness of methanolic (MEMK) and aqueous extract (AEMK) of *M. koenigii* against cyclophosphamide-induced renal injury in male Wistar rats.

2. Materials and methods

2.1. Plant collection

The selection of plant was based on traditional use such as nephroprotective [14]. The leaves of *M. koenigii* were collected in the month September from the regional area of Hata Dist.-Damoh and identified by 'Department of Botany' Safia College of Science, peer gate, Bhopal (M.P). A voucher specimen was deposited there no. 417/Bot/Safia/16.

2.2. Preparation of extract

The leaves were washed, dried under shade condition, crushed them and stored in air-tight container for further use. The dried leave powder of *M. koenigii* was subjected to successive solvent hot extraction using soxhlet apparatus with various organic solvents in increasing order of polarity. Firstly, the drug was defatted with petroleum ether (40–60 °C) then the Marc subjected to successive extraction using methanol and water, respectively. Both the extracts were stored for phytochemical investigation and assessment of nephroprotective activity.

2.3. Phytochemical screening

Phytochemical screening test of the methanolic and aqueous extracts was performed to ascertain the presence or absence of phytoconstituents such as flavonoids, tannins, carbohydrates, protein, glycosides, alkaloids and steroids using standard procedure [15].

2.4. Animal

Animal model consisted of male Wistar rats [weight (180 ± 20) g; age 2–3 months]. The rats were housed in standard polypropylene cages under standard lab environment of 12 h light-dark cycle, temperature [(20 ± 2) °C], relative humidity [(50 ± 15)%], standard diet and water *ad libitum*. The animal experiments were conducted in the Department of Pharmacology, VNS Faculty of Pharmacy Bhopal, M.P., India with due permission from the Institutional Animal Ethics Committee (CPCSEA Protocol No. PH/IAEC/VNS/2K14/003).

2.5. Toxicity study

Adult male Wistar rats weighing (160–200 g) were used for acute toxicity studies. The rats were divided into control and test group containing 6 animals in each. The rats were administered intraperitoneally (*i.p.*) with MEMK and AEMK at a dose of 1 000 mg/kg (high dose) and 200 mg/kg (low dose). Normal control rats received the same amount of vehicle (saline) only. Rats were observed carefully for 24 h after extract administration and then for the next 7 days. End of experimental period the rats were observed for a sign of toxicity, mortality and morphological behavior. Toxicity was evaluated based on the previous study [13].

2.6. Experimental method

The rats were randomly assigned into six groups ($n = 6$). The group-I was kept as normal treated with normal saline *i.p.* for 7 days. Other five groups of animals were treated with a single dose of cyclophosphamide 150 mg/kg administered *i.p.* for 7 days. Group-II animals were treated with cyclophosphamide alone and kept as negative control. The animals of group-III and group-IV were administered with 100 mg/kg, 200 mg/kg of methanolic extract, respectively. Group-V and group-VI were administered with 100 mg/kg, 200 mg/kg of aqueous extract of *M. koenigii i.p.* for 7 days, respectively.

2.7. Analysis of renal function parameters

2.7.1. Measurement of antioxidant enzyme activities

Activities of the antioxidant enzymes were determined by UV spectroscopy. Superoxide dismutase (SOD) was determined using the method established by Weydert and Cullen [16]. The concentration of reduced glutathione (GSH) in the kidney was also estimated [17]. The protein level in the kidney was estimated with the help of bicinchoninic acid kit. The lipid peroxide (LPO) level was estimated [18].

2.7.2. Biochemical parameters

Serum Creatinine (Cr) and blood urea nitrogen (BUN) concentrations were analyzed by Alkaline picrate method and Dam method in a pathology center (Bhopal) [19].

2.8. Histopathology

For the histological examination, the kidneys were fixed in 10% formalin for at least 24 h. Then, kidney tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut into 5 μM sections, and stained with Hematoxylin and Eosin dye (H&E stain) and histopathological analysis was carried out.

2.9. Statistical analysis

The result data are expressed as mean ± SEM for BUN, Cr, GSH, SOD, LPO and analyzed by one way analysis of variance followed by Tukey's test. The statistical significance was performed by using Graph Pad software and accepted at $P < 0.01$.

