

Nephroprotective effect of Methanolic Fraction on Bark of *Pithecellobium dulce* Roxb. in Rats

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Abstract

For centuries, herbal medicines have been used as a more vital resource for sustaining life. Kidney failure is a critical global health concern that significantly impacts the lives of millions of people worldwide. A kidney injury is an abrupt loss of renal function that causes the body to accumulate waste products like urea, creatinine and albumin. Numerous substances elicit nephrotoxic effects by means of unique morbidic defense mechanisms. Common nephrotoxicity agents include paracetamol (a non-steroidal anti-inflammatory drug), gentamicin (an aminoglycoside antibiotic) and cisplatin (a chemotherapeutic agent). The range of natural chemicals utilized to treat renal problems has increased recently. Considering the numerous studies that have been conducted on the ability of various medicinal plants to prevent drug-induced nephrotoxicity. Beta-sitosterol, flavonoids, triterpenoids, saponin glycoside and the catechol type of tannin are found in the dried bark of *Pithecellobium dulce* Roxb (*Leguminosae*). The medication is successfully assessed as having diabetes protective properties; it presumably works by scavenging free radicals and preventing the production of reactive oxygen species. The present study was aimed to evaluate the nephroprotective activity of methanolic fraction of the bark of *Pithecellobium dulce* which belong to family *Leguminosae* by using in rat model.

Keywords: *Pithecellobium dulce*, catechol, diabetes, creatinine, nephroprotective.

I. INTRODUCTION

A vital part of the natural environment are herbal plants. Following a number of surveys and investigations, numerous medicinal plants were shown to be essential sources of medication. Since prehistoric times, medicinal plants have been utilized to treat a wide range of illnesses. *Pithecellobium dulce* also referred to as Madras thorn or in Hindi Vilayati babul, Vilayati imli or Jangle jalebi is well renowned for its medicinal properties against a number of illnesses including diabetes. This plant is tiny to medium-sized, spiky, evergreen and can reach a height of 18 meters. It is grown over the plains of Mexico, India and Central America all the way to Colombia¹.

The kidney is a vital organ in the human body that supports several physiological processes, such as excretion of harmful metabolites, regulation of extracellular fluids, detoxification and homeostasis. Numerous chemicals have the potential to be nephrotoxins. Substances that cause nephrotoxicity are known as nephrotoxins. Nephrotoxicity is a fast decline in renal function brought on by the harmful effects of some drugs and substances². Nephrosis is a chronic illness caused by poisons from the environment, toxic chemicals and medications such as antibiotics and cisplatin which is used in chemotherapy. These substances can seriously damage the kidney, heart, pancreas, intestine and other internal organs³. The kidneys are essential organs in the human body that eliminate harmful substances thus they must be protected in order to maintain good health. As previously mentioned, it is important to take the incidence of kidney problems seriously. *P. dulce* (*Leguminosae*), Whereas the leaves yield dulcitol, afezilin, kaempferol, quercetin and the bark yield 37% catechol type tannins⁴. It has been discovered that the bark lowers the blood glucose levels in rats with diabetes caused by alloxan⁵. Accordingly, the present investigation was undertaken to evaluate the nephroprotective potential of methanolic fraction of *Pithecellobium dulce* Roxb. (*Leguminosae*) bark. The outcomes of this study may provide a scientific basis for the development of therapeutic agents aimed at preserving renal function an aspect of considerable importance in contemporary healthcare.

II. LITERATURE SURVEY

The kidney is a vital organ responsible for detoxification, maintenance of fluid balance, electrolyte regulation, and excretion of metabolic waste products. Impairment of renal function, either acute or chronic, constitutes a major public health concern worldwide. Chronic kidney disease (CKD) and end-stage renal disease (ESRD) are particularly alarming due to their progressive and irreversible nature⁶⁻⁷. According to the World Health Organization, nearly 80% of the population in developing countries relies on traditional medicine, primarily plant-derived formulations, for primary health care needs. Over 13,000 plant species have been studied globally for therapeutic potential, and more than 12,000 phytochemicals have been isolated, many of which exhibit promising nephroprotective properties⁸. Medicinal plants serve as a rich source of secondary metabolites with therapeutic efficacy. Ayurveda, Unani, and Siddha systems of medicine have historically utilized plants for managing disorders such as diabetes, hypertension, asthma, and hepatic dysfunction. Several herbal formulations have demonstrated nephroprotective effects, often linked to antioxidant and anti-inflammatory properties. For example, *Astragalus* and its combinations with *Angelica*, *Ligusticum*, *Triptolide*, and *Rhubarb* have been reported to slow the progression of renal failure by reducing proteinuria and correcting dyslipidaemia without significantly altering systemic blood pressure⁹.

