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Original Article

Exploring the Nephroprotective Efficacy of *Leucaena leucocephala* Seeds in Mitigating Cisplatin-Induced Renal Damage

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ABSTRACT

Objectives: The study aimed to evaluate the nephroprotective effects of the methanolic extract of *Leucaena leucocephala* seeds against Cisplatin-Induced Nephrotoxicity (CIN) in rats through biochemical, antioxidant and histopathological assessments. **Materials and Methods:** 2 experimental models were employed, using 30 Wistar rats in each model, divided into 5 groups of 6 animals each. In Model 1, the normal control group received normal saline (10 mL/kg, p.o.), the disease control group received cisplatin (2 mg/kg, i.p.) on alternate days, the standard group was treated with Vitamin E (250 mg/kg, p.o.) and the test groups were administered *Leucaena leucocephala* methanolic extract at doses of 250 mg/kg and 500 mg/kg. Model 2 followed the same group allocation, but the disease control group received a single dose of cisplatin (8 mg/kg, i.p.). **Results:** In both models, cisplatin administration caused significant nephrotoxicity, characterized by elevated serum creatinine, urea, uric acid, Blood Urea Nitrogen (BUN) and electrolyte levels (Na^+, K^+, Cl^- and Ca^{++}). Additionally, it reduced antioxidant enzyme levels (SOD, CAT and GSH) and increased pro-inflammatory cytokines such as TNF-α. Histopathological analysis revealed marked structural damage, including tubular dilation, glomerular inflammation and disrupted renal architecture. Treatment with the methanolic extract of *Leucaena leucocephala* showed dose-dependent nephroprotective effects. Both doses (250 mg/kg and 500 mg/kg) significantly reduced renal function markers, improved antioxidant enzyme levels and decreased pro-inflammatory cytokines. Histological improvements were evident, with reduced inflammation, tubular dilation and preservation of glomerular and tubular structures, especially at the higher dose of 500 mg/kg. **Conclusion:** These findings suggest that the methanolic extract of *Leucaena leucocephala* effectively mitigates cisplatin-induced nephrotoxicity by normalizing biochemical markers, enhancing antioxidant defense and protecting renal histology. The study highlights its potential as a natural therapeutic agent for managing cisplatin-induced renal damage.

Keywords: Cisplatin, *Leucaena leucocephala*, Nephroprotective, Nephrotoxicity.

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INTRODUCTION

Nephrotoxicity refers to a medical condition in which the kidneys are damaged owing to exogenous intoxication, most commonly drugs or chemicals. Such nephrotoxic exposure can immediately cause deterioration in renal function, especially in individuals who suffer from impaired kidney function. It is estimated that up to 20% of nephrotoxicity is drug-induced and that rate tends to be greater in the elderly due to their longer life spans and use of multiple drugs. Renal tubular toxicity, glomerular damage, inflammation, crystal deposition in the kidneys and thrombotic microangiopathy are some of the key mechanisms involved in nephrotoxicity. These processes can lead to impaired kidney function by damaging different parts of the kidneys, including the renal tubules, glomeruli and blood vessels, which can result in a variety of kidney-related complications.[1] Cisplatin is a platinum-based chemotherapy drug that was approved in 1978. It is widely used to treat various cancers, either alone or in combination with other therapies, especially in cases of more aggressive cancers like osteosarcoma and squamous cell carcinoma, as well as cancers of the breast, cervix, oesophagus, bladder, lung (small cell) and testicles. Although the precise approach of cisplatin-provoked cell death remains unclear, it may work by significantly impairing DNA repair. Cisplatin forms crosslinks between purine bases on DNA strands, disrupting the cell's ability to replicate DNA properly, halting the cell cycle and ultimately leading to cell death. Cisplatin is known

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to accumulate in kidney tissues, which is believed to be the cause of Cisplatin-Induced Acute Kidney Injury (CIAKI). This accumulation triggers the release of TNF-α and ROS, provoking inflammation, oxidative stress, vascular damage and the initiation of cell death mechanisms, all of which contribute to kidney tissue damage.[2]

Leucaena leucocephala is typically from southern Mexico and northern parts of Central America, namely Belize and Guatemala and has spread to tropical regions of the globe, including Asia.[3]

It has been confirmed by earlier studies that *Leucaena leucocephala* possesses various pharmacological activities namely anti-diabetic, anti-inflammatory, anticancer, antibacterial, anti-apoptotic and antioxidant activity.[4-6] These activities are attributed to its rich phytochemical profile, which comprises of terpenes, flavonoids, coumarins, phenols and sterols. Out of these compounds, phenols are considered to be especially essential to favour the plant's antioxidant activity that is pivotal in countering free radicals and defending cells from oxidative stress. Present investigation is based on cisplatin toxicities caused during cancer chemotherapy. Cisplatin, an extensively used chemotherapy drug, while effective in treating various cancers, has significant nephrotoxic side effects. CIN has also been known as a critical barrier in cancer therapy due to its destructive influence on renal function, by promoting oxidative stress, inflammation and apoptosis in renal cells. These toxicities result from ROS and TNF-α accumulation since they interfere with renal tubules, glomerular filtration barrier and overall renal dysfunction.[7]

The rationale for this study lies in addressing the significant challenge of Cisplatin-Induced Nephrotoxicity (CIN), a major limitation in cancer therapy caused by oxidative stress, inflammation and apoptosis. Currently, few effective nephroprotective agents are available, creating a need for safe and complementary strategies. *Leucaena leucocephala*, known for its antioxidant, anti-inflammatory and anti-apoptotic properties, holds promise in mitigating these effects due to its rich phytochemical profile. This study explores its potential to alleviate renal damage caused by cisplatin, aiming to improve chemotherapy outcomes.