Table 1
Phytochemicals screening of various extracts of *M. koenigii* (leaves).

S. no.	Phytoconstitute	Methanolic extract	Aqueous extract
1	Alkaloids	+	+
2	Glycosides	+	+
3	Flavonoids	+	+
4	Protein	+	+
5	Carbohydrates	–	+
6	Tannins	–	+
7	Steroids	+	–

+, Present; –, Absent.

3. Results

3.1. Phytochemical studies

The study was done to confirm the presence of phytochemicals, which are considered active medicinal chemical constituents as shown in Table 1. Important phytochemicals such as carbohydrates, flavonoids, tannin, alkaloids, glycosides, protein and steroids were found to be present in the extract.

3.2. Toxicological studies

The animals did not show any sign of toxicity even after administration of MEMK and AEMK at the highest dose (1000 mg/mL) in the first 24 h or during the experimental period (7 days) and the activity of serum was found to be normal. In addition, there were no histopathological changes in the kidney. *M. koenigii* was tested at two dosage levels (100 mg/kg and 200 mg/kg) as they represented 1/10th and 1/5th of the highest dose.

3.3. In vivo studies

The study measured the renal function markers like BUN and Cr level, which was found to be significantly ($P < 0.01$) higher in the cyclophosphamide alone treated animals when compared to that of normal animals. This increased level was found to be decreased significantly by *M. koenigii* extract treatment as shown in Table 2.

3.4. Ex vivo studies

The enzyme activities of renal SOD, GSH, and LPO were determined. The levels of SOD and GSH were found to be decreased and LPO level was increased in cyclophosphamide treated group as compared to the normal group. But there was an increase in SOD and GSH level in cyclophosphamide and extract of *M. koenigii* treated groups while LPO level was decreased as shown in Table 3.

3.5. Histopathological study

These sections were examined under a photomicroscope for the presence of glomerulus, proximal convoluted tubule (PCT), and distal convoluted tubule (DCT), tubular degeneration, mononuclear/polymorphonuclear cell infiltration and narrowing of Bowman's capsule. Histological study of the kidney tissues indicated that normal cytoarchitecture of the glomerulus, PCT, DCT, and tubular degeneration was maintained in group-I (Normal) while cellular necrosis and glomerular hypercellularity were observed in group-II (cyclophosphamide-treated group). Rats which were administered with *M. koenigii* extract showed nearly normal glomerulus, PCT, DCT structures and renal tubules (Figure 1).

Table 2
Effect of *M. koenigii* on some biomarkers of cyclophosphamide induced nephrotoxicity.

Groups	BUN (mg/dL)		Creatinine (mg/dL)	
	Mean ± SEM	% Inhibition	Mean ± SEM	% Inhibition
Vehicle	52.16 ± 2.86	–	1.58 ± 0.26	–
CP	62.65 ± 1.45***	–	2.76 ± 0.47***	–
MEMK 100 (mg/mL) + CP	38.90 ± 0.10***	38	1.25 ± 0.05***	47.2
MEMK 200 (mg/mL) + CP	43.80 ± 0.10***	30	1.54 ± 0.05***	32.4
AEMK 100 (mg/mL) + CP	43.45 ± 0.55***	30	1.55 ± 0.05***	30.3
AEMK 200 (mg/mL) + CP	49.30 ± 1.90***	27	1.42 ± 0.05***	22.0

***Significantly different with normal saline and CP at $P < 0.01$. CP: Cyclophosphamide.

Table 3
Effect of *M. koenigii* on tissue GSH, LPO, SOD level in cyclophosphamide induced nephrotoxicity.