Nephrotoxicity induced by synthetic drugs also poses a significant challenge. Aminoglycosides (e.g., gentamicin), anticancer agents (e.g., cisplatin), and analgesics (e.g., acetaminophen) are known to trigger oxidative stress, apoptosis, and tubular necrosis in renal tissues. Cisplatin, though a highly effective chemotherapeutic agent, is limited by dose-dependent nephrotoxicity mediated through reactive oxygen species (ROS) generation and mitochondrial injury. Similarly, acetaminophen overdose depletes glutathione stores, forming toxic intermediates that impair renal and hepatic function. These mechanisms highlight the urgent need for safer nephroprotective interventions¹⁰⁻¹². In Ayurveda, plants such as milk thistle, drumstick, ginger, and tamarind are recognized for their nephroprotective properties, largely attributed to bioactive constituents like flavonoids, isoflavonoids, and phenolic compounds, while *Pithecellobium dulce* has similarly been employed in the management of chronic ailments including diabetes¹³. *Pithecellobium dulce* has been reported to possess diverse pharmacological activities, including anti-diabetic, antimicrobial, antioxidant, hepatoprotective, and anti-inflammatory effects. Despite these findings, there remains a paucity of research on its potential nephroprotective properties which the present study seeks to evaluate using in vivo models

III. MATERIALS AND METHODS

Collection and authentication of plant materials:

Pithecellobium dulce bark (family: *Leguminosae*) was collected in the Chopda area of Jalgaon district between April and June. The bark was authenticated by the Maharashtra Association for the Cultivation of Science, Department of Biodiversity and Palaeobiology, Agharkar Research Institute, Pune - 411004 (Certificate number AUTH 19 – 129 and Reference No – COPS/2661/2018-19).

Drugs and chemicals

The following chemicals were used for the study, Cisplatin injection (Cismax 50 GLS Pharma limited), Gentamicin injection (Gentalab, Laborate Pharmaceutical India Ltd), Paracetamol (Paracip500, Cipla) Urea estimation kit (Yucca Diagnostics), Creatinine estimation kit (Delta Lab) and Albumin kit (Pathozyme diagnostics).

Preparation of the extract and fraction

For weeks, the barks were shade-dried. The dried barks were reduced to a powder by further chopping them into tiny pieces. Powdered *P. dulce* bark (250g) was extracted with petroleum ether at 60–80°C to defatted it, and then it was extracted with methanol using a Soxhlet extractor. A rotary flash evaporator was then used to concentrate the methanolic extract until it reached a syrupy consistency. The residual solvent was removed by drying the extract in vacuum oven (yield - 23.40gm) and stored in air-tight, amber-colored containers at room temperature for separation of fraction by using column chromatography and further experimental studies¹⁴⁻¹⁵.

Phytochemical Screening

The extract obtained was subjected to qualitative tests for identification of different phytoconstituents like alkaloids, tannins, glycosides, phenolic, flavonoids, proteins and steroids¹⁶.

Fractionation of the Methanolic fraction by column chromatography¹⁷⁻¹⁸

Using the wet slurry method, a solution of silica gel and distilled water was packed into a column measuring 2 x 25 cm. This entails making a silica gel solution in a beaker—in this case, with distilled water—and then pouring it into the column until it is around three-fourths full. The solution was stirred for dispersal and quickly added to the column before the gel settles. This method was used to prevent the trapping of air bubbles. A ball of wool was pushed into the column to settle atop the packed silica gel. A substantial amount of ethyl acetate, toluene, formic acid, and water (40:5:2.5:2.5) was poured continuously into the column and allowed to drain but prevented from reaching the cotton wool. Then 10 ml of the methanol extract of TA bark was introduced into the column. The quantity collected was poured back into the column. Fractions are eluted and collected in dry glass bottles.

Acute toxicity studies¹⁹

Acute oral toxicity studies were carried out in accordance with OECD Guideline 423. Female rats were administered methanolic fractions orally at doses of 300 mg/kg and 2000 mg/kg body weight. The animals were carefully observed at 30, 60, 120, 180 and 240 minutes post-dosing and subsequently monitored once daily for 14 days. Throughout the study period, no clinical signs of toxicity were observed, and no mortality occurred in any of the treated animals.