Based on the earlier findings demonstrated by *Leucaena leucocephala* contains anti-inflammatory, antioxidant and anti-apoptotic properties, this current work aimed to investigate whether its methanolic seed extract can attenuate the damaging effects of cisplatin on renal tissues. Through evaluating the nephroprotective efficacy of *Leucaena leucocephala* in a CIN model in rats, this study intends to ascertain whether the plant can alleviate oxidative stress, inflammation and renal tissue damage. It has been an approach to find a compound that can alleviate the noxious effects of cisplatin and enhance the safety profile of chemotherapy drugs. This study is particularly important given that only a few nephroprotective agents are available that can be co-administered with cisplatin.

MATERIALS AND METHODS

Drugs and chemicals

Leucaena leucocephala seeds were obtained from a local seller in Bangalore, Karnataka, India. Dr. Noorunnisa Begum of the Foundation for Reservation of Local Health Traditions (FRLHT) Bengaluru India identified and authenticated the plant specimen and specimens were retained for reference under FRLHT Acc. No 5887. Cisplatin and Vitamin E were obtained from a local medical supply store in Bangalore. The chemicals used for the study were the entire highest analytical standard and created fresh on the day of the examination.

Preparation of the extract of *Leucaena leucocephala*

The seeds of *Leucaena leucocephala* were rinsed with tap water and then parched in shade at 25°C. Once dried, seeds were crushed and ground into a fine powder. This powdered material was subsequently passed through a 1 mm sieve to prepare for extraction.

Method of Extraction

A total of 50 g powder was dissolved in 500 mL of methanol and stirred at 25°C utilizing a magnetic stirrer set at 1000 rpm and the mixture was clarified through filter paper. The resulting extract was then evaporated to dryness, yielding a constant extract weight of 7.7%. The final extract was stored at refrigerator until further use.

Experimental animals

In this study, Wistar rats weighing 150-200 g was utilized. They were maintained in standard laboratory environment with access to regular feed pellets and water. Experimental techniques cohered the guidelines set forth by CPCSEA, India and received approval from the IAEC.

Dose selection for pharmacological activity

The selected doses for evaluating the nephroprotective activity of *Leucaena leucocephala* and Vitamin E were determined based on findings from acute toxicity studies available in the literature. The doses were set at 250 mg/kg and 500 mg/kg,[8] while Vitamin E was administered at 250 mg/kg.[9]

Experimental Design

2 models of CIN were employed. In Model 1, rats obtained cisplatin (2 mg/kg, i.p) on every other day (three times per week) for 10 days. The groups were: Group I: received saline (10 mL/kg/day, orally) for ten days; Group II: received cisplatin (2 mg/kg, i.p) on every other day; Group III: cisplatin and Vitamin E (250 mg/kg, orally) 2 hr after each cisplatin dose; Group IV:

treated with cisplatin and *L. leucocephala* (250 mg/kg, orally) 2 hr after each cisplatin dose; and Group V: received cisplatin plus *L. leucocephala* (500 mg/kg, orally) in the same manner. In Model 2, a single shot of cisplatin (8 mg/kg, i.p) was given on 8th day and the groups were treated for 10 days as follows: Group I obtained saline (10 mL/kg/day, orally); Group II obtained saline with a single cisplatin dose on the 8th day; Group III received Vitamin E (250 mg/kg, orally) and cisplatin on the 8th day; Group IV: *L. leucocephala* (250 mg/kg, orally) and cisplatin; and Group V: *L. leucocephala* (500 mg/kg, orally) and cisplatin.

Biochemical and Tissue (Kidney) Histology Assessments

Over the 10th day, blood samples were taken by means of cardiac puncture after anaesthetizing the animals for serum separation. Kidneys were removed, with one snap-frozen for antioxidant studies and partly retained in 10% formalin for histopathological analysis.[10,11] Renal functions were assessed and the key parameters measured included Blood Urea Nitrogen (BUN), uric acid, creatinine and urea, as well as electrolyte levels such as sodium, potassium, chloride and calcium. The study aimed to assess serum antioxidant enzymes and their effects on inflammation. Lipid peroxidation was measured using *Ohkawa et al's* method, which involved adding Thiobarbituric acid, SDS and acetic acid to a 10% tissue homogenate. Absorbance of malondialdehyde was assessed at 532 nm and indicated as nmol/mg of protein.[12] SOD activity was measured by adding adrenaline and observing shift in absorbance at 480 nm. Sucrose served as a blank and SOD was indicated in units per milligram of protein[13] and pro-inflammatory cytokines like TNF- α and IL-6 were studied by a Sandwich ELISA.[14,15] Plasma samples were collected under mild anaesthesia and protein content was normalized using the *Lowry* method. IL-6 and TNF-α concentrations were studied by antigen capture ELISA. Histopathological analysis involved preserving a small portion of each kidney in neutral buffered formalin, dehydrating it in ethanol, clearing it with xylene and embedding it in paraffin wax. Portions were cut, processed, stained with H and E and examined in a microscope for histopathological evaluation.[16]

Statistical Analysis

The outcomes were articulated as Mean±SEM, with each group consisting of 6 rats (n=6). Statistical analyses were performed using GraphPad Prism (version 10). Group differences were assessed through ANOVA, followed by Tukey's test. Statistical significance was established by comparing the untreated control group with the other groups, where p-values lower than 0.001 were regarded highly significant.

