ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

NEPHROPROTECTIVE EFFECT OF *DIOSPYROS MALABARICA* (DESR.) KOSTEL AGAINST CISPLATIN INDUCED NEPHROTOXICITY IN RATS

Laxmikant Maruti Purane*[1], Suryadevara Vidyadhara[2]

[1]*Pharmacology Department, Shivnagar Vidya Prasarak Mandal's, College of Pharmacy, Malegaon Bk, Baramati, Pune, Maharashtra, India*

[2]*Pharmacology Department, Chebrolu Hanumaiah, Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, Andhra Pradesh, India*

**Corresponding author: laxmikant.anuphd@gmail.com*

ABSTRACT

The plant *Diospyros malabarica* (Desr.) Kostel are such plant, which are abundantly grown and various parts are traditionally used for treating wound, cancer, diabetes, ulcer, diarrhea, and renal diseases. Further, it was observed that there is no pharmacological standard established on fruits of *Diospyros malabarica* (Desr.) Kostel. The present investigation was designed to evaluate the ability of ethanolic extract of fruits of *Diospyros malabarica* (Desr.) Kostel to combat cisplatin (7.5 mg/kg, i. p.) induced nephrotoxicity. The protective property of ethanolic extract of fruits of *Diospyros malabarica* (Desr.) Kostel was assessed by measuring the body weight of animals before and after experiments, calculating change in body weight of animals, weight of kidney, urine output and estimating urine creatinine, serum creatinine, blood urea, blood urea nitrogen and histopathological observations of kidney. Our data showed that, cisplatin administration resulted in significant increased biochemical parameters levels. Pretreatment with ethanolic extract of fruits of *Diospyros malabarica* (Desr.) Kostel normalized the cisplatin induced biochemical parameters levels, protected the rat kidneys from cisplatin induced histopathological changes, restored body weight reduction and prevent increase in weight of kidney of animals. The ethanolic extract of fruits of *Diospyros malabarica* (Desr.) Kostel offered commendable protection against cisplatin induced nephrotoxicity and possible mechanism underlying this effect is mediated collectively through presence of polyphenols like flavonoids, tannins, higher urine output and antioxidant effect. The present study gives the idea that when we use the plant along with the cisplatin, will reduce the incidence of drug induced nephrotoxicity.

Keywords: Cisplatin, *Diospyros malabarica* (Desr.) Kostel, Nephrotoxicity, Nephroprotective effect.

1. INTRODUCTION

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals. This results on functional consequences causing glomerular or tubular dysfunction attenuation of blood pressure and/or kidney, endocrine function [1]. Approximately, 19 million adults have chronic kidney disease and an estimated 80,000 persons have chronic kidney failure diagnosed annually in India. Kidney disease is the 9th leading cause of death in India [2]. According to WHO, about 75-80 % of the world populations still rely mainly on herbal remedies; because it is safe and without any side effects [3]. Nephrotoxicity is attributed to wide spectrum of drugs like antibiotics, anticancer drugs, nonsteroidal anti-inflammatory drugs [4]. CIS is currently one of the most important chemotherapeutic agents used in the wide range of solid tumors including those of the head, neck, testis, ovary and breast. The nephrotoxicity of CIS limits the efficacy of this important anticancer drug as its pathogenesis induced acute renal failure [5]. Nephrotoxicity due to CIS is mainly due to generation of various free radicals like hydroxyl radical, superoxide radical [6]. Many plants are known to exhibit credible medicinal properties for the treatment of kidney ailments and need to be explored to identify their potential application in prevention and therapy of human ailments. DMDK, (Family - Ebenaceae) is a tree distributed throughout India. The plant used in the traditional system for various clinical conditions such as liver diseases, snake bites, diabetes, diarrhea, urinary diseases and renal stone [7-10]. The plant possesses flavonoids, tannins, terpenoides, sugars, diospyrin, napthoquinones, hydrocarbons and steroids [11]. Further, there was

report that, DMDK showed antioxidant [12], antiurolithiatic [13] and antistress effects [14]. These facts drove us to anticipate that the EEFDMDK could be useful in counteracting the free radical medicated renal injury induced by CIS. Thus, the present study was designed to assess the nephroprotective ability of EEFDMDK against CIS induced nephrotoxicity in rats.

