International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

Evaluation of Nephroprotective Effects of Cajanus Cajan Leaves Extract: In-Silico and In-Vivo Studies

Riya Thapa[1], M Arockia Babu[2], Piyush Mittal[3], Arcot Rekha[4], Imam Shaik[5], Deepthi Kuturu[6], Saumya Das[7], Mohit Rana[8], Kavita Goyal[9], Lakshmi Thangavelu[10], Lakshmana Bendre[11], Gaurav Gupta[12,13]

1. *School of Pharmacy, Suresh Gyan Vihar University, Mahal Road, Jaipur, India*
2. *Institute of Pharmaceutical Research, GLA University, Mathura, UP 281406, India*
3. *Department of Pharmacy Practice, Teerthanker Mahaveer of College of Pharmacy, Teerthanker Mahaveer University, Moradabad, India*
4. *Dr. D.Y. Patil Medical College, Hospital and Research Centre, Pune 411018, Mahārāshtra, India*
5. *Department of Pathology, School of Medicine Taylor's University,No.1, Jalan Taylor's - 47500, Subang Jaya, Malaysia*
6. *Bojjam Narasimhulu Pharmacy for Women, Vinay Nagar, IS Sadan Cross roar, Saidabad, Hyderabad-500059, Telangana, India.*
7. *Noida Institute of Engineering and Technology(Pharmacy Institute), 19 Knowledge Park 2 Greater Noida, India*
8. *Uttaranchal Institute of Pharmaceutical Sciences, Uttaranchal University, Dehradun, India*
9. *Department of Biotechnology, Graphic Era (Deemed to be University), Clement Town, Dehradun 248002, India*
10. *Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India*
11. *Government Ayurvedic and Unani Medical College Hospital, Banglore, Karnataka, India*
12. *Centre for Research Impact & Outcome-Chitkara College of Pharmacy, Chitkara University, Punjab, India*
13. *Centre of Medical and Bio-allied Health Sciences Research, Ajman University, Ajman 346, United Arab Emirates*

Date of Submission: 05-07-2025 Date of Acceptance: 15-07-2025

ABSTRACT

Background: This study aimed to assess the nephroprotective effects of Cajanus cajan leaf extract against gentamicin-induced nephrotoxicity in experimental mice. **Methods:** Leaves were extracted with ethanol and the constituents identified by GC-MS, then subjected to in silico molecular docking and ADMET analyses. The extract (100 mg/kg) was administered alongside gentamicin to mice, after which blood was analyzed for renal markers (creatinine, total protein, blood urea nitrogen, albumin) and kidneys were examined histologically and assayed for antioxidant indices (MDA, MPO, CAT, GSH, SOD). **Results:** GC-MS detected 17 compounds—most abundant were 2,2′-Bi-1,4-Dioxane (21.511%), 9,12-Octadecadienoic Acid (27.419%), 9-Eicosyne (18.047%), and 2,4,4-Trimethyl-3-hydroxymethyl-5a-cyclohexane (7.946%). Treatment reduced urine output, protein, and sodium levels; alleviated oxidative stress by lowering MDA and MPO and elevating CAT, GSH, and SOD; histology revealed improved glomerular and interstitial architecture with minimal congestion, necrosis, and hemorrhage; in silico data indicated strong binding affinities and favorable safety profiles for most extract components. **Conclusion:** The ethanolic extract of C. cajan is safe and exerts significant nephroprotective effects against gentamicin-induced renal injury, likely owing to its flavonoid and phenolic constituents

Keywords: Cajanus cajan, molecular docking, ADMET analysis, gentamicin-induced nephrotoxicity, nephro

I. INTRODUCTION

Gentamicin is used to treat certain gram-negative infections, although its usefulness is limited by kidney damage in approximately 60% of cases reported in hospitalized patients [1]. It typically manifests as a rapid decline in kidney

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

function, partly due to tubular destruction, changes within the glomeruli, the buildup of harmful substances, and inflammation in the renal tissue [2]. Because of their many antioxidant and anti-inflammatory phytochemicals, medicinal plants have, in the past, been important in producing nephroprotective agents. Flavonoids, phenolics, and stilbenes, including cajaninstilbene acid and pinostrobin, found in Cajanus cajan (pigeon pea), protect against free radicals and serve as cytoprotectants in various test models [3]. However, the ability of C. cajan leaf extract to prevent gentamicin-induced kidney injury is poorly understood.