RESULTS

In Model 1 (Tables 1 and 2; Figures 1 and 2), the nephroprotective potential of the Methanolic Extract of *Leucaena leucocephala* (MeLL) was assessed against CIN by analyzing various biochemical parameters. Serum creatinine (p<0.0001), urea (p<0.0001), uric acid (p<0.0001), Blood Urea Nitrogen (BUN) (p<0.0001), sodium (p<0.01), potassium (p<0.001), chloride (p<0.0001) and calcium (p<0.0001) were all significantly raised in contrast to untreated group during cisplatin administration. Therapy involving Vitamin E (250 mg/kg) and the MeLL at 500 mg/kg significantly lowered serum creatinine (p<0.0001), urea (p<0.001), uric acid (p<0.0001), BUN (p<0.0001), sodium (p<0.001), potassium (p<0.001), chloride (p<0.0001) and calcium (p<0.0001) showing improvement against the cisplatin group. Contrarily, 250 mg/kg methanolic extract did not demonstrate a considerable variation in comparison with cisplatin control group. Additionally, cisplatin treatment resulted in increased lipid peroxidation (p<0.001, Table 2) and a decrease in the antioxidant defence system, as evidenced by lower levels of SOD (p<0.05, Table 2) correlative to untreated group. Both Vitamin E (250 mg/kg) and the 500 mg/kg MeLL considerably lowered LPO (p<0.01) and restored SOD levels (p<0.001) contrasted to the cisplatin group. Furthermore, TNF-α and IL-6 were substantially raised in cisplatin-treated group (p<0.001, Figures 1 and 2) comparative to untreated group. Therapy with Vitamin E and MeLL 500 mg/kg effectively prevented the increase in these serum cytokine levels (p<0.0001) corresponding to the cisplatin group. Conversely, the lower 250 mg/kg methanolic extract did not demonstrate a pivotal difference.

In Model 2 (Tables 3 and 4; Figures 3 and 4), the nephroprotective effects of the MeLL were assessed against CIN by evaluating various biochemical parameters. Cisplatin administration led to significant increases in renal parameters, including creatinine (p<0.001), urea (p<0.0001), uric acid (p<0.001), Blood Urea Nitrogen (BUN) (p<0.0001), sodium (p>0.05), potassium (p<0.001), chloride (p<0.0001) and calcium (p<0.0001), comparative to untreated group. Preliminary therapy with Vitamin E (250 mg/kg), MeLL at 500 mg/kg significantly lowered these renal parameters, including serum creatinine (p<0.01), urea (p<0.0001), uric acid (p<0.0001), BUN (p<0.0001), potassium (p<0.001), chloride (p<0.0001) and calcium (p<0.0001), relative to the cisplatin group, while sodium levels did not reveal notable variations (p>0.05). 250 mg/kg methanolic extract did not yield significant differences compared to the cisplatin control group. Furthermore, CIN remarkably enhanced LPO (p<0.001, Table 4) and reduced the antioxidant defence system, indicated by decreased Superoxide Dismutase (SOD) levels (p<0.01, Table 4) comparative to untreated group. Treatment with Vitamin E (250 mg/kg) and the 250 mg/kg methanolic extract markedly decreased LPO (p<0.01) and restored SOD levels (p<0.001) corresponding to cisplatin group. Notably, the 500 mg/kg MeLL showed even more significant effects against the cisplatin group. Cytokines TNF-α and IL-6 (Figures 3 and 4) were notably enhanced in cisplatin-treated animals (p<0.001) in respect to the untreated group. Treatment with Vitamin E (250 mg/kg) and the 500 mg/kg methanolic extract notably lowered the levels of these serum

Table 1: Effect of MeLL on biochemical parameters of kidney.

Group	Serum creatinine (mg/dL)	Urea (mg/dL)	BUN (mg/dL)	Uric acid (mg/dL)	Sodium (m mol/L)	Potassium (meq/dL)	Chloride (m mol/L)	Calcium (mg/dL)
NC (Untreated Saline 10 mL/kg).	0.6333± 0.02333	21.44± 0.6312	19.74± 0.6465	1.785± 0.09387	136.0± 2.324	3.565± 0.09301	97.00± 1.310	3.622± 0.06529
DC Cisplatin (2 mg/kg i.p) alternative (thrice per week) Administration.	0.9600± 0.01155$^{\#\#\#\#}$	27.52± 0.4896$^{\#\#\#\#}$	29.49± 0.5471$^{\#\#\#\#}$	3.745± 0.1048$^{\#\#\#\#}$	145.7± 1.145$^{\#\#}$	5.902± 0.04206$^{\#\#\#\#}$	113.1± 1.172$^{\#\#\#\#}$	8.770± 0.07987$^{\#\#\#\#}$
Std. Drug-Vitamin E (250 mg/kg).	0.7317± 0.02414****	24.69± 0.6647*	21.73± 0.5494****	2.468± 0.1351****	141.2± 1.138^{ns}	4.408± 0.04799****	101.0± 2.113****	5.687± 0.1094****
MeLL (250 mg/kg).	0.7883± 0.02167***	26.76± 0.4612^{ns}	25.00± 0.9309***	2.865± 0.04938****	142.2± 1.493^{ns}	4.867± 0.06200****	105.3± 1.382**	6.893± 0.05892****
MeLL (500 mg/kg).	0.6733± 0.02860****	23.16± 0.8714***	19.12± 0.3800****	2.340± 0.06121****	135.3± 0.9888***	3.447± 0.04807****	98.08± 0.9867****	4.900± 0.1071****