2. MATERIAL AND METHODS

2.1. Plant material and Preparation of extract

Fruits of DMDK was collected in bulk quantities from the college campus area and authenticated by Department of Botany, S.S.M.M. Baramati, Maharashtra. The specimen of collected material was deposited in Herbarium of Department of Pharmacognosy at S.V.P.M's College of Pharmacy, Malegaon Bk, Baramati, Pune, Maharashtra (Voucher specimen number DPG:B-03/08). The fruits were shade dried separately at room temperature and powder was obtained. The powder of fruits of DMDK was subjected to successive soxhlet extraction with solvents of increased polarity. The ethanolic extract was selected for the present study. The extract was concentrated using rotary flash evaporator and stored at room temperature.

2.2. Dose and route of administration

According to earlier report [14], the EEFDMDK was found to be safe and no mortality of the animals observed. Hence 2500 mg/kg was considered as LD_{50} cut off value as per fixed dose method of CPCSEA. So, the 250 mg/kg p. o. and 500 mg/kg p. o. doses were selected for the nephroprotective effect.

2.3. Animals

Healthy, Wistar rats each weighing between 150 to 210 g were used for the study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60% humidity). They were feed with standard rat feed and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee of S.V.P. M's College of Pharmacy, Malegaon Bk II, Baramati, registered under CPCSEA, India (Registration No. 1214/ac/08/CPCSEA).

2.4. Evaluation of nephroprotective effect [15]

The wistar rats were divided in to four groups and each group contained 6 rats. Group I animals were administered normal saline 1 ml/kg, p. o. throughout the course of the experiments and served as normal control. Group II animals were administered a single dose of CIS 7.5 mg/kg, i.p. on the 5th day and served as positive control. Group III animals were administered 250 mg/kg p. o. EEFDMDK for 4 days, on 5th day single dose of CIS 7.5 mg/kg, i.p. was given, one h after CIS administration, 250 mg/kg p. o. EEFDMDK was administered to the group, and the treatment was continued for four days after 5th day. Group IV animals were administered 500 mg/kg p. o. EEFDMDK for 4 days, on 5th day single dose of CIS 7.5 mg/kg, i.p. was given, one h after CIS administration, 500 mg/kg p. o. EEFDMDK was administered to the group, and the treatment was continued for four days after 5th day. On the 9th day all group of animals were kept in separate metabolic cages for 24 h for urine collection to determine urine output and UCRE level. On the last day (10th day) CBW of animals were recorded i.e. final weight of animals (on 10thday of experiments) – initial weight of animals (on 1st day of experiments).

2.5. Histopathological Analysis

The blood sample was collected via retroorbital puncture. The serum was rapidly separated and processed for determination of SCRE, BU, BUN using commercially available kits, Span Diagnostic Ltd. Surat, India (on 10th day). The rats were killed by high dose of ether, abdomen was opened and the kidneys were removed and WK was taken (on 10th day). The kidneys were stored in 10% neutral formalin solution, fixed in bouin liquid, soaked in paraffin and section were taken using a microtome. The sections were stained with H and E and observed under a computerized microscope (100X and 400X).

2.6. Statistical analysis

The data were presented as men ± standard error of mean (SEM) and analyzed using one way analysis of variance (ANOVA) followed by Dunnett's and $P < 0.05$ was considered statically significant. Statistical Package for social Science (SPSS 20.0) version software was used for statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Effect of EEFDMK on biochemical parameters of rat

Administration of single injection of CIS at 7.5 mg/kg i. p. caused a marked reduction in renal function, as characterized by significant increase in SCRE, BU, BUN levels and WK in positive control rats; whereas such effects was reduced at 250 mg/kg and 500 mg/kg p.

o.doses of EEFDMDK as shown in (Table 1). There was found to be significant decrease in CIBW and increase in WK of animals in positive control group; whereas treatment with EEFDMDK restored BW reduction of animals and normalized the weight of kidney as shown in (Table 1). The rats treated with EEFDMDK significantly increases urine output and decreases UCRE level as compared to positive control group as shown in (Table 1).

Table 1: Effect of EEFDMK on biochemical parameters of rats

Groups	CIBW (g)	WK (g)	Urine Volume (ml)	UCRE (mg/dl)	SCRE (mg/dl)	BU (mg/dl)	BUN (mg/dl)
I	5.00±0.25	0.63±0.00	4.75±0.07	103.50±0.76	0.73±0.01	42.00±0.57	19.61±0.26
II	- 12.33±0.21	0.76±0.00	3.98±0.06	198.66±1.28	2.26±0.00	82.50±0.76	38.52±0.35
III	- 9.50±0.42[b]	0.69±0.00[b]	4.93±0.12[b]	158.83±0.47[a]	1.44±0.01[a]	63.50±0.76[a]	29.65±0.35[a]
IV	- 5.16±0.30[a]	0.67±0.00[b]	6.13±0.06[a]	110.50±0.76[a]	0.87±0.00[a]	56.33±0.49[a]	26.30±0.23[a]

Values are expressed as mean ± SEM, n=6, [c]P<0.05, [b]P<0.01, [a]P<0.001 compaired with positive control (one –way ANOVA followed by Dunnett's test).

