EVALUATION FOR NEPHROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *DIOSPYROS MALABARICA* GENTAMICIN -INDUCED NEPHROTOXICITY IN RATS

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Abstract*- The present study reveals the acute toxicity of *Diospyros malabarica* fruit extract. No morbidity and mortality were observed at a higher dose of2000 mg/kg throughout the 14 days observation period. This acute study helps to predict that it does not contain any type of toxicityand it it full safe. Gentamicin induced nephroxicity is characterized by elevated levels of urea, creatinine, uric acid, total protein sodium and potassium in serum as well as urine urea and creatinine, severe proximal tubularnecrosis, renal failure were found to be significantly increased in rats treated with only gentamicin. Similar pattern of changes were also observed in this study following gentamicin treatment. DM supplemation to GM treated rats recorded decrement in levels of urea, creatinine, uric acid, total protein, sodium and potassium in serum and also in urine urea and creatinine. These observations indicate an improved in renal function. GM administration to control rats produced a typical pattern of nephrotoxicity which was manifestated by marked increase in serum BUN. DM supplementations to GM treated rats recorded decrement in levels of blood urea nitrogen in plasma. Histopathological results demonstrating structural changes n renal tissue of aminoglycoside antibiotics such as GM were reported by some researches. Histopathological view of renal sections in GM treated groups showed the degeneration, desquamation and necrosis in tubules, blood vessel congestion and swelling in glomerulus, as compared to control groups. Groups treated with GM + DM 200 mg/ kg showed tubular necrosis, necrotic changes, karyopicnosis, glomeruli showed mesangeal matrix expansion. Glomerular and tubular epithelial changes were considerably mild in groups treated with GM + 400 mg/kg and GM + 600 mg/kg i.e animal treated with DM 400 mg/kg showed mild glomerular mesangeal matrix expansion, mild tubular epithelial changes and no congestion inblood vessels while in case of animal treated with DM 600 mg/kg showed regeneration in tubular epithelial cells. Thus, morphological changes in kidneys were because of GM administration, but these changes tended to be mild in GM + DM treatment.

***Key words*: Nephroprotective Activity, Ethanolic Extract, Diospyros Malabarica, Gentamicin -Induced Nephrotoxicity**

INTRODUCTION

The kidneys play an important role in human physiology, maintaining fluid homeostasis, regulating blood pressure, erythrocyte production and bone density, regulating hormonal balance, and filtering and removing nitrogenous and other waste products [1,2]. Chronic kidney disease (CKD) is characterized by a progressive loss of functions while acute kidney injury (AKI) is an abrupt reduction in kidney function. Both CKD and AKI have increased worldwide and are considered one of the leading public health problems [2,3]. A projection of health concerns by 2040 ranked CKD as the fifth leading cause of death worldwide [3]. In addition, kidney diseases have been recognized as risk factors for severe forms of COVID-19 [4]. The increase of their prevalence is associated with the increase of diabetes Mellitus (DM) and hypertension as the main reported causes of kidney dysfunctions, although various factors can trigger this physiopathology. Factors are classified, based on the pathway they led to kidney damage, as pre-renal, intrinsic, and post-renal factors.

According to clinical criteria, pre-renal diseases are related to a decrease in renal perfusion or alteration in the systemic circulation, which will first compromise the glomerular filtration rate (GFR) and secondly lead to more severe alterations in the kidney structure. These dysfunctions are reflected in clinical analyses by changes in biomarker levels; for example, an increase in serum creatinine; and fluctuations in urine flow [5,6]. Several diseases have been identified as pre-renal factors such as bleeding, trauma, shock, hypertension, cirrhosis, diabetes, systemic infections, hypotension, autoimmune diseases, rhabdomyolysis, disorders of the gut microbiota, liver damage, and intravascular volume depletion [7]. The factors that directly cause kidney damage are intrinsic, whether heavy metals, trauma, Wegener's granulomatosis, proteinuria, congenital abnormalities, drug toxicity, renal atheroembolic disease, arthralgias, lupus erythematosus, or kidney cancer. Histologically, the main diagnoses are ischemic acute tubular necrosis, nephrotoxic acute tubular necrosis, and glomerulonephritis [8]. Any other health disorders which can indirectly induce kidney failures that occur after the physiological action of the kidney are post-renal diseases. Among these post-renal factors, mainly related to urinary flow disorders, are the ureter and urethra obstruction due to blood clots, lithiasis, or tumor growth, which can lead to increased pressure inside tubules, and as a consequence, the GFR is compromised, and a urinary tract infection (UTI) [9] is caused.