Values are expressed as Mean±SEM. (n=6). ##p<0.0.01, ####p<0.0001 compared with Normal Control (NC),*p<0.05,**p<0.01,***p<0.001, ****p<0.0001 compared with DC using one-way ANOVA followed by Tukey's multiple comparison test.

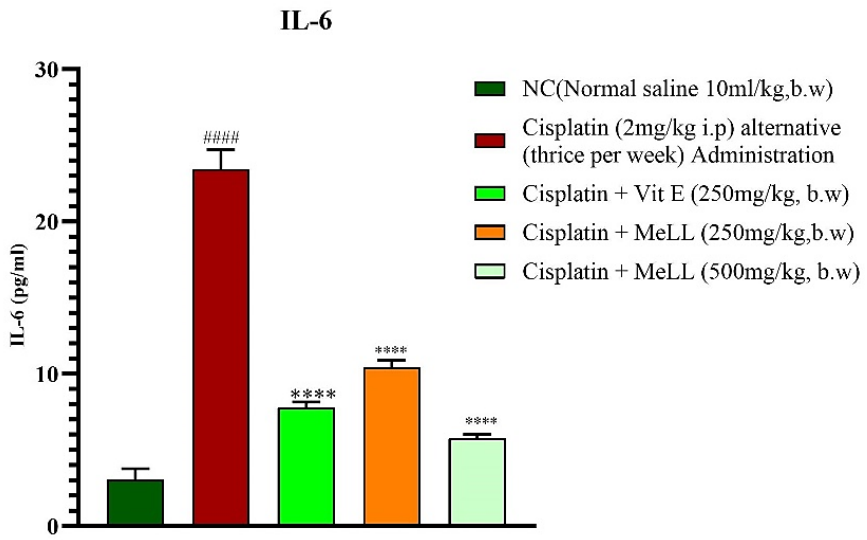

Figure 1: Effect of MeLL on Interleukin-6 levels in CIN. Values are expressed as Mean±SEM. (n=6). ####p<0.0001 compared with NC, ****p<0.0001 compared with DC.

cytokines (p<0.0001) comparative to cisplatin group, while 250 mg/kg of the extract did not demonstrate remarkable differences.

Histopathological examination (Figures 5a-5e) of the kidneys from animals treated with cisplatin revealed significant glomerular degeneration, characterized by capillary loss around Bowman's capsule. The proximal tubules, distal DCT and interstitial renal tissue exhibited marked dilation and inflammation in cisplatin group compared to untreated group. In contrast, animals that received Vitamin E treatment and those treated with the MeLL showed a notable restoration of glomerular architecture, with well-preserved tufts of capillaries surrounded by Bowman's capsule. Most tubules maintained typical morphology and demonstrated signs of healing. However, a small fraction of tubules exhibited moderate degeneration, indicated by the aggregation of inflammatory cells across tubular lumen. Overall, the treatment effectively reduced abnormalities in the proximal tubules and DCT, with diminished dilation observed following the interventions.

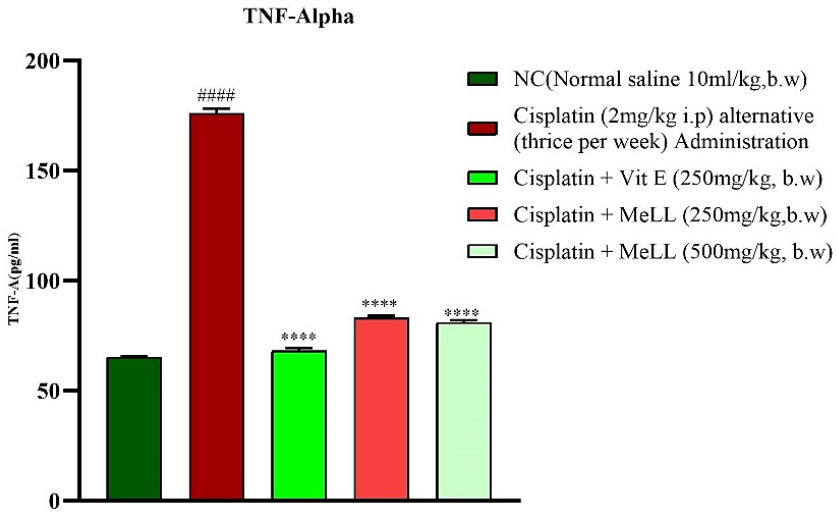

Figure 2: Effect of MeLL on TNF-Alpha levels in CIN. Values are expressed as Mean±SEM. (*n*=6). ####*p*<0.0001 compared with NC, ****p<0.0001 compared with DC.