Pharmacological Study

Animals

Healthy female Wistar rats (180-200gm each) were used for the study. The animals were housed in polypropylene cages with stainless steel grill top and maintained under standard environmental conditions such as temperature ranging from 22 ± 3°C, relative humidity 55 ± 5% and illumination cycles set to 12 hours dark/light cycle. All the animals were fed with rodent pellet diet and water *ad libitum* under strict hygienic conditions. All procedures were performed in accordance to CPCSEA guidelines after approval from the Institutional Animal and Ethics Committee (IAEC) of the Crystal Biological solution [No. CRY/2021/2030/PO/RcBiBt/S/18/CPCSEA].

Evaluation of Nephroprotective activity

Cisplatin- induced nephrotoxicity²⁰⁻²¹

Nephrotoxicity was induced by administering a single dose of Cisplatin 5mg/kg b.w.i.p. for seven consecutive days.

Gentamicin – induced nephrotoxicity²²

Nephrotoxicity was induced by administering dose of Gentamicin 100 mg/kg b.w.i.p. for seven consecutive days.

Paracetamol- induced nephrotoxicity²³

Nephrotoxicity was induced by administering dose of paracetamol (2g/kg) per orally on 7th day.

Treatment Schedule: The female Wistar rats (180-200g) were divided into groups (n=6). The animals are given the following treatment in the study.

Group I (Normal control): The vehicle (gum acacia mucilage 2% w/v) was given to the once a day for 7 days.

Group II (Standard): Received the nephroprotective medication Lipoic acid (50 mg/kg, p.o) diluted in dimethyl sulfoxide (DMSO) for 7 days.

Group III (disease control or toxicant): A single dose of Cisplatin (5 mg/kg, b.w.i.p.) was received on day 1.

Group IV (test): 100 mg/kg b.w.p.o. of methanolic fraction once a day starting from day 1 to day 7 along with 5 mg/kg b.w.i.p. of Cisplatin on day 1.

Group V(test): 200mg/kg b.w.p.o. of methanolic fraction once a day starting from day 1 to day 7 along with 5mg/kg b.w.i.p. of Cisplatin on day 1.

Group VI (Standard): The nephroprotective drug Lipoic acid (50 mg/kg, p.o) soaked in dimethyl sulfoxide (DMSO) was given for 7 days.

Group VII (Disease control or toxicant): Gentamicin (100 mg/kg, i.p) was given to the rats in this group for seven days in a row.

Group VIII (test): Methanolic fraction (100 mg/kg b.w.p.o.) and Gentamicin (100 mg/kg i.p) were given to rats in this group for seven days in a row.

Group IX (test): Methanolic fraction (200 mg/kg b.w.p.o.) and Gentamicin (100 mg/kg i.p) were given to rats in this group for seven days in a row.

Group X (Standard): The nephroprotective drug Lipoic acid (50 mg/kg; p.o) diluted in dimethyl sulfoxide (DMSO) was given for 7 days.

Group XI (Disease Control group): Paracetamol (2 g/kg b.w.p.o.) was given to the on day 1.

Group XII (test): For seven days, rats in this group were given 100 mg/kg b.w.p.o. of the methanolic fraction of *P. dulce*. On the last day, they were given 2 g/kg b.w.p.o. of paracetamol.

Group XIII (test): Methanolic fraction of *P. dulce* (200 mg/kg b.w.p.o.) was given to the rats in this group for seven days in a row and then Paracetamol (2g/kg b.w.p.o.) was given on the last day.

Biochemical and Histopathological studies²⁴

On 7th day, blood was withdrawn from retro-orbital plexus and serum was separated. The serum samples were used for biochemical estimations like serum creatinine, urea, and albumin. All biochemical analysis was carried out using standard test kits and the measurements were performed with a Smart 5 semi-auto biochemistry analyzer. After end of the study, the animals from each group were sacrificed and kidneys were isolated. The rat kidney identified and carefully dissected for histopathological examination.

Estimation of Serum Creatinine²⁵

Creatinine levels were estimated using the standardized kits which followed the Jaffe's reaction. Creatinine reacts with alkaline picrate (in ratio of 1:1) a colored creatinine picrate complex produces reddish colour. The absorbance of the colour complex is directly proportional to creatinine concentration in sample. It was measured at 492 (480-520 nm).

Estimation of Urea²⁶

Urea reacts with Urease GLDH reagent and then urea decomposes to form ammonia, which further reduces to form NAD⁺. The rate of formation of NAD⁺ is directly proportional to the amount of urea present in the sample and it is estimated by monitoring absorbance change within fixed time at 340 nm.

Estimation of Serum Albumin²⁷

Albumin reacts with a dye bromo cresol green in a buffered medium to form a green coloured complex. Intensity of the colour formed is directly proportional to the amount of albumin present in the sample. The absorbance of sample was measured at 546 nm.

