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Research Article

Nephroprotective Activity of *Diospyros Malabarica* (Desr.) Kostel against Gentamicin induced Nephrotoxicity in Rats

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ABSTRACT

Diospyros malabarica (Desr.) Kostel (DMDK) plant is abundantly grown, and various parts are traditionally used for treating diarrhea, wound, cancer, diabetes, ulcer, and renal diseases. However, its medicinal importance is not assessed. Further, it was observed that there is no pharmacological standard established on fruits of DMDK. Therefore, the present study investigates the nephroprotective activity of DMDK fruits against the gentamicin (GEN) induced nephrotoxicity model. Ethanolic extract of fruits of DMDK (EEFDMDK) was screened for its nephroprotective activity against GEN (100 mg/kg i. p.) induced nephrotoxicity in rats by standard method. The protective property of EEFDMDK was assessed by measuring the body weight (BW) of animals before and after experiments and calculating a change in body weight (CIBW) of animals, the weight of kidney (WK), urine output, estimating biochemical parameters, and histopathological observations of a kidney. Our data showed that GEN administration resulted in significantly increased levels of biochemical parameters. Pretreatment with EEFDMDK normalized the GEN-induced increased levels of biochemical parameters. We also observed that EEFDMDK significantly protect the rat kidneys from GEN-induced histopathological changes. Similarly, EEFDMDK restored BW reduction of animals and prevented an increase in WK of animals. The possible mechanism underlying this effect is mediated collectively through the presence of polyphenols like flavonoids, tannins, and the diuretic effect of the DMDK.

Introduction

Medicinal or herbal plants are the willpower of popular medicine schemes and are highly valuable and incredible sources for the treatment of numerous deteriorating diseases in the form of medicines.[1] The term renal failure primarily denotes failure of the kidney's excretory function, leading to retention of nitrogenous waste product of metabolism in the blood. In addition, there is a failure in the regulation of fluid and electrolyte balance along with endocrine dysfunction. Renal failure is fundamentally categorized into acute and chronic renal failure (CRF). Various causes of acute renal failure like antibiotics, chemotherapeutic agents, organic solvents acetaminophen, and chronic renal failure have been attributed to hypertension, DM, and chemotherapeutic agents like cyclophosphamide vincristine and cisplatin.[2] Gentamicin is an important aminoglycoside antibiotic commonly used in treating life-threatening gram-negative infections.[3] However, its usefulness is limited by signs of nephrotoxicity, which may occur in 13–30% of patients.[4] Lipid peroxidation and generation of free radicals may occur in Gentamicin administration which is highly toxic to tissue.[5-7] In the present scenario, kidney-related different diseases like acute renal failure, chronic renal failure, nephrotic syndrome, end-stage renal disease, acute streptococcal glomerulonephritis, golmerular nephritis, and non-streptococcal glomerulonephritis are the direct causes like lymphatic nephropathy, diabetes nephropathy, and as well as infectious nephrotoxicity are the vital complication across the world.[8] Some secondary

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metabolites[9] of plants include tannins, alkaloids, flavonoids, triterpenoids, and phenol compounds. These phytoconstituents are used as natural or herbal medicine, and their extracted products are used for a long time to treat various chronic diseases in the world. Because plant products are frequently considered to be less toxic and free from side effects than synthetic products. 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 the prevention and therapy of human ailments. DMDK (Family-Ebenaceae) is a tree distributed throughout India. The plant is used in the traditional system for various clinical conditions such as liver diseases, snake bites, diabetes, diarrhea, urinary diseases, and renal stone.[10-13] The plant possesses flavonoids, tannins, terpenoids, sugars, diospyrin, naphthoquinone, hydrocarbons, and steroids.[14] Further, there were reports that DMDK showed antioxidant[15], Antistress,[16], and antiurolithiatic[17] effects. Therefore, the present study aimed to evaluate the nephroprotective ability of fruits of DMDK against GEN-induced nephrotoxicity in rats.

Materials and Methods

Preparation of Extract

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 a rotary flash evaporator and stored at room temperature.

Dose and Route of Administration

According to an earlier report,[17] the EEFDMDK was safe, and no mortality of the animals was observed. Hence 2500 mg/kg was considered as LD_{50} cut-off value as per the fixed dose method of CPCSEA. So, the 250 mg/kg p.o. and 500 mg/kg p.o. doses were selected for the nephroprotective activity.