Table 2: Effect of MeLL on anti-oxidants enzyme study level.

Groups	LPO (n moles of MDA/g protein)	SOD (units/min/mg of protein)
NC (Untreated Saline 10 mL/kg).	1.122±0.1635	24.13±1.705
DC Cisplatin (2 mg/kg i.p) alternative (thrice per week) Administration.	2.970±0.2984####	6.0±0.5#
Std. Drug-Vitamin E (250 mg/kg).	1.365±0.1588****	27.50±3.04**
MeLL (250 mg/kg).	1.965±0.1335*	42.3±2.82***
MeLL (500 mg/kg).	1.328±0.1800****	47.38±3.26***

Values are expressed as Mean±SEM. (*n*=6). #*p*<0.05 compared with NC, **p<0.01, ***p<0.001 compared with DC using one-way ANOVA followed by Tukey's multiple comparison test.

DISCUSSION

Acute renal failure is more likely to occur in hospitalized individuals, especially those who are critically ill, depletion of electrolytes and require intensive care. Globally, renal failure affects 8-10% of the adult population and every year, millions face early mortality due to complications related to this condition. As kidney failure is becoming a principal factor of fatality globally, developing effective drugs to treat renal illnesses has become an important goal. However, the main limitation of cisplatin's clinical application is its nephrotoxicity. Cisplatin's intracellular actions trigger tubular destruction and dysfunction, along with sodium, potassium and magnesium wasting. Nephroprotective approaches to counter cisplatin-induced damage includes, reduced cisplatin absorption by renal cells, cisplatin metabolic inhibition, inhibition of cell death pathways, inhibition of cyclin-dependent kinase and genetic p53 inhibition, precise MAPK inhibition. Cisplatin nephrotoxicity is driven by ROS, which leads to lipid peroxidation, protein damage and DNA fragmentation. ROS can generate free radicals, initiating chain reactions that result in cellular damage. Antioxidants, particularly those derived from natural sources, are of interest due to their potential to detoxify ROS without interfering with cisplatin's anticancer efficacy. Thus, antioxidants may have therapeutic potential in mitigating CIN.

Thus, interest has sparked in natural products with high antioxidant content, particularly *Leucaena leucocephala*.[17] The conclusions of the present study depicted that *Leucaena leucocephala* seeds at 250 mg/kg and 500 mg/kg possess nephroprotective activity due to their antioxidant properties. Prior phytochemical screening has demonstrated that the MeLL seeds contain phenols, sterols, flavonoids, coumarins and triterpene derivatives.[18] These phytocontsituents are noted for their antioxidant activity and the phenolic content is likely responsible for this effect.[6] Thus, when administered to rats with CIN, the MeLL revealed considerable nephroprotective effects. Acute toxicity testing revealed, methanolic extract of *Leucaena leucocephala* seeds did not cause fatality up to 2000 mg/kg and the LD50 was found to be 5000 mg/kg. For therapeutic purposes, doses of 1/10th and 1/20th of 5000 mg/kg were selected as high and low doses, respectively.[8] In nephrotoxicity models, cisplatin was administered to rats in 2 different protocols: 2 dose levels have been established, a low-dose regimen of 2 mg/kg on every other day and a high-dose of 8 mg/kg as an individual injection. Cisplatin treated group showed elevated serum creatinine, urea, uric acid, BUN, sodium, potassium, chloride, calcium. These changes were correlated with tubular dysfunction, necrosis and lowered GFR. Also, cisplatin raised TNF-α and IL-6 and promoted extrinsic apoptotic cascade. Pre-treatment with the MeLL at 250 mg/kg and 500 mg/kg for ten days significantly regulated LPO and oxidative stress in diseased

Table 3: Effect of MeLL on biochemical parameters of kidney.

Group	Serum creatinine (mg/dL)	Urea (mg/dL)	BUN (mg/dL)	Uric acid (mg/dL)	Sodium (m mol/L)	Potassium (meq/dL)	Chloride (m mol/L)	Calcium (mg/dL)
NC (Untreated Saline 10 mL/kg).	0.6333± 0.02333	21.44± 0.6312	19.74± 0.6465	1.785± 0.09387	136.0± 2.324	3.565± 0.09301	97.00± 1.310	3.622± 0.06529
DCCisplatin (8 mg/kg; i.p) Single Administration.	0.7717±0.01939$^{\#\#\#}$	25.71±0.5256$^{\#\#\#}$	27.45±0.5740$^{\#\#\#\#}$	2.942±0.05986$^{\#\#\#\#}$	139.0±1.915^{ns}	5.612±0.07436$^{\#\#\#\#}$	109.6±1.463$^{\#\#\#\#}$	6.592±0.02701$^{\#\#\#\#}$
Std. Drug -Vitamin E (250 mg/kg).	0.6867±0.01520*	19.65±0.7506****	23.71±0.4854**	2.152±0.1106****	138.8±1.922 ^{ns}	3.395±0.1143****	98.08±0.9867****	4.593±0.08110****
MeLL (250 mg/kg).	0.6900±0.01592*	21.39± 0.4979***	25.51± 0.5253^{ns}	2.468± 0.1351*	138.8± 1.922 ^{ns}	4.507± 0.02275****	99.03± 1.321****	5.808± 0.06973****
MeLL (500 mg/kg).	0.6633± 0.01783**	19.38± 0.8233****	21.40± 0.6523****	2.085± 0.08273****	137.8± 1.424 ^{ns}	3.363± 0.05965****	97.00± 1.310****	4.278± 0.03692****

Values are expressed as Mean±SEM. (n=6). ###p<0.0.01, ####p<0.0001 compared with NC, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 compared with DC using one-way ANOVA followed by Tukey's multiple comparison test.