3.2. Effect of EEFDMDK on histopathological features of rat kidney

The architecture of the kidney was significantly disturbed with CIS administration in positive control group i.e. significant GLMP, CI, TND and DT in kidney sections. The treatment with EEFDMDK showed amelioration of such effects in kidney sections induced by CIS. The results are shown in Table 2 and Fig. 1, Photomicrographs A-H.

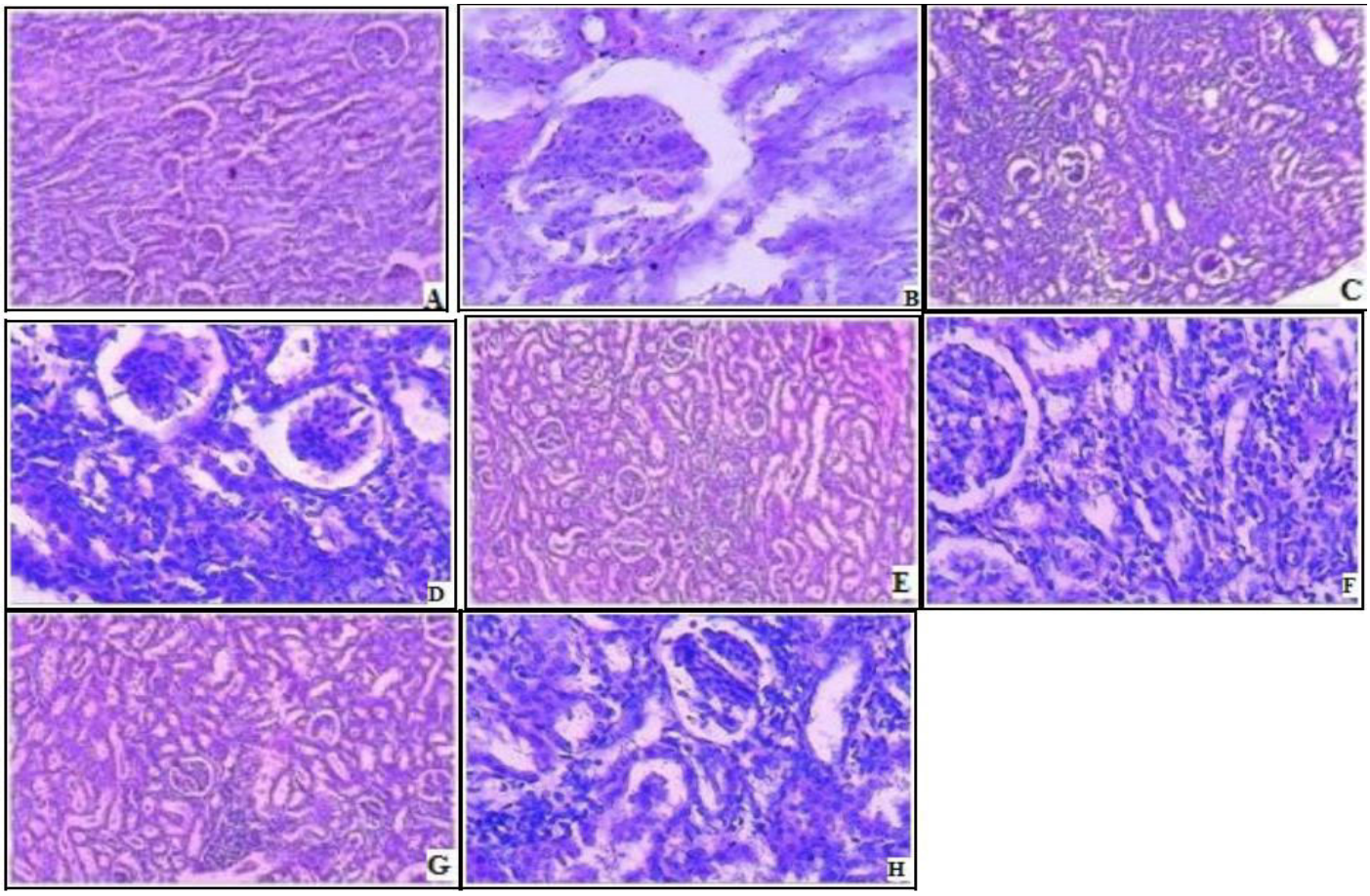
Table 2: Effect of EEFDMK on histopathological features of rat kidney