The kidney is the primary organ in the human body that accomplishes several vital processes, such as detoxification, control of extracellular fluids, maintenance of homeostasis, and elimination of harmful compounds [4]. Nephrotoxicity refers to the rapid onset of kidney problems following drug or toxin consumption. Various types of medications and multiple methods can influence kidney function [5]. In clinical medicine, acute kidney injury due to drugs can occur in up to 60 percent of cases. Drug-induced nephrotoxicity is a complex process that often involves changes in blood flow inside the kidney, impaired secretion in the tubules, inflammation, uric acid buildup, muscle breakdown, and formation of small blood clots in the kidney [6]. Aminoglycosides exhibit prolonged post-antibiotic action and kill bacteria in a concentration-dependent manner. Frequent antibiotic administration can cause nephrotoxicity, which builds up in the renal parenchyma [7]. Among aminoglycosides, neomycin is the most nephrotoxic, followed by gentamicin, tobramycin, and amikacin. Plants have historically been used as remedies for several ailments, including conditions categorized as pre-, intra-, and post-renal factors [8]. The therapeutic properties of plants are ascribed to their secondary metabolites, which can provide protection against infections or confer significant physiological advantages in preventing specific illnesses. In addition, plant-derived nephroprotective medicines alleviate conditions such as interstitial nephritis, modified intraglomerular hemodynamics, tubular necrosis, and glomerulonephritis [9].

Cajanus cajan, often known as pigeon pea, is a plant that belongs to the Fabaceae or Leguminosae family [10]. It is endemic to the Eastern Hemisphere. The primary bioactive components found in C. cajan are categorized into three main groups: flavonoids, phenolics, and stilbenes [11]. The plant's root contains genistin, genistein, hexadecenoic acid, α-amyrin, β-sitosterol, pinostrobin longistylin A, longistylin C, Cajanus lactone, betulinic acid, vitexin, and orientin. In contrast, the leaves are abundant in 2'-2' methyl cajanone, 2'-hydroxygenistein, isoflavones, cajanin, pinostrobin, cajaninstilbene acid, and vitexin [12].

Additionally, it includes amino acids such as tryptophan, threonine, isoleucine, and cysteine, as well as vitamins such as thiamine, riboflavin, niacin, and B6. Historically, it has been documented that leaves and seeds are externally applied to the breast to stimulate breastfeeding [13]. The leaves are ingested orally to alleviate food poisoning, colic, and constipation. A paste from the leaves treats oral malignancies, whereas the juice extracted from the leaves is orally administered for its laxative properties [14]. It has been documented to demonstrate antimicrobial, hypocholesterolemic, antidiabetic, neuroactive, antioxidant, anticancer, hepatoprotective, anthelmintic, and antiviral properties [15]. Therefore, Cajanus canus, which possesses many therapeutic qualities and contains phytoconstituents such as flavonoids, phenolics, and stilbenes, may be a promising candidate for further research as a nephroprotective agent. The chemical may be identified using GCMS analysis, examination of the docking interaction, evaluation of the ADMET profile of the crude extract, and assessment of its nephroprotective ability against gentamicin-induced nephrotoxicity in experimental animals.

Accordingly, the present study aimed to evaluate the nephroprotective efficacy of an ethanolic extract of C. cajan leaves against gentamicin-induced nephrotoxicity in mice. We combined GC-MS–based chemical profiling and in silico docking/ADMET analyses to identify the bioactive constituents. We assessed renal function, oxidative stress markers, proinflammatory cytokines, and histopathology in the treated animals to elucidate the underlying protective mechanisms of the extract.

II. MATERIALS AND METHODS

C. cajan leaf (local market Hyderabad), Ammonium molybdate, Acetic anhydride, Bromothymol, Barfoed's reagent, Dragendorff's reagent, Magnesium, Picrate were purchased from S.D Fine chemicals, Mumbai; ELISA kit, Formalin, Mayr's reagent, Methanol were purchased from R & D systems incorp, USA; Eosin stain and Wagner's reagent was purchased from Himedia,

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

Mumbai; Carageenan, Gallic acid, Rutin were purchased from Sigma Aldrich, USA; Aluminium chloride was purchased from Finner chemicals; ELYTE 2 kit and Accurex biomedical kit were purchased from Mahim, Mumbai.

Plant material

The leaves of C. cajan were gathered between August and September from several sites in Hyderabad and conserved as a herbarium. The plant was certified by Dr. Vijaya Bhasker Reddy, an Assistant Professor in the Department of Botany at Osmania University in Hyderabad. A voucher was gathered as specimen OUAS-151.