Kidney diseases can be addressed at several levels according to the physiological pathway of the original cause. Each pre-renal and post-renal disease currently has pharmacological treatments; however, most of the drugs used cause adverse effects and sometimes lead to intrinsic kidney damage. Among them are non-steroidal anti-inflammatory drugs (NSAID), proton pump inhibitors, antibiotics, and chemotherapy [10,11,12,13]. Nephrotoxicity of drugs administrated as treatments for pre-renal and post-renal diseases, as well as for other diseases, is now considered as a risk factor of acute and chronic kidney conditions. To avoid the adverse effects caused by medication, different alternatives have been sought to treat these pathologies.
Plants have been traditionally used as treatments for various diseases, among them several pathologies identified as pre-, intra-, and post-renal factors. The medicinal characteristics of plants have been attributed to their secondary metabolites, which can protect against pathogens or have important physiological benefits to prevent some diseases [14,15]. Plants provide a wide range of bioactive compounds which act as antioxidants, anti-inflammatory, diuretic, anticancer, and antimicrobial [16,17,18]. Further, nephroprotective agents from plants mitigate processes such as interstitial nephritis, altered intraglomerular hemodynamics, tubular necrosis, or glomerulonephritis [19]. Previous works have already reviewed the usages of plants and phytochemicals as nephroprotective agents providing an important understanding of how extracts or single compounds interfere with molecular pathways to mitigate kidney diseases [2,20]. However, they usually focused on intrinsic damage such as nephrotoxicity, omitting the pre-renal and post-renal factors, and to our knowledge, no classification of nephroprotective plants according to pre-, intra-, and post-renal diseases have been reported.

Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to drug or toxin. A number of therapeutic agents can adversely affect the kidney resulting in acute renal failure, chronic interstitial nephritis, and nephritic syndrome because increasing number of potent therapeutic drugs like aminoglycoside antibiotics, chemotherapeutic agents and NSAIDS. Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants like *Diospyros malabarica* traditionally use to protect nephrotoxicity by boiling three to four piecesof dried fruit pulp of the plants and taken it after cooling it. Thus in this study i have plan to use the dried fruit pulp of *Garcinicia pedunculata* which possess nephroprotective activity on Gentamicin induced nephrotoxicity.

MATERIAL AND METHODS

COLLECTION OF THE PLANT
The fresh and matured fruits was collected from Chittoor district, Andra Pradesh.

CHEMICAL AND REAGENTS
Matured fruits of *Diospyros malabarica*, Ethanol, Mayers reagent, Fehling's A and B solution, ferric chloride, sodium hydroxide, sulphuric acid, hydrochloride acid, mercuric chloride, nitricacid, gentamicin, (Himedia labs Pvt. Ltd.) ethyl ether and formalin.

PREPARATION OF THE EXTRACT
The freshly fruits were collected and cut it into small pieces and was dried for two weeks underthe sunlight. The dried fruit was pulverised to fine powder and 150gm was extracted with 1000ml ethanol in Soxhlet apparatus for two days. The extract was concentrated by distillationand then the solvent was evaporated to dryness on water bath.

YIELD AND COLOUR DETERMINATION
The colour of the extract was observed by naked eye. The yield of the extract was determinedusing the following formula

% yield = (weight of the extract / weight of powder taken)× 100

PRELIMINARY STUDIES
Small amount of the ethanolic extract of dried fruit pulp of *Diospyros malabarica*was investigated to find the presence of different phytochemicals. To determine the presence of phytochemicals standard methods are used.

Table I: Phytochemical Analysis

Sl.No	Phytoconstituents	Name of the test	Procedure	Inference
1	Alkaloids	Mayer'stest	1ml of extract + add 1ml of Mayer's reagent and add far drop of iodinesolution	Formation of yellow colour

2	Terpenoids	-	1ml of extract + 1mlof conc. H2SO4, water bath for 2- 4 mins	Formation of greyish colour
3	Phenol and tannins	Ferric chloride test	1ml of extract + 1mlof ferric chloride	Formation of blue green or black colour
4	Carbohydrates (sugar)	Fehling'stest	1ml of extract + 1ml of Fehling's A and B solution, water bath for 2 - 4 mins	Formation of red colour
5	Saponins	Froth formation test	1ml of extract + 1ml or 2ml of distill water, shake well	Formation of 1cm foam layer
			few drops of conc.HCL	
7	Quinines	-	1ml of extract, add 1ml of 2 % sodium hydroxide	Formation of blue green orred colour
8	Proteins	Millon'stest	1ml of extract, add far drop of mercuric acid or nitric acid	Formation of yellow colour
9	Steroids	Salkowskitest	1ml of extract, add 1ml of chloroform + 1ml of conc. H2SO4siderwise	Red colour produce at the lower chloroform layer

EXPERIMENTAL ANIMALS AND THEIR CARE

Young adult Wistar rat (120- 150 gm) of either sex and Swiss albino mice of either sex (25 -30gm) were procured from the small animals breeding station, Mannuthy, Kerala, India. The animals were housed in a polyethylene cages under standard environmental conditions (12h dark / 12H light cycles, temperature, 25^0C 35-60% humidity, air ventilation), and fed with standard rat chow and water *ad libitum.* The animals were acclimatized to the environment fortwo weeks prior to experiment use. The animal experiments were conducted according to the guidelines prescribed by Animal Welfare Board and with prior approval of animal ethical committee.