Groups	Histopathological features of kidney section			
	GLMP	CI	TND	DT
I	-	-	-	-
II	+++	++++	++++	++++
III	++	+++	++	+++
IV	+	++	+	++

Impression: - : No abnormality detected; +: damage/ active changes up to less than 25 %; ++: damage/active changes up to less than 50 %; +++: damage/ active changes up to less 75 %; ++++: damage/ active changes up to more than 75 %

CIS are an extensively used anticancer agent for the management of germ cell tumors, head and neck cancer, bladder cancer. Although higher doses of CIS are more efficacious for the suppression of cancer and high dose therapy manifests irreversible renal damage [16]. CIS inhibits antioxidant enzymes like catalase, superoxide dismutase and peroxidase in kidneys. It's directly interacted with kidney and generates superoxide anion in cell surface system. There are reports that CIS increases the activity of Ca^{++} independent nitric oxide synthase and increase the production of nitric oxide through arginine metabolism [17]. All these findings confirm that generation of oxidative stress and increased nitric oxide is due to the renal damages induced by CIS [18]. In the present study, nephrotoxicity has been induced in experimental animals by administrating CIS and identified by estimating the biochemical and histopathological features like UCRE, SCRE, BU, BUN, CIBW, WK, GLMP, CI, TND and DT. The results of present study demonstrate that, the EEFDMDK possesses potent nephroprotective effect against CIS induced nephrotoxicity model. In the present investigation CIS significantly increases such biochemical parameters levels in treated rats; whereas EEFDMDK reduces such biochemical parameters levels significantly at 250 mg/kg and 500 mg/kg p. o. ([a]P<0.001) doses. Subsequently, significant decrease in CIBW of animals and increase in WK of animals in positive control group in CIS induced nephrotoxicity model; whereas such effect of CIS was found to be significantly reduced by EEFDMK at 250 mg/kg ([b]P<0.01) and 500 mg/kg ([a]P<0.001) p. o. doses i.e. EEFDMDK restored BW reduction and prevents increase in WK of animals. Urine volume ([b]P<0.01, [a]P<0.001) was significantly increased and UCRE was significantly ([a]P<0.001) decreased in rats treated with EEFDMK at 250 mg/kg and 500 mg/kg p. o. doses and there are reports that, reduced renal damage may be related to the higher urine output [19]. Similarly, histopathological features of kidney of rats in positive control group showed significant GLMP, CI, TND and DT in kidney sections; whereas rats treated with EEFDMK at 250 mg/kg and 500 mg/kg p. o. doses restored such histological features of kidney in dose dependent manner. All these finding confirms that, EEFDMDK possesses potent

nephroprotective effect. In the present demonstration nephroprotective effect of EEFDMDK against CIS induced nephrotoxicity model is, therefore mediated by normalization of reactive oxygen species (ROS) production and inhibition of nitric oxide release and this might be due to the presence of polyphenols like flavonoids, tannins, higher urine output i.e. diuresis and antioxidant property of the DMDK. In the near future DMDK could constitute a lead to drug discovery a novel drug which will be useful in treatment of drug induced nephrotoxicity.



A: Photomicrograph of Group I rat kidney, H and E stain, 100X; B: Photomicrograph of Group I rat kidney, H and E stain, 400X; C: Photomicrograph of Group II rat kidney, H and E stain, 100X; D: Photomicrograph of Group II rat kidney, H and E stain, 400X; E: Photomicrograph of Group III rat kidney, H and E stain, 100X; F: Photomicrograph of Group III rat kidney, H and E stain, 400X; G: Photomicrograph of Group IV rat kidney, H and E stain, 100X; H: Photomicrograph of Group IV rat kidney, H and E stain, 400X.

Fig. 1: Photomicrograph of histopathological analysis of rat kidney

4. CONCLUSION

In the present investigation, it was observed that all the biochemical and physical parameters were brought back to the normal levels with the EEFDMDK treatment. The histopathological reports of kidney sections concluded that the EEFDMDK has mark improvement in the renal damage occurred due to CIS. To conclude, it was clear from the results of the investigation that the EEFDMDK offered commendable protection against CIS induced nephrotoxicity at both doses. The possible mechanism underlying this effect is mediated collectively through presence of polyphenols like flavonoids, tannins, higher urine output and antioxidant effect of the DMDK. The present study gives the idea that when we use the plant along with the CIS will reduce the incidence of drug induced nephrotoxicity.

