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Original Research Article

Experimental evaluation of beneficial potential of *Rhizophora mucronata* Lam. leaves in Cisplatin-induced nephrotoxic animal model

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Abstract

Objective: Chronic kidney disease typically evolves over many years, with a long latent period when the disease is clinically silent. Diagnosis, progression and treatment of renal impairment are based mainly on biomarkers that assess kidney function. There are a number of drugs used in the therapeutics which possesses nephrotoxic properties. But till date there is no nephroprotective drug available in therapeutics. In recent years the popularity of the herbal drugs is increased for their safety, efficacy and cost effectiveness. They have also gain importance in the drug development research. Present study has been focused to evaluate the preventive effect of the ethanolic extract of Sunderban mangrove *Rhizophora mucronata* Lam. leaves (RME) against Cisplatin induced nephrotoxicity in experimental animal model.

Methods: *In-vivo* nephrotoxic model was developed using a single dose of Cisplatin (5mg/kg i.p.) in rats. The extract (RME) was administered in different doses orally for consecutive 14 days and different biochemical parameters were evaluated at the end of the study.

Results: The extract (RME) significantly reduced the urine output, kidney weight, urinary microprotein, microalbumin, elevated serum urea, creatinine levels than the nephrotoxic control rats. RME at the doses 200mg/kg and 400mg/kg body weight orally had significant nephroprotective activity.

Conclusion: Present study results indicated the protective effect of the ethanolic extract of the mangrove *Rhizophora mucronata* Lam. leaves in Cisplatin induced nephrotoxic model in rats. The amelioration of the nephrotoxicity might be mediated by the secondary metabolites of the plant extract, possessing significant antioxidant property.

Keywords: Cisplatin, nephrotoxicity, mangrove, Rhizophora, antioxidant.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum II) is a very popular anticancer drug but has a number of therapeutic limitations due to its toxicity, specifically injury in the proximal tubule of kidney.[1] Kidney diseases including acute renal failure, obstructive nephropathy and glomerular damage lead to severe morbidity and mortality worldwide.[2,3] It is difficult to study kidney ailments in animal models. Acute renal failure model can be developed *in-vivo* by induction of nephrotoxicity with Cisplatin. Apoptosis can be induced by Cisplatin, producing acute tubular necrosis in the renal tubular cells in rats. [4,5] Till date there is no renoprotective agents available in the therapeutics, in spite of long research.

Rhizophora mucronata is widely distributed mangrove worldwide including Sunderban region, which have traditional therapeutic use. Several traditional applications and ethnopharamcological research pointed out the potential medicinal activity of different metabolites present in the plant.[6,7] Different parts of varieties of mangrove have shown bioactive potentials in research. This

plant has been used for treating diabetes, diarrhoea, hepatitis, inflammation, wounds, ulcers etc. in South Asian countries including India. Studies revealed the potential antioxidant, anti-diabetic and anti-inflammatory action of the leaves of *Rhizohora mucronata* mangrove. [8-10] There is no study still now on this mangrove plant in the management of renal impairment. Present study evaluated the preventive effect of the ethanolic extract of Sunderban mangrove *Rhizophora mucronata* Lam. leaves (RME) in Cisplatin induced neohrotoxic model in rats.

2. Materials and methods

2.1 Collection and preparation of Test Sample

The *Rhizophora mucronata* leaves were collected from Sunderban mangrove, West Bengal, India and the leaves were identified by Botanical Survey of India, Howrah, West Bengal as *Rhizophora mucronata* Lam. (CNH/55/2013/Tech.II/19 dated 02.12.2013). Leaves were shed dried in room temperature, pulverized with grinder and extracted with 50% aqueous-ethanol in Soxhlet apparatus. Thereafter the extract was dried by solvent evaporation in room temperature.

2.2 Experimental Animals

The study was approved by the Institutional Animal Ethics Committee of R. G. Kar Medical College, Kolkata (RKC/IAEC/13/17/1). *Wistar albino* rats both male and female, 150-200gm body weight were used for this study. The animals were kept in the Institutional animal house, maintaining proper condition, diet and water *ad libitum*. The animal experiments were conducted in accordance with the accepted principles for laboratory animal use and care by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.3 *In-vivo* study in Cisplatin induced nephrotoxic model

In-vivo protective effect of the *Rhizophora mucronata* leaf ethanolic extract (RME) was evaluated against Cisplatin induced acute nephrotoxic model.[11,12] Previous research indicated the extract is safe upto 2gm/kg body weight in rats.[10] The experimental animals were divided into five groups (n = 6/group) and treated as follows.