APPROVAL OF PROTOCOL

All the experimental procedures and protocols used in this study were reviewed and approvedby the Institutional Animal Ethical Committee (IAEC) of RVS College of Pharmaceutical Sciences, Sulur, Coimbatore constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests Government of India (Reg. No. 1012/PO/c/CPCSEA). Ethical guidelines were strictly followedduring all the experiments.

TOXICOLOGICAL EVALUATION

Acute toxicity studies

Acute toxicity studies was performed according to Organisation for Economic Co- operation and Development (OECD) guideline 423. Swiss albino mice of either sex were divided into three groups with six animals each. Dried fruit extract of *Diospyros malabarica* was administered orally as a single dose to mice at different dose levels of 50, 200 and 2000 mg/kg body weight. Animals were observed periodically for general behavioral changes, symptoms of toxicity and death within the first four (crucial) hours and within 24hours and then daily for14 days.

EXPERIMENTAL INDUCTION OF GENTAMICIN AND THEIR TREATMENTWITH THE EXTRACT

Thirty Wistar rats (120 - 150gm) were divided randomly into 5 groups of 6 animals each.

- **Group I** : Normal animals, orally received distill water for 10 days.
- **Group II** : gentamicin treated rats, orally received gentamicin (80 mg/kg body weight) for 10 days
- **Group III** : treated rats, orally received gentamicin (80 mg/kg body weight) and (200 mg/kg body weight) of dried fruit pulp extract of *Diospyros malabarica* for 10 days.
- **Group IV** : treated rats, orally received gentamicin (80 mg/kg body weight) and (400 mg/kg body weight) of dried fruit pulp extract of *Diospyros malabarica* for 10 days.
- **Group V** : treated rats, orally received gentamicin (80 mg/kg body weight) and (600 mg/kg body weight) of dried fruit pulp of *Diospyros malabarica* for 10 days.[2]

BIOCHEMICAL ASSAYS

Rats of each group were individually housed in metabolic cages for 24 hours and urine was collected on the 11th day after the treatment. Urea and creatinine in urine were assayed andblood samples were collected from the overnight fasted animals through retro orbital undermild ethyl ether anaesthesia. Blood samples were collected for urea, creatinine, Uric acid, Blood urea nitrogen (BUN), potassium and sodium in blood and total protein and they were collected into plain sample bottles and then the animals from every group were sacrificed. Serum and urine parameters were assayed by using various biochemical laboratory analyzing methods.

HISTOPATHOLOGICAL STUDIES OF RAT KIDNEYS

After the animals were sacrificed, the rat kidneys were identified and carefully dissected out for histopathological examination. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formalin solution, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into serial sections stained with hematoxylin - eosin and examined under light microscope with the help of a veterinary pathologist.

STATISTICAL ANALYSIS

All the results are expressed as mean SEM (standard error mean). Data obtained was analyzedby using one way ANOVAfollowed by dunnet's and $p< 0.05$ was considered as statistically significant

RESULTS

YIELD OF ETHANOLIC EXTRACT

150gm of powdered form of *Diospyros malabarica* fruits was extracted with ethanol by using Soxhlet apparatus. The extraction process was continue for 48 hours. Solvent was evaporated to get the solvent free extract. Yield of the extract was found to be 5.46 % w/w. The extract was blackish and sticky in nature.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Qualitative phytochemical analysis test was carried out using several test and results shows that ethanolic extract of *Diospyros malabarica* fruits contain alkaloids, terpenoids,carbohydrates, saponins, quinines, proteins and steroids and do not contain phenol and tannins(glycosides) and flavonoids.

ACUTE TOXICITY STUDIES

Acute toxicity studies on albino rats shows no mortality at a dose of 2000mg/kg during a timeperiod of 14 days. This acute study helps to predict that it does not contain any type of toxicityand it it full safe. Therefore, one tenth of the maximum non mortality dose were selected as therapeutic lower dose 200 mg/kg b.w, then 400 mg/kg b.w and 600 mg/kg b.w respectively, in this study.

EFFECT OF *GARCINICINIA PEDUNCULATA* EXTRACT ON PLASMA UREA,CREATININE AND URIC ACID

The effect of various doses of *Diospyros malabarica* were studied in urea, creatinine and Uric acid in gentamicin intoxicated animals. Renal injury induced by gentamicin caused significant changed in renal marker in plasma as urea by 86.39%, creatinine by 160.24% and Uric acid by 57.5% respectively compared to control group. The percentage protection in renal marker in treated groups at 200 mg/kg as urea is 34.26% ($P< 0.05$), creatinine 27.77% ($P<0.05$)and Uric acid 13.83% ($P<0.01$) then at a dose of 400 mg/kg the percentage protection for urea is 38.21% ($P<0.01$), creatinine 71.75% ($p<0.05$) and Uric acid 31.29% ($P<0.01$)respectively when compared to toxic group while maximum percentage protection in renal markers is at a dose of 600 mg/kg as urea by 51.96% ($P<0.01$), creatinine by 83.79%($P<0.01$) and Uric acid by 51.02% ($P<0.01$) respectively.(Table II).