Animals

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

Experimental Design: Evaluation of nephroprotective activity in GEN Induced Nephrotoxicity in Rats.[17,18]

Twenty-four male Wistar albino rats were assigned to four groups, and each group contains six rats. Group I was treated with normal saline 1 mL/kg, p. o. for 8 days and served as normal control. Group II was treated with 100 mg/kg/day GEN by i. p. route for 8 days and served as a positive control. Group III were treated with 250 mg/kg p.o. EEFDMDK and 100 mg/kg/day GEN by i. p. route for 8 days. Group IV was treated with 500 mg/kg p. o. EEFDMDK and 100 mg/kg/day GEN by i. p. route for 8 days. After dosing on the 8th day, rats were placed in separate metabolic cages for 24 hours for the urine collection to determine urine output and urine creatinine (UCRE) level. After 24 hours (on the 9th day), changes in body weight of animals were recorded i.e. final weight of animals (on 9th day of experiments) – initial weight of animals (on 1st day of experiments).

Histopathological Analysis

On 9th day blood sample was collected via retroorbital puncture and serum was rapidly separated and processed for determination of blood urea (BU), blood urea nitrogen (BUN) and serum creatinine (SCRE) using commercially available kits, Span Diagnostic Ltd. Surat, India. On 9th day rats were killed by a high dose of ether, the abdomen was opened, and the kidneys were removed and weighed. 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 hematoxylin (H) and eosin (E) and observed under a computerized microscope (100X and 400X).

Statistical Analysis

The data were presented as men ± standard error of the 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.

Results

Effect of EEFDMDK on Biochemical Parameters in Rats

As shown in Table 1, the CIBW of rats treated with GEN was significantly reduced compared to EEFDMDK treated rats. SCRE, BU, BUN, and WK were significantly increased in rats treated with only GEN, whereas treatment with the EEFDMDK at 250 mg/kg and 500 mg/kg p. o. doses were found to protect the rats from such effect of GEN. We also observed a significant increase in urine output and decrease in the UCRE level of rats treated with EEFDMK compared to the positive control group, as shown in Table 1.

Histopathological examination of normal control rats showed normal tubular histology in kidney sections; whereas positive control rats were found to cause significant glomerulopathiea (GLMP), cellular infiltration (CI), tubular necrosis, and degeneration (TND) and dilation of tubules (DT) in kidney sections. The treatment with

Table1: Effect of EEFDMDK on biochemical parameters in rat

Groups	*CIBW (gm)*	*WK (gm)*	*Urine Volume (mL)*	*UCRE (mg/dL)*	*SCRE (mg/dL)*	*BU (mg/dL)*	*BUN (mg/dL)*
I	4.83 ± 0.30	0.56 ± 0.00	3.68 ± 0.06	94.00 ± 0.57	0.77 ± 0.01	48.16 ± 1.01	22.499 ± 0.47
II	-15.83 ± 0.30	0.71 ± 0.00	3.28 ± 0.06	216.0 ± 0.89	1.65 ± 0.00	135.33 ± 1.62	62.80 ± 0.83
III	-12.16 ± 1.47[c]	0.65 ± 0.00[b]	4.48 ± 0.11[b]	147.0 ± 1.08[a]	1.14 ± 0.00[a]	85.16 ± 1.51[a]	39.77 ± 0.70[a]
IV	-6.16 ± 1.47[a]	58 ± 0.00[a]	5.81 ± 0.06[a]	104.0 ± 0.70[a]	0.86 ± 0.00[a]	60.50 ± 0.76[a]	28.25 ± 0.35[a]