Statistical Analysis

The experimental data was obtained from the biochemical estimations were expressed as Mean $\pm$ SEM for each group. The statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Dennett's t-test. Values $p > 0.05$ were considered non-significant, $p < 0.05$ as significant, $p < 0.01$ as highly significant and $p < 0.001$ as very highly significant respectively. All experimental data was computed for statistical analysis by using Graph Pad PRISM Software.

RESULTS AND DISCUSSION

Phytochemical Screening: Phytochemical screening results showed the presence of carbohydrates, flavonoids, proteins, phenol, steroid and tannin.

Acute Toxicity Studies: Acute oral toxicity for methanolic fractions of *Pithecellobium dulce* Roxb. Bark using wistar rats, according to the OECD Guidelines, 423, was found to be in GHS Category 5, $> 2000 - 5000$ mg/kg body weight, with a LD₅₀ cut off at 5000 mg/kg body weight. Animal did not cause any mortality during the study. All the animals showed no any clinical

signs of toxicity immediately after dosing and appeared normal up to four hours and showed no clinical signs of intoxication at daily observations up to 14 days. As the oral LD₅₀ cut off value of the fraction of was found to be safe for use.

Table 1. Effect of administration of methanolic fraction of *P. dulce* bark on various biochemical parameters in Cisplatin, Gentamicin and Paracetamol induced renal damage.

Sr. no	Group	Urea (mg/ dl)	Creatinine (mg/ dl)	Albumin (g/ dl)
I	Vehicle	30.372 ± 2.278	0.608 ± 0.110	3.648 ± 0.253
II	Cisplatin + Lipoic acid (Std- 50mg/kg)	36.943 ± 2.859***	0.689 ± 0.027***	2.752 ± 0.136***
III	Disease Control- Cisplatin	57.117 ± 4.988***	1.360 ± 0.272***	2.308 ± 0.177***
IV	Protective group - Cisplatin + 100 mg/kg Fraction	52.911 ± 8.893	0.670 ± 0.093***	2.502 ± 0.208***
V	Protective group - Cisplatin + 200 mg/kg Fraction	44.054 ± 5.550**	0.606 ± 0.062***	2.907 ± 0.182***
VI	Gentamicin + Lipoic acid (Std-50mg/kg)	33.545 ± 3.079***	0.674 ± 0.033***	3.102 ± 0.232***
VII	Disease Control - Gentamicin	49.668 ± 5.857***	0.835 ± 0.119***	2.415 ± 0.213***
VIII	Protective group -Gentamicin + 100 mg/kg Fraction	40.936 ± 1.909***	0.587 ± 0.047***	2.655 ± 0.245***
IX	Protective group - Gentamicin + 200mg/kg Fraction	34.714 ± 2.430***	0.554 ± 0.035***	2.692 ± 0.142***
X	Paracetamol + Lipoic acid (50mg/kg)	34.292 ± 2.978***	0.597 ± 0.026***	2.822 ± 0.205***
XI	Disease control - Paracetamol	41.519 ± 2.353***	0.679 ± 0.031***	2.368 ± 0.263***
XII	Protective group- Paracetamol +100 mg/kg Fraction	31.805 ± 1.267***	0.561 ± 0.025***	2.372 ± 0.099
XIII	Protective group- Paracetamol + 200 mg/kg Fraction	28.407 ± 2.885***	0.532 ± 0.014***	2.635 ± 0.170***

Values are expressed as Mean ± S.E.M. (n=6). The Disease control group is compared with vehicle group and the treated groups are compared with the Disease control group by One way ANOVA test followed by Dunnette's multiple comparison test ***P<0.0001 **P< 0.001, *P<0.05.

Cisplatin (CP) Induced Nephrotoxicity

Biochemical markers including urea, serum creatinine and reducing serum albumin were used to confirm development of renal failure in the treated rats. Rats given cisplatin showed a dramatic elevation in urea (***P<0.0001) to reach 57.117 ± 4.988 mg/ dl when as compared with vehicle group (30.372 ± 2.278 mg/dl). After treatment of fraction at low dose showed there is no significantly decreased urea level (52.911 ± 8.893 mg/ml) and high dose treated showed significantly decreased urea level (44.054 ± 5.550 mg/ml) when as compared with disease control rats. Disease control group plasma creatinine levels were also considerably higher at 1.360 ± 0.272 mg/dl compared to the vehicle group. Both low-dose (100 mg/kg) and high-dose (200 mg/kg) fraction-treated rats saw a considerable reduction in plasma creatinine levels, to 0.670 ± 0.093 and 0.606 ± 0.062 mg/dl, respectively.