Table II: Effect of DMon serum urea, creatinine and uric acid level against GM induced nephrotoxicity in rats (n= 6)

GROUPS	UREA (mg/dL)	CREATININE (mg/dL)	URIC ACID (mg/dL)
CONTROL(CON)	31.83 ±1.92	0.838± 0.05	2.8 ±0.14
GENTAMICIN (GM)	59.33± 1.05*	2.16± 0.06*	4.41± 0.09*
GM + DM 200	39± 0.57**	1.56 ±0.11**	3.8± 0.09***
GM + DM 400	36.33± 0.66***	0.61 ±0.06**	3.03± 0.07***
GM + DM 600	28.5± 1.56***	O.35± 0.03***	2.16± 0.06***

Values are expressed as mean ± SEM. * P<0.001 when compared with respective control groupCON. **P<0.05, *** P<0.01 were considered significant when compared with gentamicin group (GM)

EFFECT OF *DIOSPYROS MALABARICA* EXTRACT ON TOTAL PROTEIN, SODIUM AND POTASSIUM IN SERUM

The effect of various doses of *Diospyros malabarica* were studied in total protein, sodium and potassium in gentamicin intoxicated animals. Renal injury induced by gentamicin caused significant changed in serum total protein by 47.49%, sodium 21.43% and potassium by 82.8% respectively compared to control group. The percentage protection in renal marker in treated groups at 200 mg/kg as total protein 7.01% (P<0.05), sodium 6.31% (P<0.05) and potassium by 7.99% (P<0.05) then at a dose of 400 mg/kg the percentage protection for total protein is 18.38% (P<0.01), sodium by 14.23% (P<0.01) and potassium by 7.25% (P<0.05) respectivelywhen compared to toxic group while maximum percentage protection in total protein, sodiumand potassium is at a dose of 600 mg/kg as total protein 34% (P<0.01), sodium by 19.33% (P<0.01) and potassium by 53.71% (P<0.01) respectively. (Table III).

Table III: Effect of DM on total protein, sodium and potassium level in serum against GMinduced nephrotoxicity in rats (n=6)

GROUPS	TOTAL PROTEIN (gm/dL)	SODIUM (mEq/L)	POTASSIUM (mEq/L)
CONTROL (CON)	6.38 ±0.11	136.83± 1.95	3.9 ±0.13
GENTAMICIN (GM)	9.41± 0.15*	166.16 ±1.01*	7.13± 0.10*
GM + DM 200	8.75 ±0.07**	155.66 ±1.14**	6.56± 0.11**
GM + DM 400	7.68 ±0.14***	142.5± 0.76***	5.9± 0.13**
GM + DM 600	6.21± 0.08***	134.03± 1.70***	3.3± 0.17***

Values are expressed as mean. ± SEM. *P<0.001 when compared with respective control groupCON. **P<0.05, ***P<0.01 were considered significant when compared with gentamicin group(GM)

EFFECT OF *DIOSPYROS MALABARICA* EXTRACT ON SERUM BLOOD UREANITROGEN

The effect of various doses of *Diospyros malabarica* were studied on serum blood urea nitrogen in gentamicin intoxicated animals. Renal injury induced by gentamicin causedsignificant changed in BUN plasma by 97.42% compared to control group. The percentage protection in blood urea nitrogen of treated groups at 200 mg/kg as 12.51% (P>0.05) and then at a dose of 400 mg/kg as 21.71% (P<0.01) respectively when compared to toxic group while maximum percentage protection in blood urea nitrogen is at a dose of 600 mg/kg by 41.45% (P<0.01) respectively.(Table IV).

Table IV: Effect of DM on serum BUN, urine urea and urine creatinine level against GMinduced nephrotoxicity in rats (n=6)

GROUPS	SERUM BUN (mg/dl)	URINE UREA (mg/dl)	URINE CREATININE (mg/dl)
CONTROL (CON)	12.83± 0.94	62.5 ±0.76	11.58± 0.26
GENTAMICIN (GM)	25.33± 1.22*	106.83± 3.14*	31.58± 0.47*
GM + DM 200	22.16 ±0.94**	85.16 ±0.94***	27.5± 0.76**
GM + DM 400	19.83± 0.47***	79.5 ±0.76***	18.33± 0.88 ***
GM + DM 600	14.83± 0.94***	60.66± 1.66***	10.5± 0.76***

Values are expressed as mean ±SEM.*P<0.001 when compared with respective control group CON. **P<0.05, ***P<0.01 were considered significant when compared with gentamicin group (GM)