Group I : Normal control (without Cisplatin, distilled water 0.1ml/kg, orally for 14 consecutive days)

Group II : Nephrotoxic control (Cisplatin 5mg/kg intraperitoneally on day 1, distilled water 0.1ml/kg, orally for 14 consecutive days)

Group III : Cisplatin induced 5mg/kg intraperitoneally on day 1 and treated with RME (100 mg/kg, orally for 14 consecutive days)

Group IV : Cisplatin induced 5mg/kg intraperitoneally on day 1 and treated with RME (200 mg/kg, orally for 14 consecutive days)

Group V : Cisplatin induced 5mg/kg intraperitoneally on day 1 and treated with RME (400 mg/kg, orally for 14 consecutive days)

As there is no standard drug available for prevention and therapy of nephrotoxicity, no comparable standard group was included in the study. On day 14, the animals were kept in metabolic cage for 24hrs and urine samples of the respective groups were collected and urine output volumes were noted. On the 15th day, all animals were euthanized and the kidney was removed and weighed. The relative kidney weight was calculated by dividing the sum of the both kidney weight by body weight and then multiplying it by 100. After end of the study blood was collected from the retro orbital plexus of the rats. Serum was separated from the collected blood and stored for biochemical estimation. The renal function markers including total protein, urea, creatinine in serum of the different group of rats were measured using standard diagnostic kits.

2.4 Statistical analysis

Study data were expressed as mean ± SEM. The statistical analysis was done by one-way analysis of variance (ANOVA), followed by Dunnet test for post-hoc analysis using 5% level of significance ($p<0.05$). The statistical software package used for analysis was statistical package for the social sciences (SPSS 15).

3. Results

Table 1: Estimation of volume of urine and kidney weights of different group of rats

Parameters	Groups				
	Normal control	Cisplatin control	RME 100mg/kg	RME 200mg/kg	RME 400mg/kg
Urine volume (ml)	6.5 ± 0.045	12.5 ± 0.033	5 ± 0	4 ± 0.05 *	4.5 ± 0.013 *
Kidney weight (mg/gm body weight)	0.0062 ± 0.239	0.0076 ± 0.335	0.0066 ± 0.265 *	0.0061 ± 0.12 *	0.0059 ± 0.164 *

Values were mean ± SEM (n=6). Statistical analysis were done using one way ANOVA followed by Dunnet test; *p<0.05. RME- ethanolic extract of *Rhizophora mucronata* leaves

Present study revealed that Cisplatin (5mg/kg) caused significant increase in urinary output than the normal control. Treatment with RME in different doses for consecutive 14days caused reduction in urine output, which was near normal (Table 1). The relative kidney weights in the Cisplatin induced nephrotoxic control group of rats were significantly increased, compared to the normal control groups ($p<0.05$). Treatment with RME in different doses decreased the kidney weight (Table 1). After Cisplatin induction in all animals, reduction in body weight was observed in all groups. However the extract treatment prevented the weight loss and restored the body weight.

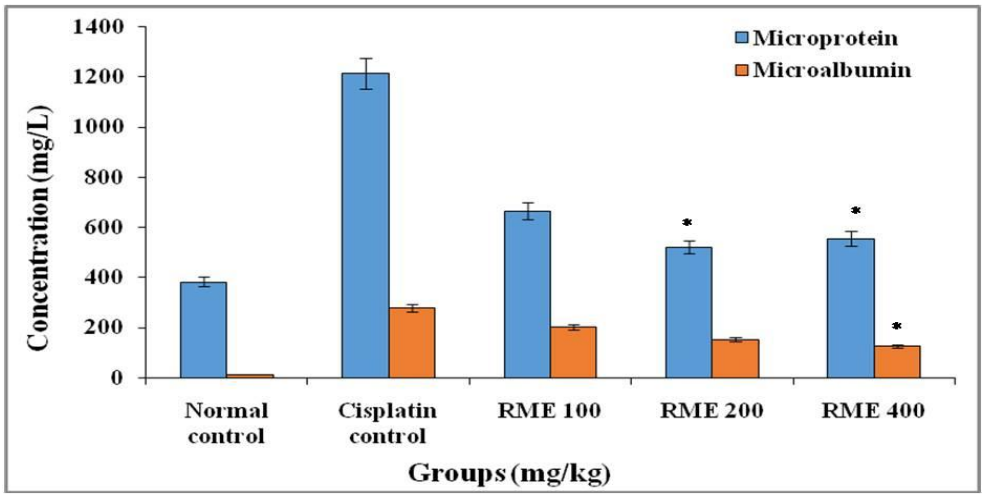

Figure 1: Microprotein and microalbumin concentration in urine samples of different groups of rats.