Groups	GSH (mg/mL)		LPO (mg/mL)		SOD (mg/mL)	
	Mean ± SEM	% Increase	Mean ± SEM	% Inhibition	Mean ± SEM	% Increase
Vehicle	78.24 ± 3.12	–	35.6 ± 0.4	–	0.42 ± 0.01	–
CP	36.00 ± 4.80***	–	61.8 ± 4.2***	–	0.14 ± 0.03***	–
MEMK 100 (mg/mL) + CP	121.20 ± 1.20***	236	27.6 ± 0.4***	55.3	0.28 ± 0.02***	0.0
MEMK 200 (mg/mL) + CP	133.20 ± 25.20***	270	47.8 ± 0.2***	22.6	0.27 ± 0.03***	28.5
AEMK 100 (mg/mL) + CP	157.20 ± 8.40***	336	39.9 ± 1.1***	35.4	0.26 ± 0.01***	23.8
AEMK 200 (mg/mL) + CP	189.60 ± 21.60***	426	46.2 ± 0.4***	25.2	0.25 ± 0.01***	19.0

***Significantly different with normal saline and CP at $P < 0.01$. CP: Cyclophosphamide.

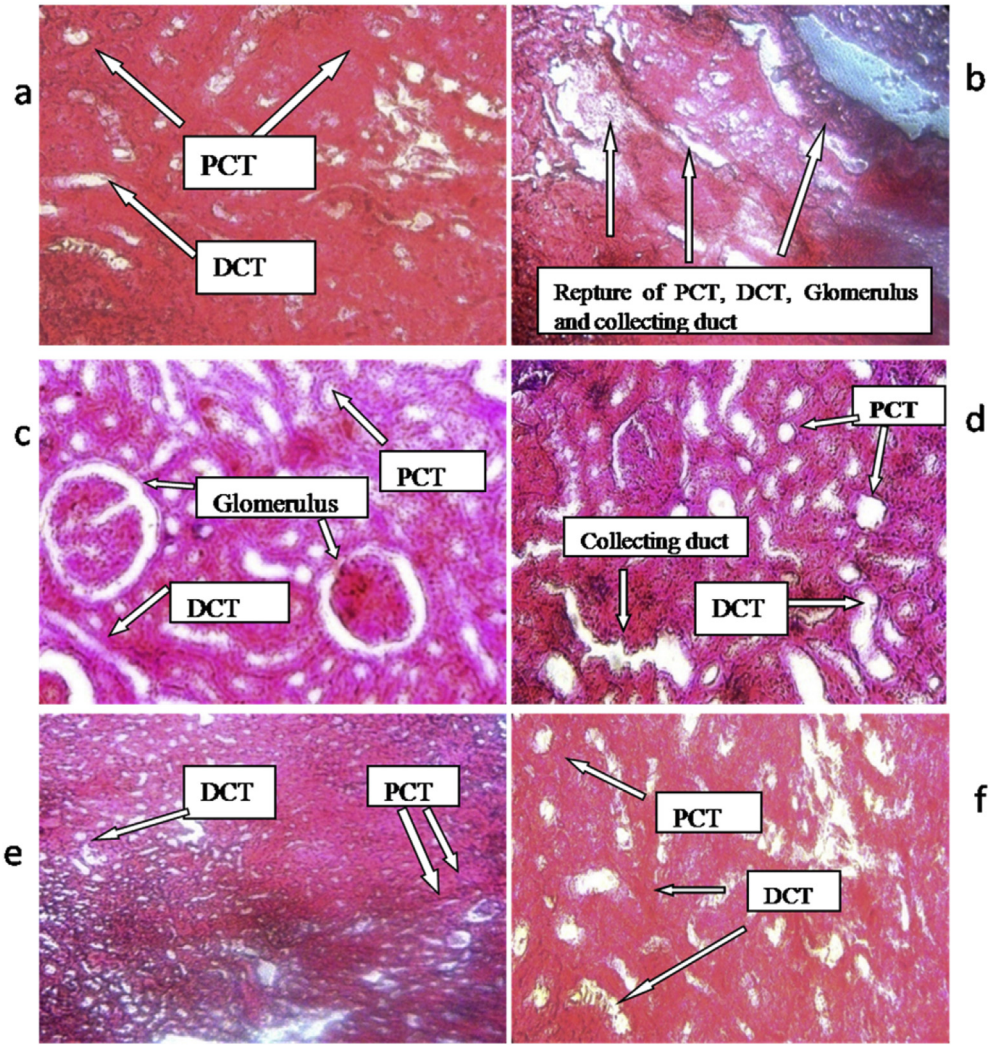

Figure 1. Histopathological results.
a. Sham-treated group shown clear structure of glomerulus PCT and DCT of nephron; b. CP-treated group shown rupture of nephrons; c. MEMK-100 mg/kg treatment group shown recovered clear structure of glomerulus, PCT, and DCT of nephron; d. MEMK-200 mg/kg treatment group shown recovered clear structure Collecting duct, PCT and DCT of nephron; e. AEMK-100 mg/kg treatment group shown recovered clear structure PCT and DCT of nephron; f. AEMK-200 mg/kg treatment group shown recovered clear structure PCT and DCT of the nephron.