Cisplatin treatment plasma albumin level was 3.648 ± 0.253g/dl, in vehicle treated, 2.308 ± 0.177 g/dl in disease control, 2.502 ± 0.208 g/dl in low dose and 2.907 ± 0.182 g/dl in high dose treatment group. Administration of Standard (lipoic acid - 50mg/kg) revealed that there is significant rise in serum albumin level (2.752 ± 0.136 g/dl) when as compared with disease control group. Outcomes obtained are shown in Table 1 and Figure 1,2 & 3.

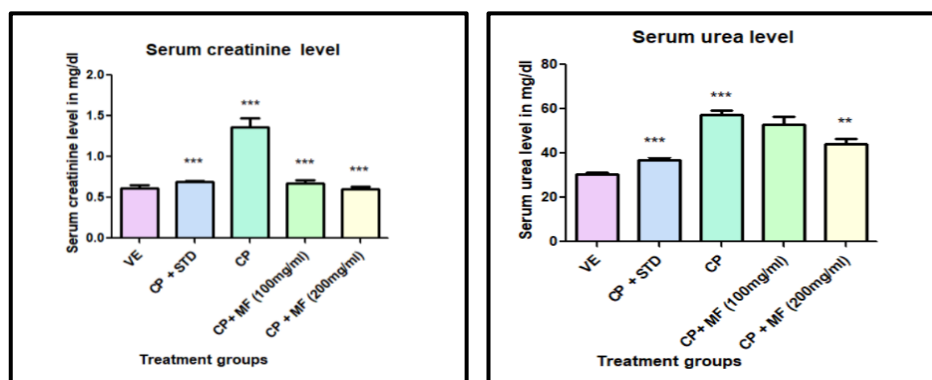


Figure 1 & 2: Effect of methanolic fraction (MEF) of *P. dulce* on serum creatinine and urea in cisplatin nephrotoxicity in rats.

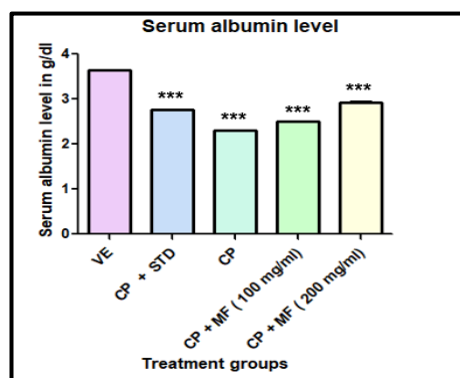


Figure 3: Effect of methanolic fraction (MEF) of *P. dulce* on serum albumin in cisplatin induced nephrotoxicity in rats.

Gentamicin (GM) Induced Nephrotoxicity

Administration of Gentamicin in dose of 100mg/kg b.w.i.p exposed that there is noteworthy rise in the serum creatinine (0.835 ± 0.119 mg/dl) and serum urea levels (49.668 ± 5.857 mg/dl) when evaluated for nephroprotective studies. After treatment with the fraction the urea and creatinine levels were reduced significantly in low and high dose fraction treated animals. In low dose treatment group urea level (40.936 ± 1.909 mg/dl) and creatinine (0.587 ± 0.047 mg/dl) were observed. In high dose treated group values for urea (34.714 ± 2.430 mg/dl) and creatinine level (0.554 ± 0.035 mg/dl) were decreased significantly as compare to disease control group. A marked reduction in serum albumin by about 2.415 ± 0.213 g/dl was observed due to administration of gentamicin (100 mg/kg). The serum albumin level (2.655 ± 0.245 & 2.692 ± 0.142 g/dl) was increased significantly ($***P < 0.0001$) in fraction treated animals when as compared with disease control group. Comparison of results in protective regimen presented in Table 1 and Figure 4, 5 & 6 respectively. It is observed that the fraction could offer nephroprotection. The effects may be due to presence of high amount of tannins in the methanolic fraction.

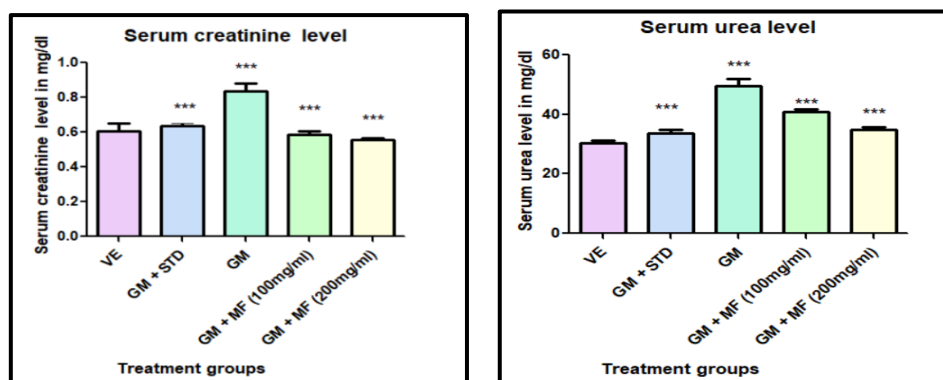


Figure 4 & 5: Effect of Methanolic Fraction (MEF) of *P. dulce* on Serum Creatinine and Urea in Gentamicin Induced Nephrotoxicity in Rats.