Abbreviations:

DMDK: Diospyros malabarica (Desr.) Kostel, EEFDMDK: ethanolic extract of fruits of Diospyros malabarica (Desr.) Kostel, CIS: cisplatin, p. o.: orally, UCRE: urine creatinine, BW: body weight, CBW: change in body weight, SCRE: serum

creatinine, BU: blood urea, BUN: blood urea nitrogen, H: Hematoxylin, E: Eosin, WK: weight of kidney, GLMP: glomerulopathiea, CI: cellular infiltration, TND: tubular necrosis and degeneration, DT: dilation of tubules, CPCSEA: The Committee for the Purpose of Control and Supervision of Experiments on Animals, $LD_{50:}$ Lethal Dose.

5. ACKNOWLEDGEMENTS

The authors are thankful to the Dr. D. S. Suryawanshi, Omega Laboratories, Pune, Management and Principal of SVPM's College of Pharmacy, Malegaon Bk II, Baramati, Maharashtra, Department of Botany, S.S.M.M. Baramati, India, Management and Principal of Chebrolu Hanumaiah, Institute of Pharmaceutical Sciences, Chowdavaram, Guntur, 522019, A. P. India for providing research facilities to carry out the work.

Conflict of interest

The authors declare that they do not have any conflicts of interest in this research.

6. REFERENCES

1. Skinner R. *Journal of Pediatric Hematology/Oncology*, 2011; **33(2):**128-134.
2. Pryadarsini G, Kumar A, Anbu J, Anjana A, Ayyasamy S. *International Journal of Life Science and Pharma Research,* 2012; **2(4):**56-62.
3. Sharma SK, Sharma SM, Saini V, Mohapatra S. *The Global Journal of Pharmaceutical Research,* 2013; **2(1):**1608-1612.
4. Hoste EA, Kellum JA. *Current Opinion in Critical Care,* 2006; **12(6):**531-537.
5. Finley RS, Fortner CL, Grove WR. *Drug Intell Clin Pharm*, 1985; **19:**362-367.
6. Murthy RLN, Nataraj HN, Ramachendra SS. *International Research Journal of Pharmacy*, 2011; 2(9):137-142.
7. Warriers PK, Nambiar UPK, Ramankutty. Indian Medicinal Plants. Vol. II; Orient Longman Private Limited, 160 Anna Sali, Channani, 600002, 1994; 337-41.
8. Kirtikar KR, Basu BD. Indian Medicinal Plants. Vol. II; International Book distributors, Bookseller and Publisher 9/3, Rajpur Road, Dehradun, India, 1987; 1502-505.
9. Asima C, Pakrashi SC. The Teratise On Medicinal Plant. Vol. IV; National Institute of Science, Communication and information Resources, New Delhi, 1995; 68-69.
10. Nadkarni KM. The Indian Materia Medica. Vol. I; Popular Prakashan Private LTD, 35 C, Tardeo Road, Bombay, 400034, 1976; 452-453.
11. Sinha BN, Bansal SK. *Journal of Natural Remedies*, 2008; **(8):**11-17.
12. Mondal SK, Chakraborty G, Gupta M, Mazumder UK. *Indian J. Exp. Biol*, 2006; **44:**39-44.
13. Laxmikant MP, Suryadevera V. *Pharmacophore*, 2015; **6:**299-305.
14. Laxmikant MP, Suryadevera V. *International Journal of Pharmaceutical Sciences and Research,* 2016; **7(8):**3299-3305.
15. Nairuti MP, Vrushbendraswamy BM, Archanaswamy P, Ramu R. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2012; **3:**1236-1246.
16. Mohana LS, Ushakiran RT, Ashokkumar CK, Sateesh D, Prathyusha S. *Innovare Journal Of Life Science*, 2013; **1:**35-39.
17. Devi Priya S, Shyamala Devi CS. *Indian J Pharmacol*, 1999; **31:**422-426.
18. Minoru S, Naoki K, Sohachi F. *The J of Pharmacology and Experimental Therapeutics*, 2003; **305(3):**1183-1189.
19. Hans CK, Monika SK. The Benefits/Risk Ratio. A Handbook for the Rational Use of Potentially Hazardous Drugs. CRC press LCC, USA, 1999; 198.