Data are mean ± S.E.M. Statistical analysis was done by one way ANOVA followed by Dunnet Test.
* denotes significance, p<0.05

Acute nephrotoxicity caused significant elevated level in urinary microprotein and albumin in Cisplatin induced control groups of rats compared to the normal control. The treatment with RME significantly reduced the urinary microalbumin and microprotein level (Figure 1).

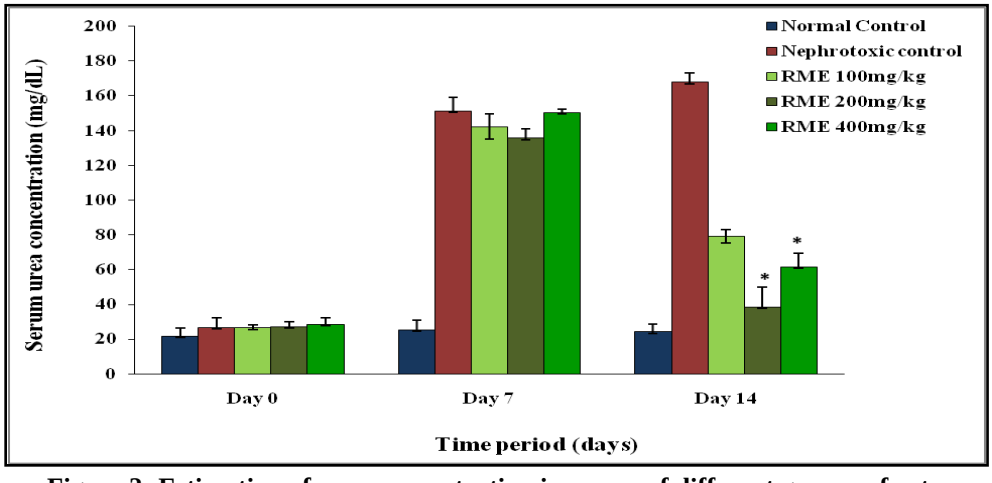

Figure 2: Estimation of urea concentration in serum of different groups of rats.

Values are mean ± SEM (n=6 per group). Statistical analysis was done by One way ANOVA followed by Dunnet test.
* denotes significant change with respect to Cisplatin induced nephrotoxic control p<0.05

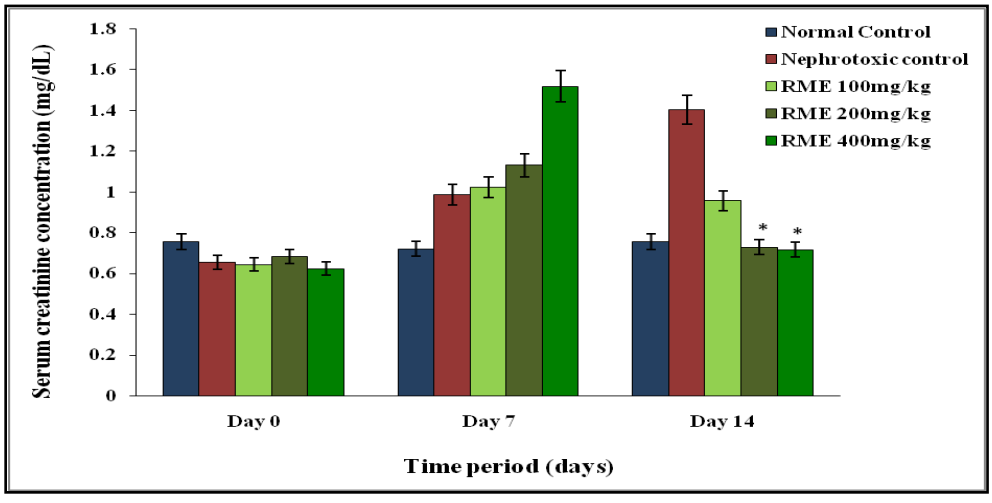

Figure 3: Estimation of creatinine concentration in serum of different groups of rats.