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

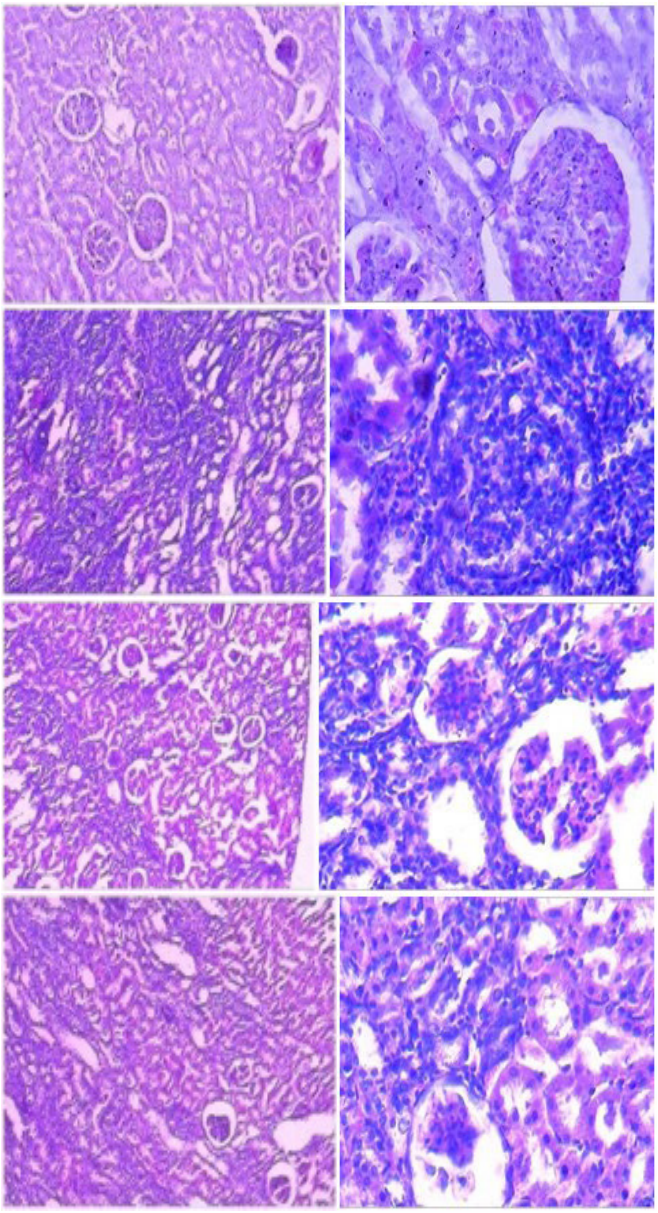

Fig. 1: Photomicrograph of Histopathological Analysis of rat kidney
A: Photomicrograph of Group I rat kidney, H and E stain, 100X;
B: Photomicrograph of Group I rat kidney, H and E stain, 400X;
C: Photomicrograph of Group II rat kidney, H and E stain, 100X;
D: Photomicrograph of Group II rat kidney, 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

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

	Histopathological features of the kidney section			
Groups	*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%

EEFDMK was found to reduce such changes in kidney histology in a dose-dependent manner. The results are shown in Table 2 and Fig. 1 (Photomicrograph A, B, C, D, E, F, G, and H).

Discussion

In the present study nephrotoxicity has been induced in experimental animals by administrating GEN, and nephrotoxicity is identified by estimating the biochemical and histopathological features like UCRE, SCRE, BU, BUN, CIBW, WK, GLMP, CI, TND, and DT. In the present study, the possible mechanism for GEN-induced nephrotoxicity may be due to inhibition of protein synthesis in a renal cell or oxidative stress generated through the induction of superoxide anions.[19] The present study demonstrates that the EEFDMDK possesses potent nephroprotective activity against the GEN-induced nephrotoxicity model. Drug-induced nephrotoxicity of many drugs mainly depends on their accumulation in the renal cortex and kinetics. The nephrotoxicity of many drugs is mainly associated with significant elevation of SCRE, UCRE, BU, and BUN. GEN significantly increases such biochemical parameters in treated rats, whereas EEFDMDK reduces such biochemical parameters significantly at 250 mg/kg and 500 mg/kg p. o. ([a]P<0.001) doses. Subsequently, a significant decrease in CIBW of animals and increase in WK of animals in the positive control group; whereas such effect 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 prevented the 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 some reports reduced renal damage may be related to the higher urine output.[20] Similarly, histopathological features of kidneys of rats in the positive control group showed significantly GLMP, CI, TND and DT in kidney section; whereas rats treated with EEFDMK at 250 mg/kg and 500 mg/kg p. o. doses restored such histological features of a kidney. All these findings confirm that EEFDMDK possesses potent nephroprotective activity against GEN model. In the present demonstration, the nephroprotective effect of EEFDMDK against the GEN-induced nephrotoxicity model is mediated by the normalization of reactive oxygen species (ROS) production and inhibition of nitric oxide release. The commendable nephroprotection offered by EEFDMDK due to the presence of polyphenols like flavonoids, tannins[21] and might be due to the diuretic effect of EEFDMDK. The present investigation gives the idea that when we use the plant along with the GEN, will reduce the incidence of drug-induced nephrotoxicity.

Conclusion

To conclude, our study has shown that the EEFDMDK possesses a significant nephroprotective effect. 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 that occurred due to GEN. The possible mechanism underlying this effect is mediated collectively through the presence of polyphenols like flavonoids, tannins, and higher urine output, i.e., the diuretic and antioxidant effect of the DMDK. In the near future, DMDK could lead to drug discovery a novel drug that will be useful in treating drug-induced nephrotoxicity.

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