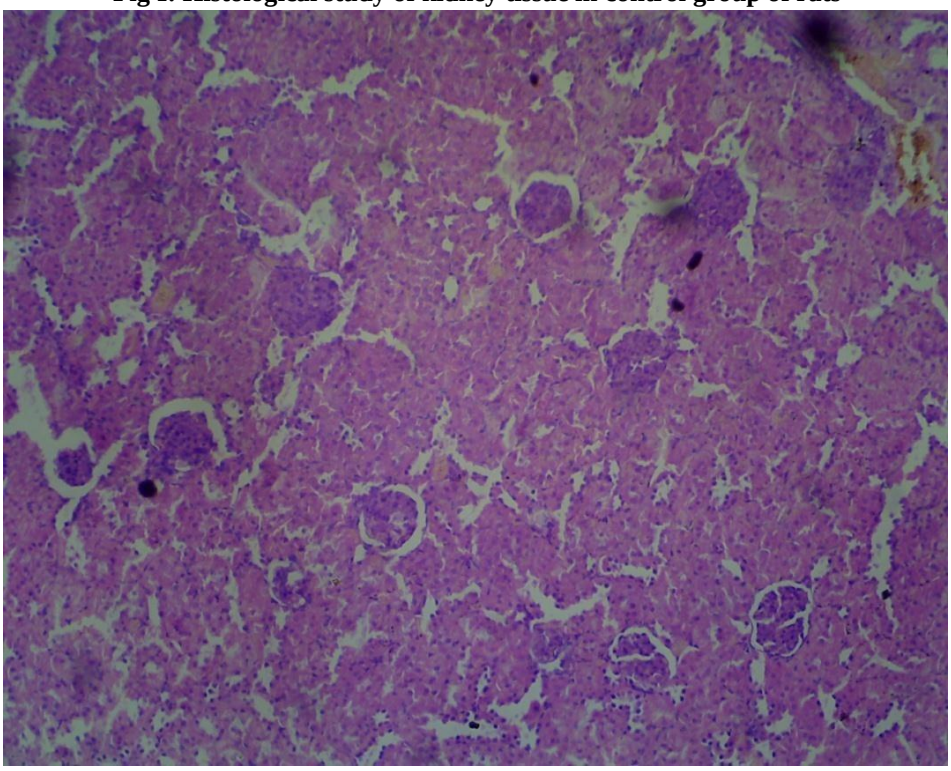
EFFECT OF *DIOSPYROS MALABARICA* EXTRACT ON URINE UREA ANDCREATININE

The effect of various doses of *Diospyros malabarica* were studied on urine urea and creatinine n gentamicin intoxicated animals. Renal injury induced by gentamicin caused significant changed in urine urea by 70.92% and creatinine by 172.71% compared to control group. The percentage protection of treated groups at 200 mg/kg as urea 20.28% (P<0.01) andcreatinine 12.91% (P<0.05) and then at a dose of 400 mg/kg the percentage protection for urea25.58% (P<0.01) and creatinine 41.95% (P<0.01)respectively when compared to toxic group while maximum protection in urine urea and creatinine is at a dose of 600 mg/kg as urea 43.21% (P<0.01) and creatinine as 66.75% (P<0.01) respectively. (Table III).

HISTOPATHOLOGICAL OBSERVATIONS

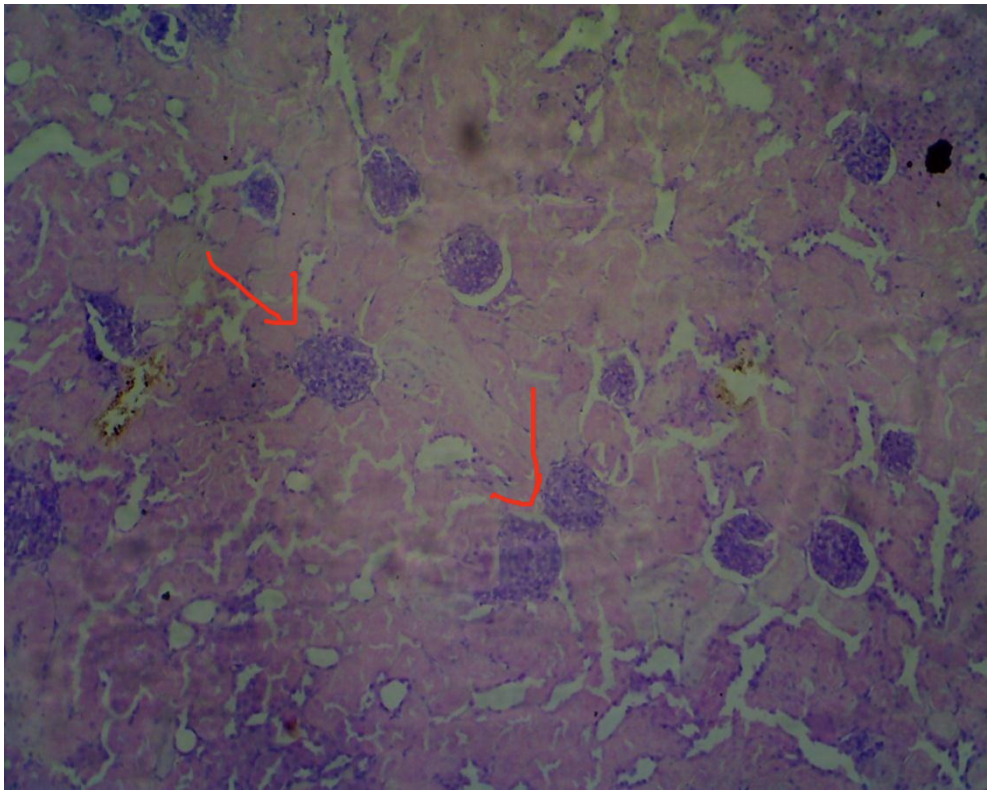
The nephrotoxicitywere confirmed by evaluating the pathological symptoms such as degeneration, desquamation, necrosis in tubules, blood vessel congestion and swelling in glomerulus. Treatment with DM extract 200, 400 and 600 mg/kg b.w ameliorated the toxicmanifestations in the kidney. The histopathological observations supported this conclusion