Values are mean ± SEM (n=6 per group). Statistical analysis was done by One way ANOVA followed by Dunnet test.
* denotes significant change with respect to Cisplatin induced nephrotoxic control p<0.05.

In the present investigation, it has been observed that the Cisplatin induction in rats resulted in a significant increase in serum urea and creatinine levels compared to the healthy normal control rat group. Figure 2 and 3 showed the increase in serum urea and creatinine levels respectively on 7th and 14th day of Cisplatin induction in different groups of rats. The consecutive treatment with the ethanolic extract of *Rhizophora mucronata* leaves (RME) for 14 days significantly lowered the elevated serum urea, creatinine levels in compared to the nephrotoxic control group.

4. Discussion

Cisplatin being one of the most potent anticancer drugs selectively accumulates in the kidney in higher amount than in other organs. Research proved that in animals a single dose of Cisplatin injection can develop nephrotoxicity, which can lead to acute renal failure. Therefore nephroprotective effect of any compound can be evaluated using this experimental animal model. [13,14] Some terrestrial plants have been evaluated for their renoprotective activities. *Morus alba* (white mulberry) leaves were found to be effective against Cisplatin induced nephrotoxicity. The flavonoids present in this might be responsible for the protective effect.[14] Another popular plant is *Azadirachta indica* (neem), leaves of which have potential nephroprotective effect.[15] Very few studies have been done for evaluation of nephroprotective effect in the marine halophytes. Recent research indicated protective effect of hydroalcoholic leaf extract of the mangrove *Avicennia marina* against carbon tetrachloride induced renal toxicity.[16] Earlier studies revealed that *R. mucronata* leaves fresh juice as well as ethanolic extract content rich amount of phenolic acids and flavonoids.[10,17] Present study evaluated the effect of the 50% ethanolic extract of the mangrove leaves against Cisplatin induced nephrotoxicity in experimental animal model.

Present study revealed that a single injection of Cisplatin in 5mg/kg body weight induced nephrotoxicity in rats. Kidney dysfunction in Cisplatin induced rats caused gradual decrease in body weights. It has been also observed that Cisplatin caused an increased volume of urine with loss of protein.

Excretion of protein in urine, which is called proteinuria, results in nephrological disorders. Estimation of urinary microprotein and microalbumin contents are important biomarkers for the determination of nephrological status.[18] These are useful marker of acute kidney injury and concomitant proximal tubular cell damage. Microalbuminuria can be defined as albumin excretion through urine, which is an indicator of progressive renal disease. Structural changes in the glomerular basement membrane (podocytes) affect the intra glomerular pressure, which also alters the glomerular filtration. This may be assumed as a cause of microproteinuria and microalbuminuria. Albuminuria may also occur in normal glomerular filter, due to the failure of the proximal tubular reabsorption function.[18,19] In the present study, the urinary microprotein and microalbumin contents were significantly elevated in the Cisplatin induced nephrotoxic control group of rats than the normal rats at the end of the study period. These levels were reduced significantly after administration of RME in different doses for 14days. The extract in 200mg/kg and 400mg/kg doses were found to be effective.

Cisplatin toxicity causes destruction of proximal and distal tubules, suppresses the tubular reabsorption and increases vascular resistance. As a result, significant elevation in renal markers like serum urea and creatinine levels was observed after Cisplatin induction. [12,14,20] On day 7 of the Cisplatin induction, the marked elevations in the renal markers than the basal level were observed in Cisplatin induced groups. Daily administration of the ethanolic extract of *Rhizophora mucronata* leaves in different doses reduced the levels significantly than the nephrotoxic control rats. On day 14, after end of the study, renal markers decreased significantly near normal in the RME treated groups than the Cisplatin induced nephrotoxic control group of rats. Among the extract treated groups, the 200mg/kg dose for 14 days lowered the serum level of creatinine and urea most significantly. Therefore, present study indicated that the ethanolic extract of *Rhizophora mucronata* leaves possesses renoprotective potential. Research is ongoing to elucidate the protective mechanism of action of this plant extract. The ethanolic extract *Rhizophora mucronata* leaves ameliorated Cisplatin induced nephropathy in rats, as evidenced from the change in biochemical parameters in the present study. Significant effects were observed at 200 mg/kg and 400 mg/kg doses of the extract. The mangrove *Rhizophora mucronata* is a good source of bioactive phytoconstituents including flavonoids and polyphenolic compounds, which may be responsible for its pharmacological activity. Further research is required to clarify the mechanism of action.

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