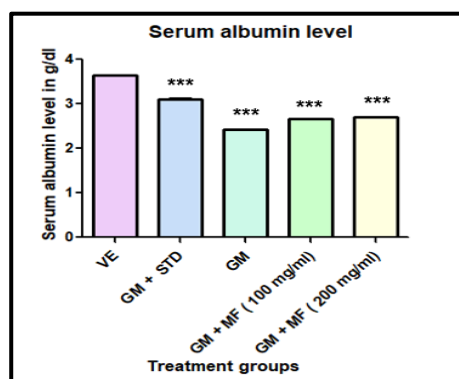


Figure 6: Effect of Methanolic Fraction (MEF) of *P. dulce* on Serum Albumin in Gentamicin Induced Nephrotoxicity in Rats.

Paracetamol (PC) Induced Nephrotoxicity

Biochemical indicators such as serum urea, serum creatinine, and serum albumin were assessed in wistar rats, where kidney damage was induced by paracetamol and the methanolic fraction of *Pithecellobium dulce* bark extract was administered at doses of 100 and 200 mg/kg. Compared to a disease control group, urea and creatinine levels dropped dramatically after treatment with the fraction. In low dose treated animals showed urea (31.805 ± 1.267 mg/dl) and creatinine (0.561 ± 0.025 mg/dl) were observed. Serum albumin level increased significantly at dose 200 mg/kg (2.635 ± 0.170 g/dl) than low dose of fraction (100 mg/kg) were observed when compared with disease control group (2.368 ± 0.263 g/dl). Whereas in high dose treated groups values for urea (28.407 ± 2.885 mg/dl) and creatinine (0.532 ± 0.014 mg/dl) were decreased as compare to disease control there is much significant difference was observed in disease and treated animals. Diseased control animals showed 41.519 ± 2.353 mg/dl urea and 0.679 ± 0.031 mg/dl creatinine and significantly decreased 2.368 ± 0.263 g/dl of albumin when as compared with vehicle group. Results obtained are shown in Table 1 and Figure 7,8 & 9.

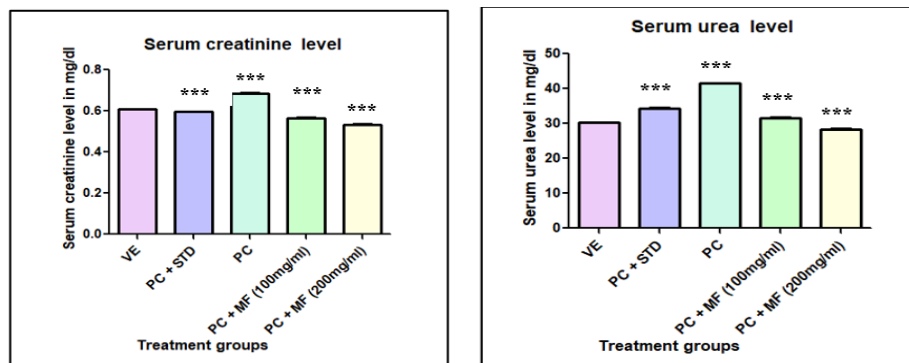


Figure 7 & 8: Effect of Methanolic Fraction (MEF) of *P. dulce* on Serum Creatinine and Urea in Paracetamol Induced Nephrotoxicity in Rats.

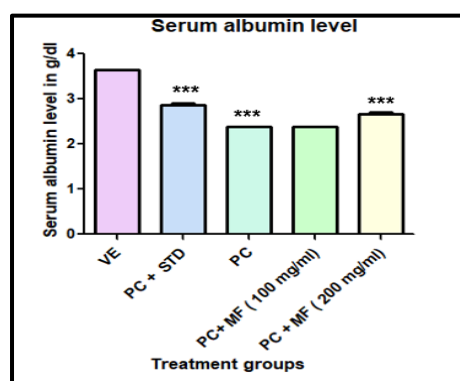


Figure 9: Effect of Methanolic Fraction (MEF) of *P. dulce* on Serum Albumin in Paracetamol Induced Nephrotoxicity in Rats.