Fig I: Histological study of kidney tissue in control group of rats



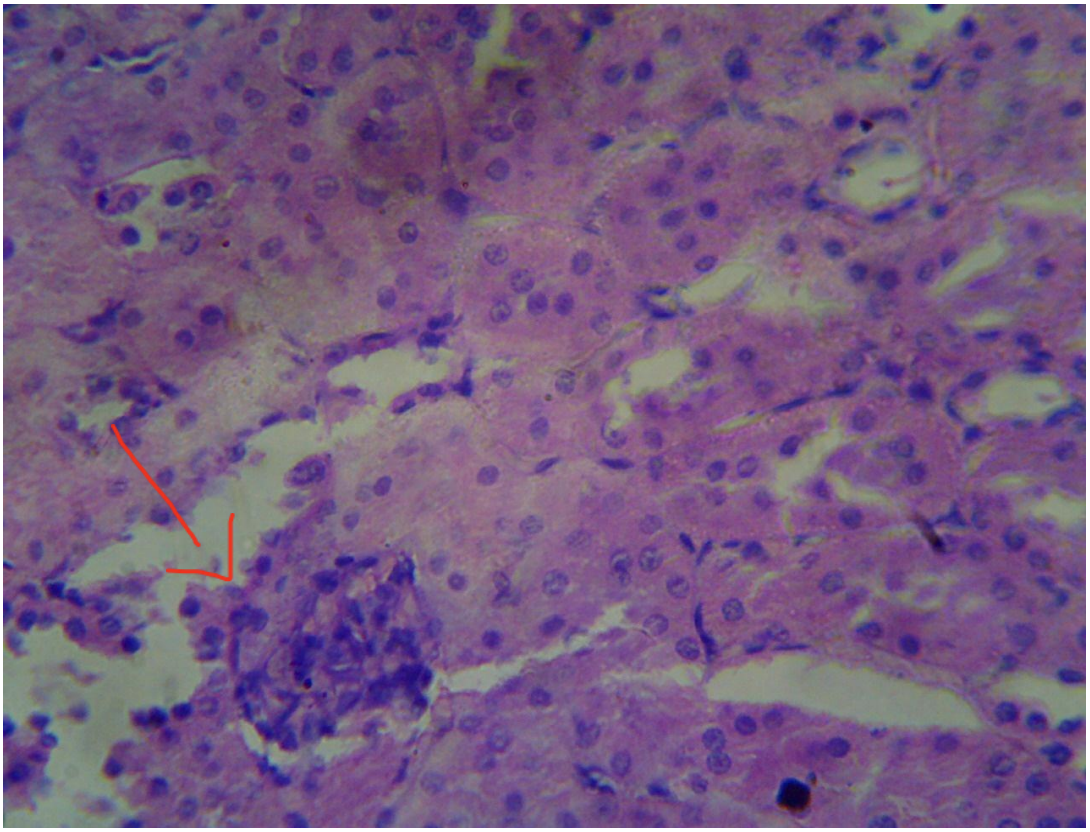
- No degeneration, necrosis, desquamation or any inflammation
- Blood vessels shows unremarkable
- Glomeruli show normal morphology