Extraction of leaves

The 3 kg leaves were collected, cleaned with flowing water, and dried in the shade for two weeks. Once dried, they were crushed into coarse powder using a No.22 mesh. The powder was obtained and placed into a Soxhlet column, and then extracted using ethanol (90%) at 40 °C. The extract was condensed using decreased pressure in a rotary flash evaporator, producing a crude semisolid substance at a temperature of 25°C. The desiccated portions were placed in hermetically sealed receptacles in a refrigerator. Preliminary phytochemical screening of the fractions: Phytochemical analysis was performed using the extracts that were qualitatively analyzed [16].

GC-MS Analysis

A Clarus 680 Gas Chromatograph (GC) with a column packed with Elite-5MS made from a mixture of 5% biphenyl and 95% dimethylpolysiloxane was used for the study. The design involved a 30 m long column, wall thickness of 0.25 mm, and film thickness of 250 m. Helium, which was used at a constant flow rate of 1 ml/min, enabled the separation of the components. An injector temperature of 260 °C was consistently used during the chromatographic process. The instrument contained one µL of extract, and the oven temperature was programmed as follows: the temperature rose to 60 °C and remained there for 2 min. A 10 °C rise to 300 °C was applied, with each increase taking one minute. Subsequently, the temperature remained steady at 300 °C for 6 min throughout the process. The mass detector was operated using the following settings: transfer line temperature, 240 °C; ion source temperature, 240 °C; and electron impact, 70 eV. The scanning process lasted 0.2 s, and each repetition ended after only 0.1 s. Chromatograms record the sizes of these chromophore species, which range between 40 and 600 Da. The spectra of all components were compared with the catalog of known component spectra found in the GC-MS NIST (2008) database [17].

In silico molecular docking analysis: Compounds identified from GC-MS extract analysis were subjected to molecular docking.

(a) Protein Preparation and Receptor Grid Generation: The binding of cilastatin to human serum albumin (PDB ID: 1ITU) was discovered based on data from the Protein Data Bank (PDB). The Protein Preparation Wizard was used to organize the protein structure. The Receptor Grid panel in Glide v 9.1 built a grid in the active site of the protein.

(b) Ligand Preparation: Ligprep was used to create the structure using the OPLS 5 force field to minimize the geometry of the molecule.

(c) Ligand-based docking: Ligand-based docking was performed using the Ligand Docking Module provided by GLIDE. Initially, the Receptor Grid (zip file) was selected, and LigPrep (outfile) was selected. After choosing the XP mode, the job was run. Once XP docking was complete, we checked the dock scores in the project table table. Examining the ligand interaction diagram revealed how the ligand and protein interacted.

(d) MM-GBSA: To assess the relative binding energy of specific ligands, we applied Prime/MM-GBSA. I used the pv. maegz file from XP docking, and I set the protein active site to move by itself to accommodate ligands as far as 5 Å.

ADMET analysis

ADME characteristics were calculated using QikProp v6.8, and toxicity prediction was performed using ProTox-II59 for the best score obtained from the MM-GBSA trial.

PHARMACOLOGICAL STUDIES

Experimental Animals

For this study, Albino Wistar rats of both sexes weighing 150-180 gm were used to examine the pharmacological effects. Food and animals were provided by Mahaveera Enterprises, Hyderabad. The laboratory employed standards set forth by experts, setting the temperature at 25 °C and following a 12-hour light-dark cycle. Food and water were available to the experimental animals at all times. The Animal Ethics Committee of the

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

Deccan College of Pharmacy, Hyderabad (protocol number DC-24- 211), approved all protocols involving animals in this study.

Dose selection

The extract of C cajan leaves was evaluated for nephroprotective pharmacological activity, and doses were selected based on previously reported studies. The chosen doses of C. cajan were 400 and 600 mg/kg bw per oral [18].

Nephroprotective activity Induction of Gentamicin-induced nephrotox

The six rats in each group (n=6) randomly chosen among 150-180 gms group received treatment for 8 days, as explained below in the table

Group I: Mice received normal saline (10 ml/kg repeated by oral gavage)

Group II: Treatment with GM (100 mg/kg, i.p.): i. p. administration of GM (100 mg/kg).

Group III was administered C. cajan extract at 400 mg/kg (CC400) and GM at 100 mg/kg, both orally and by injection.