Histopathological Examination

Kidneys from all disease-induction groups those exposed to cisplatin, gentamicin, and paracetamol were subjected to microscopic inspection, revealing a range of abnormalities. Kidney toxicity was shown to be much higher in cisplatin and gentamicin-induced diseased groups than in paracetamol-induced healthy ones. Methanolic fractions with higher concentrations were shown to have greater recovery than those with lower concentrations. Also, it was observed that moderate pathological changes were observed in Paracetamol induced animals and which was then reduced in its treatment groups. Observations are given below for all the groups. The results are represented in Figure 10 - 22.

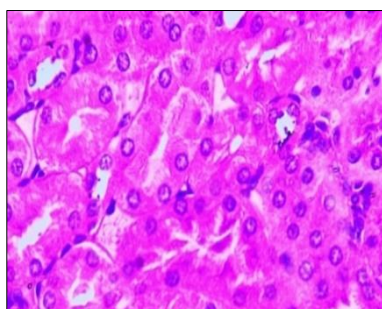


Figure 10: Group I- Vehicle showing no any pathological changes were observed in untreated animals.

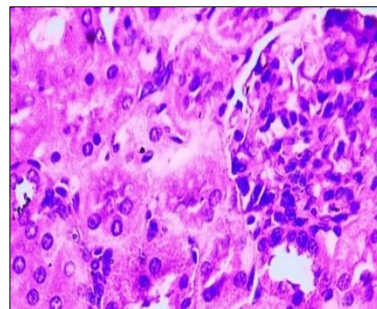


Figure 11: The group II – Lipoic acid showing no haemorrhage, no any necrotic changes, no any change in nephron and thickened basal membrane was observed.

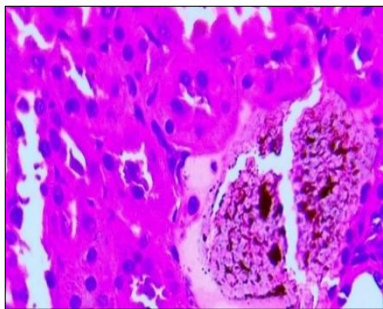


Figure 12: Group III- Disease Control- Cisplatin showing severe haemorrhage along with necrotic changes, abnormality in nephrons such as inflammation and thickened basal membrane, loss of endothelial tissue, higher glomerular fibrosis and tissue necrosis was observed.

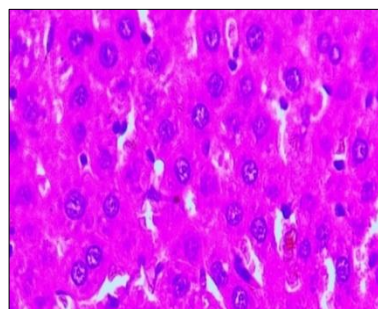


Figure 13: Group IV - Protective Group Cisplatin+ 100mg/kg Fraction showing mild haemorrhage along with inflammation and glomerular damage, thickened basal membrane was observed.

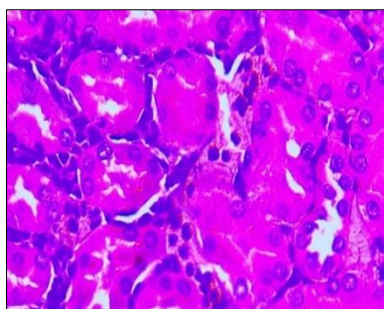


Figure 14: Group V - Protective Group Cisplatin + 200 mg/kg Fraction showing mild inflammation of nephrons along with mild necrosis in some of the animals, no damage to glomerular membrane, mild inflammation but no haemorrhage was observed.

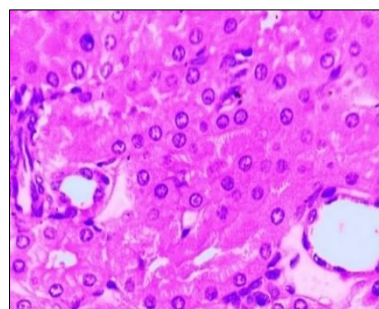


Figure 15: Group VI- Lipoic Acid showing no haemorrhage, no any necrotic changes, no any inflammation and thickened basal membrane was observed.