4. Discussion

The leaf extracts of *M. koenigii* have high antioxidant activities [20]. The present investigation was carried on MEMK for determining its antibacterial activity, antioxidant capacities and phytochemical screening [21].

Phytochemical investigation of MEMK leaves showed the presence of flavonoids, glycoside, alkaloids, proteins, steroids and aqueous extract showed the presence of alkaloids, flavonoids, glycoside, carbohydrates, tannins and proteins. The leaves of *M. koenigii* are reported to contain flavonoids, alkaloids, carbohydrates, tannin, glycoside, protein and steroids by previous researchers [22,23].

The urotoxicity may cause dose-limiting side-effects, for example, haemorrhagic cystitis [24]. The rats when given a particular dose of cyclophosphamide, intraperitoneally (150 mg/kg) for 7 days induce nephrotoxicity [25]. Cyclophosphamide leads to the toxicity of renal cells because of its toxic metabolites. The two active metabolites of cyclophosphamide are phosphoramide mustard and acrolein (ACR). The antineoplastic effects of cyclophosphamide are associated with PAM and ACR and are responsible for its toxic side effects. ACR causes cellular damage after binding with GSH and reduces its level in the cell. It impairs the GSH dependent antioxidant system and increases free radical generation. ACR interferes with the tissue antioxidant defense system and results in necrosis of tubular epithelial cells [26,27].

Herbal antioxidant agents detoxify the toxic effect of ACR. BUN and Cr are two of the conventional test indices for kidney function and renal structural integrity. In our study, increased Cr and BUN level in the cyclophosphamide-treated rat showed renal toxicity. This elevation in the Cr and BUN levels could be due to the destruction generated in the kidney tubules established by the marked changes in kidney tissues in comparison with the control group. MEMK and AEMK significantly ($P < 0.01$) decreased the BUN and Cr levels in the rats.

The nephrotoxic potential of cyclophosphamide was confirmed by the increased level of kidney function marker enzymes. As extracts also possessed a good *in-vitro* antioxidant potential, level of enzymes involved in oxidative stress was also estimated. In cyclophosphamide-treated group level of SOD and GSH was significantly less ($P < 0.01$) as compared to vehicle

treated group which was a sign of oxidative stress in the kidney. LPO level was also found to be significantly high ($P < 0.01$) as compared to vehicle treated group. In the extract treated groups at 100 mg/kg and 200 mg/kg, the level of SOD, GSH was significantly ($P < 0.01$) high and LPO was significantly ($P < 0.01$) low as compared to the cyclophosphamide treated group. Thus, protective potential of MEMK and AEMK was also found to be working against oxidative stress produced by the intoxicant.

In cyclophosphamide-treated group BUN and Cr level was significantly ($P < 0.01$) high as compared to only vehicle treated group. When the animals were administered the extracts – MEMK and AEMK at the dose of 100 mg/kg and 200 mg/kg respectively, it was observed that the level of all marker enzymes were significantly less ($P < 0.01$) as compared to that of the control treated group. *M. koenigii* significantly ($P < 0.01$) decreased the BUN and Cr levels in the cyclophosphamide-treated rat. On the basis of data obtained from experiments, the nephroprotective activity of MEMK and AEMK showed positive results are supported by decreased levels of BUN, Cr and LPO. The levels of GSH and SOD were also found high as compared to cyclophosphamide treated group. This indicates that *M. koenigii* extract is potent against cyclophosphamide-induced nephrotoxicity. Thus, the results prove the traditional use of the selected drug.

Conflict of interest statement

We declare that we have no conflict of interests.

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