Fig II: Histopathological study of kidney tissue in gentamicin treated groups



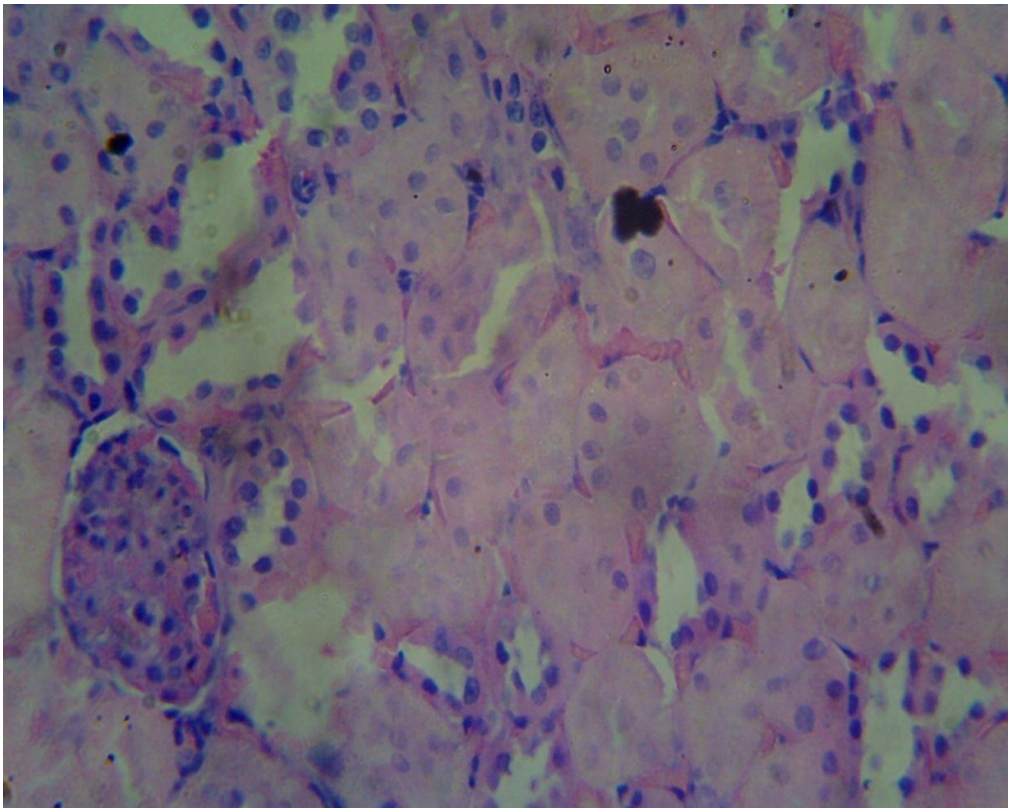
- Gentamicin treated groups showed degeneration, desquamation
- Necrosis in tubules, blood vessel show congestion
- Swelling in glomerulus (indicated by arrows)

Fig III: Histopathological study of kidney tissue in animals treated with 200mg/kgDM



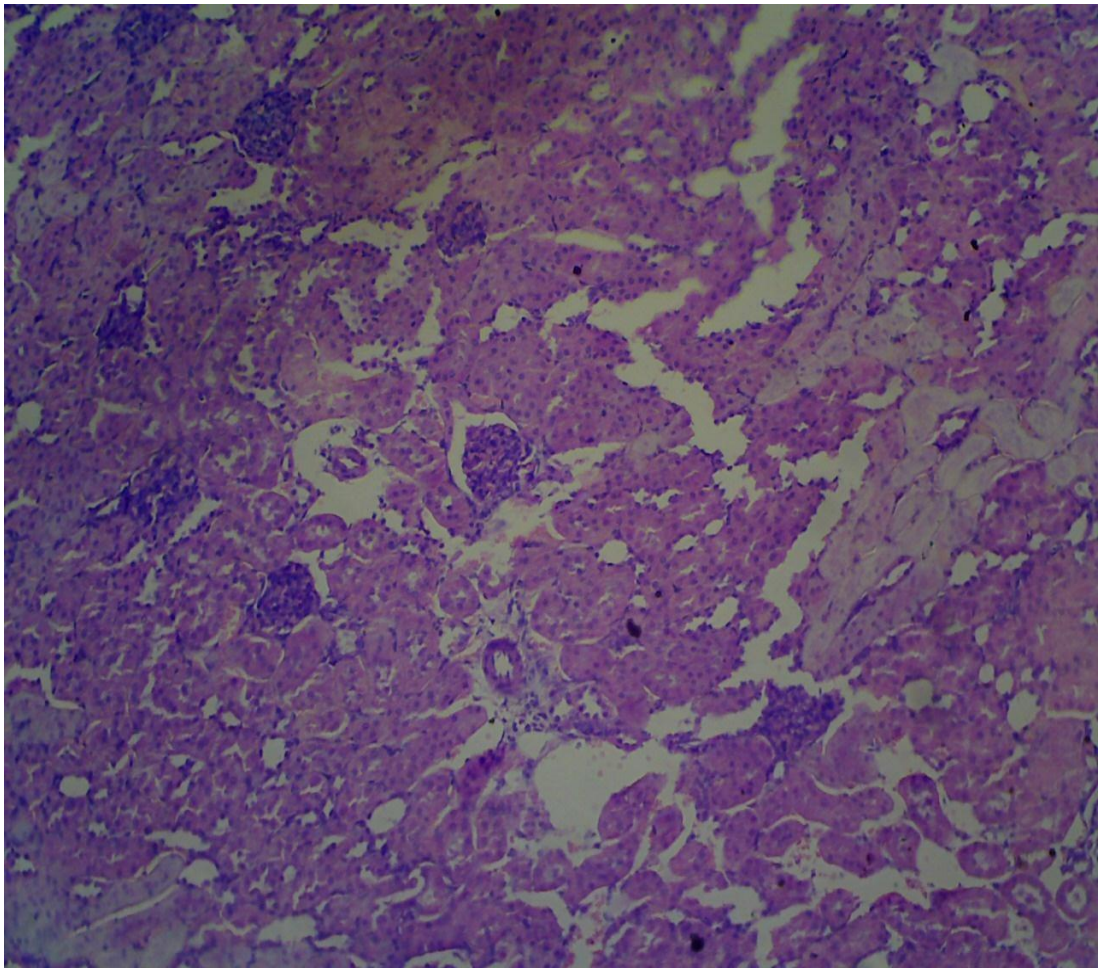
- Shows tubular degenerative and necrotic changes
- Shows karyopicnosis (indicated by arrow)
- Glomeruli shows mesangeal matrix expansion.