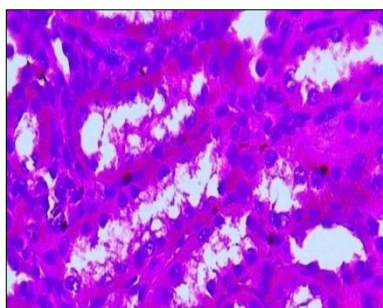


Figure 16: Group VII - Disease Control -Gentamicin severe inflammation along with moderate haemorrhage. moderate endothelial swelling and disruption, higher glomerular fibrosis and tissue necrosis was observed.

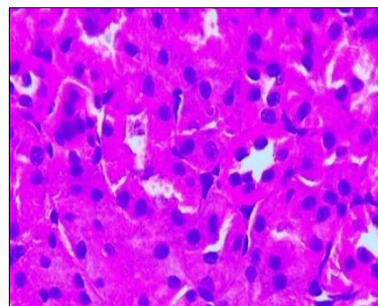


Figure 17: Group VIII - Protective Group -Gentamicin + 100 mg/kg Fraction showing mild inflammation, tissue necrosis and some endothelial loss was observed. mild fibrosis and haemorrhage were observed.

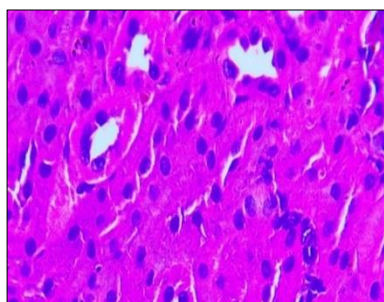


Figure 18: Group IX - Protective group - Gentamicin + 200 mg/kg Fraction showing negligible necrosis, no damage to glomerular membrane, mild inflammation but no haemorrhage was observed.

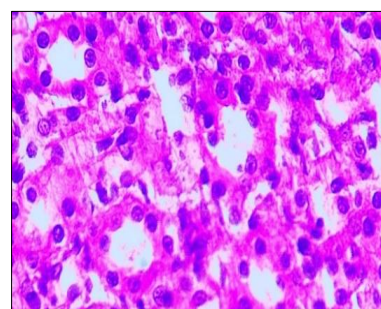


Figure 19: Group X- Lipoic Acid showing normal glomerular membrane, no any change in nephron and thickened basal membrane was observed.

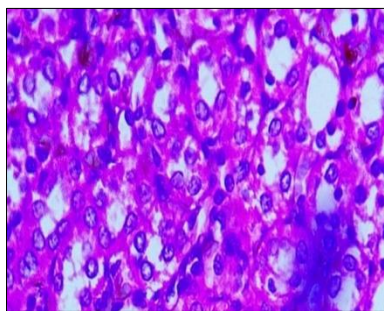


Figure 20: Group XI - Disease Control – Paracetamol showing moderate inflammation and haemorrhage observed along with loss of endothelial tissue, moderate glomerular fibrosis in some of the animals.

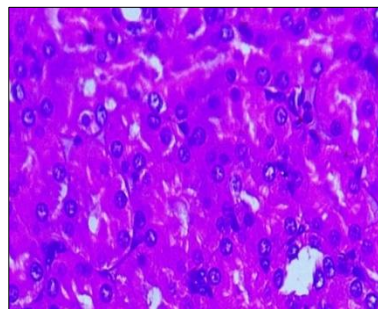


Figure 21: Group XII - Protective group-Paracetamol + 100 mg/kg Fraction showing mild hemorrhage, mild inflammation, glomerular fibrosis and tissue necrosis was observed.

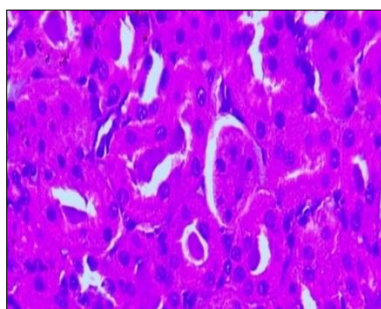


Figure 22: Group XIII - Protective Group- Paracetamol + 200 mg/kg Fraction showing no loss of endothelium but moderate tissue fibrosis in some of the animals was observed along with mild inflammation.

CONCLUSION

Pithecellobium dulce (Madras thorn) is an important Ayurvedic plant with proven nephroprotective potential. Its methanolic fraction normalized elevated serum urea, creatinine, and albumin levels in Wistar rats with cisplatin, gentamicin and paracetamol-induced nephrotoxicity. Although further toxicity studies are required for long-term use, the plant shows significant promise as a natural nephroprotective and diuretic agent, supporting its traditional use and future therapeutic potential.

FUTURE PERSPECTIVES

Further studies are needed to isolate the active compounds of *Pithecellobium dulce* bark, explore the underlying mechanisms of nephroprotection and validate its efficacy and safety through long-term toxicity studies and clinical trials.

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