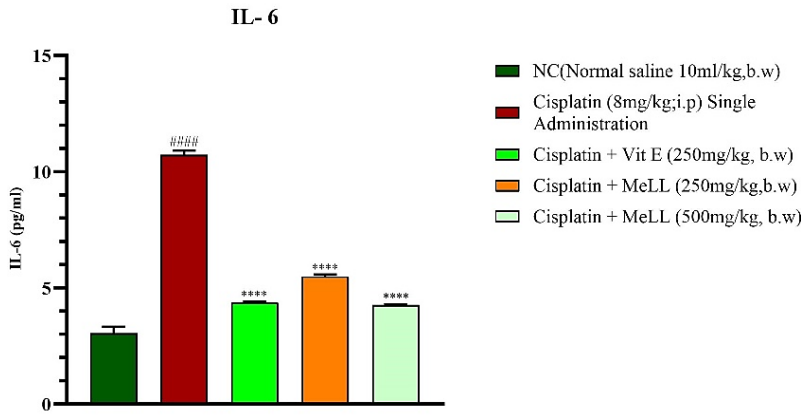

Figure 3: Effect of MeLL on Interleukin-6 levels in CIN. Values are expressed as Mean±SEM. (n=6). ####p<0.0001 compared with NC, ***p<0.001, ****p<0.0001 compared with DC.

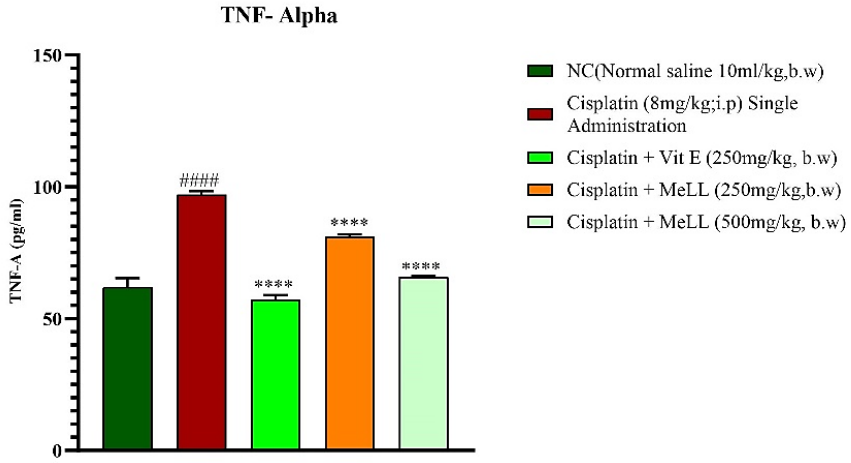

Figure 4: Effect of MeLL on TNF-Alpha levels in CIN. Values are expressed as Mean±SEM. (n=6). ####p<0.0001 compared with NC, ****p<0.0001 compared with DC.

rats. The extract also furnished an additional electron to neutralise ROS, thereby minimizing oxidative damage. Consequently, blood creatinine, urea, uric acid, BUN and electrolytes were remarkably reduced in MeLL groups in contrast to untreated group. Further, MeLL lowered TNF-α and IL-6 and minimized apoptosis, tubular destruction and oxidative stress, specifically at higher dose 500 mg/kg.

The flavonoids and polyphenols that are present in the seeds of *Leucaena leucocephala* are likely responsible for the antioxidant effects observed in this study. These compounds can cease free radicals, lower ROS generation and enhance endogenous antioxidant enzymes such as SOD. Furthermore, phytochemicals like tannins and alkaloids found in *Leucaena leucocephala* have been shown potential to hinder LPO and reduce oxidative stress.

The nephroprotective properties of *Leucaena leucocephala* align with findings from other studies on natural product-based antioxidants. For instance, silymarin derived from milk thistle has exhibited comparable free radical scavenging profile in CIN by lowering malondialdehyde levels and enhancing endogenous antioxidant enzymes. A polyphenol constituent from turmeric, Curcumin has also illustrated nephroprotective activity by governing transcription factors, which is concerned with cellular antioxidant defence system. In addition to its antioxidant properties, *Leucaena leucocephala* may also hinder inflammation by suppressing the NF-κB cascade. Similar anti-inflammatory effects have been reported in other plant-based therapies, such as *Nigella sativa* (black cumin) and *Ginkgo biloba*, which lower inflammatory cytokine levels and suppress NF-κB activation. Prior studies revealed alkaloids in *Leucaena leucocephala* may also modulate cytokine synthesis and lower leukocyte migration into inflamed tissues.