Group IV received C. cajan at a dose of 600 mg/kg (CC 600) by mouth and GM at 100 mg/kg by intraperitoneal injection.

Biochemical assessment of urine

Urine was collected in polypropylene tubes, and the quantity of urine was measured. Urine samples were collected over 24 h using conventional metabolic cages. Food availability was restricted throughout the urine collection period, but water was unrestricted. The obtained urine samples were used to evaluate the urine volume, creatinine clearance, and salt and potassium levels. The protein, salt, potassium, and creatinine clearance of the samples were determined using Accurex Biomedical kits from Mahim, Mumbai, and ELYTE 2 kits, according to the manufacturer's instructions [19].

Blood samples and tissue collection

On day 9, the rats were euthanized with anesthetic ether to obtain blood samples for biochemical analysis and investigation of renal indicators. Centrifugation was applied to the blood at 3500 revolutions per minute for 15 min to separate the serum. The kidneys were extracted for histological examination and quantification of antioxidant properties [20].

Biochemical estimation

The serum samples were analyzed for renal markers, including serum Cr, TP, BUN, urea, and albumin, as well as proinflammatory cytokines IL-6, IL-8, IL-1β, and TNF-α [21]. Accurex assay kits from Mumbai were used for the analysis, according to the manufacturer's instructions.

Preparation of tissue homogenate

The kidneys were dissected and homogenized (50 g/L) following the procedure for myeloperoxidase activity analysis. The activities of malondialdehyde, superoxide dismutase, and nitric oxide were assessed by homogenizing the kidney samples in a potassium phosphate buffer (50 g/L) with a pH of 7.4. The homogenate was centrifuged at 3000 rpm for 10-12 minutes at 4 °C. The resulting supernatant was analyzed for MDA, MPO, nitrite, and SOD [22, 23].

Histopathological analysis

Rat kidneys were collected after sacrifice for histological investigation. Each slice was cut to a thickness of approximately 4 μm, fixed in 10% neutral buffered formalin, and placed in paraffin wax. All segments were evaluated under a microscope after hematoxylin and eosin staining for cell involvement, swollen tubules, glomerular damage, crowded blood vessels, and cell death [24, 25].

Statistical analysis

All results are presented as mean and standard error of the mean. Experts used Instant Statistical Software version 17.0 for the analysis. The dataset was analyzed using Graph Pad Prism software version 8.01. I used one-way ANOVA and Dunnett's test to analyze the data. The researcher used a cutoff value of 0.05 to determine that any result with a P-value less than that was statistically significant.

III. RESULTS

Preliminary Phytochemical Screening of C Cajan

The coarsely powdered plant materials were extracted with ethanol, with a % yield of 25.7. Preliminary phytochemical studies revealed the presence of steroids, flavonoids, glycosides, terpenoids, carbohydrates, and alkaloids.

GC-MS Analysis

The GC-MS chromatogram of the C. cajan extract exhibited 17 distinct peaks, indicating the presence of 17 bioactive chemicals. The bioactive

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

chemicals in the extracts were identified based on the data gathered regarding their peak area, retention duration, molecular formula, and weight. (Figure 1 Table 1).

Molecular Docking and ADMET

Molecular Docking and ADMET: All molecules with their dock score and dG binding (PDB Id: 1ITU)

Molecular docking studies: The Protonation Wizard in the Schrödinger Maestro 12.8 software was used to prepare the protein models. The 2D structures, docking scores, and ΔG binding energies of the extract components are listed in Table 2. The study examined cilastatin as a reference compound and tested cilastatin and 17 other bioactive molecules removed from the extract. Each ligand-receptor complex was scored based on van der Waals, electrostatic, hydrogen bonding, and solvation effects. The docking score reflects the binding strength between a ligand and its receptor; the more negative the score, the stronger the interaction. Cilastatin achieved a docking score of –9.206, indicating that it firmly attaches to the target receptor, and 14 of the 17 extract-based compounds had calculable scores. The best result was observed for D-norandrostane-16-carboxylic acid (β), which had the strongest receptor attachment with a score of –10.012. The docking scores of the 14 compounds suggested a spread in their binding affinities compared to cilastatin.

The binding scores are listed in Table 2. MM-GBSA allows experts to study the binding arrangements, energy contributions, and steric details involved in drug binding, leading to the refinement of potential drug components. The ΔG_bind value was determined by calculating the difference between the protein and ligand complex and the total free energies of the unbound protein and ligand.