Fig IV: Histopathological study of kidney tissue treated with 400 mg/kg DM



- Glomeruli show mild mesangeal matrix expansion
- Blood vessel show no congestion.
- Mild tubular epithelial changes.

Fig V: Histopathological study of kidney tissue treated with 600 mg/kg DM



- Showed regeneration in tubular epithelial cells

DISCUSSION

Acute toxicity study:

Herbal products prepared from various medicinical plants have become famous in health care and obtained from natural sources.It is well known known that the herbal medicines contain more than one plant or active constituents and their therapeutic efficacy is not provided by a single group of compounds. Some of these impound act synergistically to modify the bioavailability and efficacy of active constituent. The bioactive compound from the medicinal are concluded to be safe without knowing the possible health care benefits and thus commonly used as self-medication. However, there is a defect on toxicological data of these compounds. So, acute toxicity study is required to identify the range of doses and probable clinical signs evoked by the test impound in animal under investigation. Moreover, it is a tool for calculating therapeutic index of a lead compound.

The present study reveals the acute toxicity of *Diospyros malabarica* fruit extract. No morbidity and mortality were observed at a higher dose of2000 mg/kg throughout the 14 days observation period. This acute study helps to predict that it does not contain any type of toxicityand it it full safe.

Experimental induction of gentamicin and their treatment with the extract.

Nephrotoxicity is an undesired side effect of chemotherapy in general. Most chemotherapy drugs target pathways that are essential to dividing cells . Several studies have now documented the importance of reactive oxygen metabolites in gentamicin induced renal damage. Nephrotoxicity of the drugs is usually associated with their accumulation in renal cortex, dependent upon their affinity to kidneys and on kinetics of drug trapping process. The nephrotoxicity of aminoglycoside antibiotics, and specially that of the most commonly used compound, gentamicin is well documented. Several studies have reported that oxygen free radical are considered to be important mediators of gentamicin induced renal failure.

Gentamicin induced nephroxicity is characterized by elevated levels of urea, creatinine, uric acid, total protein sodium and

potassium in serum as well as urine urea and creatinine, severe proximal tubularnecrosis, renal failure were found to be significantly increased in rats treated with only gentamicin. Similar pattern of changes were also observed in this study following gentamicin treatment. DM supplemation to GM treated rats recorded decrement in levels of urea, creatinine, uric acid, total protein, sodium and potassium in serum and also in urine urea and creatinine. These observations indicate an improved in renal function. GM administration to control rats produced a typical pattern of nephrotoxicity which was manifestated by marked increase in serum BUN. DM supplementations to GM treated rats recorded decrement in levels of blood urea nitrogen in plasma. Histopathological results demonstrating structural changes n renal tissue of aminoglycoside antibiotics such as GM were reported by some researches. Histopathological view of renal sections in GM treated groups showed the degeneration, desquamation and necrosis in tubules, blood vessel congestion and swelling in glomerulus, ascompared to control groups. Groups treated with GM + DM 200 mg/ kg showed tubular necrosis, necrotic changes, karyopicnosis, glomeruli showed mesangeal matrix expansion. Glomerular and tubular epithelial changes were considerably mild in groups treated with GM + 400 mg/kg and GM + 600 mg/kg i.e animal treated with DM 400 mg/kg showed mild glomerular mesangeal matrix expansion, mild tubular epithelial changes and no congestion inblood vessels while in case of animal treated with DM 600 mg/kg showed regeneration in tubular epithelial cells. Thus, morphological changes in kidneys were because of GM administration, but these changes tended to be mild in GM + DM treatment.

CONCLUSION

In conclusion, gentamicin treatment resulted in impairments of renal function markers, and histopathological changes in the kidneys of rats. Co-administration of GM with DM lessenedthe negative effects of GM- induced nephrotoxicity and significant decrease of all the parameters in gentamicin treated rats. The beneficial effects of *Diospyros malabarica* may be attributed to the amelioration ofrenal function. Thus the result showed that the ethanolic fruit extract of *Diospyros malabarica* offer protection against the damaging renal side effects of gentamicin. Further investigation of these promising protective effects of *Diospyros malabarica* fruit extract against gentamicin- induced renal injury may have a considerable impact on developing clinically feasible strategies to treat patients with renal failure.

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