In addition, *Leucaena leucocephala* seeds may prevent apoptosis in renal cells. Flavonoids in the seeds may hinder the mitochondrial apoptosis cascade by stabilizing mitochondrial membranes, thereby preventing the discharge of cytochrome c and lowering the indication of caspases. Moreover, phenolic compounds in *Leucaena leucocephala* may regulate the expression of cytoplasmic

Table 4: Effect of MeLL on anti-oxidants enzyme study level.

Groups	LPO (n moles of MDA/g protein)	SOD (units/ min/mg of protein)
NC (Untreated Saline 10 mL/kg).	1.122±0.1635	24.14±1.31
DC Cisplatin (8 mg/kg; i.p) Single Administration.	2.040±0.08458####	14.27±0.585##
Std. Drug -Vitamin E (250 mg/kg).	1.513±0.1092*	21.83±0.75**
MeLL (250 mg/kg).	1.657±0.08991ns	22.76±0.224**
MeLL (500 mg/kg).	1.483±0.04681**	30.75±0.81***

Values are expressed as Mean±SEM. (n=6). ####p<0.0001 compared with NC, ns p>0.05, *p<0.05, **p<0.01 ***p<0.001, and ****p<0.0001 compared with DC using one-way ANOVA followed by Tukey's multiple comparison test.

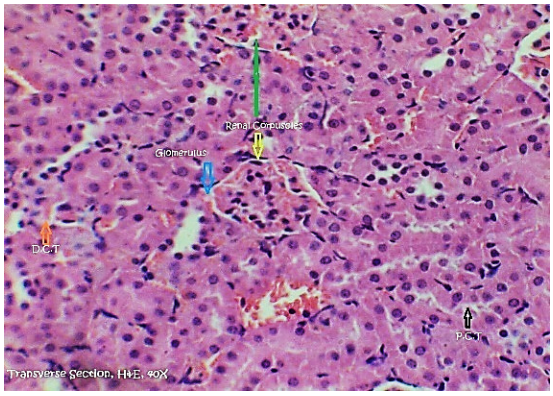

Figure 5a: Normal control Normal Saline (10 mL/kg/b.w).

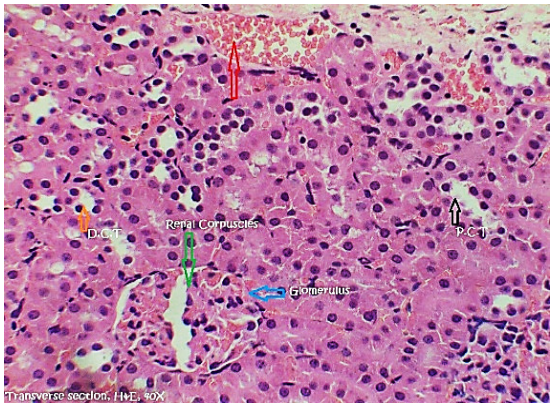

Figure 5b: Disease Control (cisplatin).

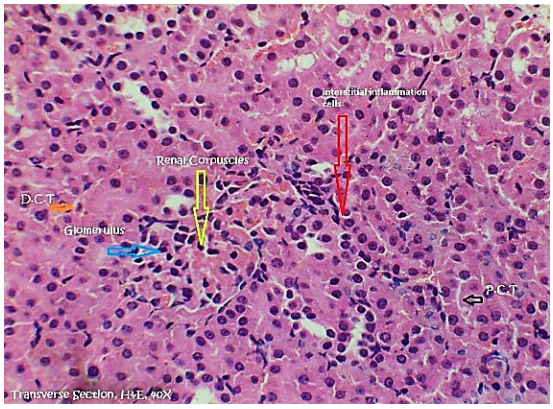

Figure 5c: Standard Drug-Vitamin E (250 mg/kg; b.w).

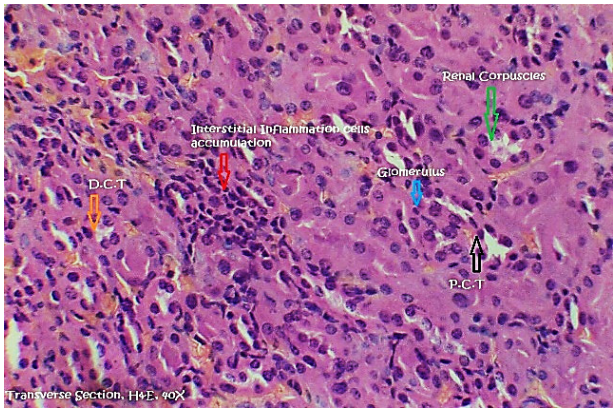

Figure 5d: Test Drug-MeLL (250 mg/kg;b.w).

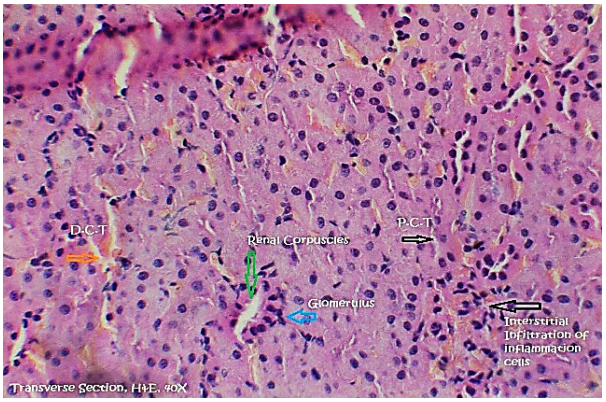

Figure 5e: Test Drug- MeLL (500 mg/kg;b.w).

Figure 5: Histology images outlined below in accordance with Normal control. **Figure 5a:** Demonstrating a healthy glomerulus (Blue Arrow) with a tuft of capillaries encircled by Bowman's capsule (Green Arrow), tubules surrounded by columnar epithelial cells and a healthy architecture. No abnormalities, such as interstitial inflammation and Proximal Tubules dilatation (PCT, Black Arrow) and Distal Convoluted Tubule (DCT, Orange Arrow) inside the renal tissue. **Figure 5b:** The image showed Bowman's capsule (Green arrow) around glomerular degeneration (Blue arrow) with capillary loss. The proximal tubules (Black arrow), DCT (Orange arrow) and interstitial renal tissue of the animal revealed prominent dilatation and inflammation (Red arrow). **Figure 5c:** Showing Bowman's capsule surrounding the glomerulus with capillary loss (Green and Blue arrow). The tubules appear to have a normal design and are recovering from toxicity, while there is some interstitial inflammation cells (Red arrow) accumulation in the tubules' center that indicates minor tubular degeneration. No abnormalities, such as Proximal Tubules dilatation (PCT, Black Arrow) and Distal Convoluted Tubule (DCT, Orange Arrow) inside the renal tissue. **Figure 5d:** Demonstrating, restoration (Green arrow) and the glomerulus' normal architecture (Blue arrow). The degenerative alterations in the kidney tissue, such as interstitial infiltration of inflammatory cells, epithelial cell dissociation (Red arrow and proximal tubule (Black arrow), DCT (Orange arrow) dilatation were reduced as a result of the treatment. **Figure 5e:** Demonstrating, restoration and normal glomerular architecture (Blue arrow) with tufts of capillaries encircled by Bowman's capsule (Green arrow). Most tubules exhibit typical morphology and healing. However, only a small number of tubules displayed moderate degeneration, which was visible as accumulation of inflammation cells (Black arrow) in the tubule center. No abnormalities in proximal tubule (Black arrow), DCT (Orange arrow) dilatation were reduced as a result of the treatment.

proteins, thereby enhancing cell survival. The nephroprotective potential of *Leucaena leucocephala* seeds suggests that they could be developed as a supplemental therapy for patients undergoing cisplatin-based chemotherapy. By reducing renal damage, *Leucaena leucocephala* seeds may enhance patient benefits without diminishing cisplatin's anticancer efficacy. Future directions include developing *Leucaena leucocephala* into a standardized nephroprotective formulation and conducting clinical trials to assess its efficacy and safety in cancer patients. Further research should investigate its molecular mechanisms and explore broader applications in other nephrotoxic conditions. The plant's accessibility and cost-effectiveness make it a sustainable option for therapeutic development, with potential for integration into combination therapies for enhanced nephro protection.

CONCLUSION

The present study confirmed the nephroprotective efficacy of *Leucaena leucocephala* seeds in Cisplatin-Induced Nephrotoxicity (CIN) in rats. Preliminary phytochemical analysis from previous studies revealed the presence of sterols, saponins, flavonoids, alkaloids and phenols, which are likely contributors to the plant's nephroprotective action. The Methanolic extract of *Leucaena leucocephala* (MeLL) administered at doses of 250 mg/kg and 500 mg/kg demonstrated significant nephroprotective effects, with the 500 mg/kg dose showing profound activity. The study highlighted MeLL's notable antioxidant, anti-apoptotic and overall nephroprotective properties. These findings encourage further research to elucidate the precise mechanisms underlying the plant's nephroprotective and antioxidant activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BUN: Blood Urea Nitrogen; **ROS:** Reactive Oxygen Species; **RPM:** Revolutions Per Minute; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethics Committee; **SOD:** Superoxide Dismutase; **TNF:** Tumor Necrosis Factor; **IL-6:** Interleukin-6; **SDS:** Sodium Dodecyl Sulfate; **SEM:** Standard Error of the Mean; **ANOVA:** Analysis of Variance; **H and E Stain:** Hematoxylin and Eosin Stain; **LPO:** Lipid Peroxidation; **MAPK:** Mitogen-Activated Protein Kinase; **DCT:** Distal Convoluted Tubules.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The protocol was approved by IAEC, Karnataka College of Pharmacy, Bengaluru - 560064 and Sl. No. (KCP/IAEC/11/22-23/12/22/12/22).

SUMMARY

- A study examining the nephroprotective effects of a plant extract (*Leucaena leucocephala)* on CIN in rats was conducted. The extract was extracted from the plant, revealing key phytochemicals like terpenes, flavonoids, coumarins, phenols and sterols.
- The study mimicked the nephrotoxicity caused by cisplatin, which is given intraperitoneally to induce kidney damage in rats. The extract was tested on serum biomarkers such as serum creatinine, uric acid, BUN, potassium, IL-6, TNF-α, chloride, calcium and SOD.
- The extract illustrated a protective effect on cisplatin-provoked kidney damage, restoring normal glomerular architecture, but some tubular degeneration and inflammation were observed. Some areas still showed signs of moderate degeneration, associated with accumulated inflammation.

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