ΔG_bind = ΔG_complex - (ΔG_ligand + ΔG_protein) kcal/mole

Compounds with more negative ΔG_bind values are expected to interact strongly with the target protein; therefore, they are welcome for further study. All 14 extract-derived molecules showed binding scores similar to or better than those of the reference compound. Overall, these energetic measurements suggest that the bioactive components of the extract help protect brain function.

3D and 2D images of each ligand-receptor complex are shown in Figures 2 and 3.

A large number of HB shape parameters from different interaction pockets are required to examine the protein-ligand HB trend. The HBs determined in the bond distance range of 1.70 – 2.00 Å were in the range of 160 – 180°, and those in the range of 2.20 – 2.40 Å were 100 – 110°. Hydrogen bonding is the primary factor controlling substrate orientation and recognition, which modifies its attraction to its attachment counterparts. An improved understanding of how HB strength responds to changes in HB length, angle, and local HB environment will aid modern drug design procedures [26].

Studies (Table 3) have shown that the hydrogen bonding of the bioactive molecules matches the hydrogen bonding reference molecule cilastatin well with amino acid residues. However, the following molecules in the extract did not show hydrogen bonding interaction: 9-EICOSYNE, CYCLOHEXENE, 4,5-diethyl-1,2, dimethylcyclohexene, cis, SQUALENE; HEXYL OCTYL ETHER.

Throughout the drug development process, ADMET analysis (http://www.swissadme.ch/index.php) or the prediction of drug behavior, encouraging its therapeutic potential, and protecting patient well-being, is indispensable.

The important ADMET characteristics that influence drug development include absorption, distribution, metabolism, excretion, and toxicity. ADMET evaluation helps determine how well a compound can be absorbed, how it moves in the body, how stable it is, how it is expelled, and whether it will cause problems in users. Without these reviews, perfecting drug uptake, efficacy, safety, and use cannot be confidently achieved for clinical use. The bioactive compounds in the extract are shown with their ADME and toxicity predictions, along with those of the reference molecules, in Tables 4 and 5.

Swiss ADME/T studies of all these drugs were performed to evaluate their pharmacokinetic characteristics by Swiss ADME. Finally, the website where it can be found is http://www.swissadme.ch/. Lipinski's rule of five was followed. According to these principles, a substance is considered safe to eat if its molecular weight is less than 500 g/mol. The molecule should have less than ten hydrogen bond acceptors and less than five hydrogen bond donors. A LogP value of equal to or less than 5 is also acceptable. In addition, toxicological qualities were assessed within an online program [27].

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

NEPHROPROCETIVE ACTIVITY

Effect of extract on GM induced in body weight and kidney weight

Results revealed that GM significantly decreased ($p < 0.001$) the body weight of the treated rats compared to the control rats. The body weights of rats treated with CC400 and 600 were significantly ($p<0.05$; $p<0.01$; $p<0.001$) better than those of rats treated with GM. The kidney weights of rats administered GM were also considerably increased ($p < 0.001$) compared to those of control rats. However, the weight of the kidney was comparable ($p < 0.05$) in rats fed with C. cajan compared to the GM group, while the weight of the kidney of rats fed with C. cajan showed a significant decrease ($p < 0.01$; $p < 0.001$) compared to the control group. Table 6 presents these data.

Effect of C. cajan on assessment of urine biochemical parameters

GM-treated animals showed an increased urine volume (ml), urine protein (mg/dl), sodium (mmol/L), and creatinine clearance (ml/min), and decreased potassium (mmol/L). Treatment with CC400 and CC600 significantly reduced urine volume (ml), urine protein (mg/dl), and sodium (mmol/L) ($p<0.0001$) compared to the treated group. Further treatment with CC400 and CC600 significantly increased potassium (mmol/L) ($p<0.01$ and $p<0.0001$) respectively(Figure 4).

Effect of C. cajan on GM induced in serum biochemical parameters

GM-treated animals showed increased serum creatinine (mg/dl), BUN (mg/dl), and urea (mg/dl) and decreased serum total protein (mg/dl) and serum albumin (mg/dl). Treatment with CC400 and CC600 significantly reduced serum creatinine levels ($p<0.01$ and $p<0.0001$, respectively) compared to those in GM-treated animals. Furthermore, CC400 and CC600 significantly decreased BUN and urea levels ($p<0.0001$) compared to GM-treated animals. Additionally, CC400 and CC600 increased serum total protein ($p<0.01$ and $p<0.0001$, respectively) and serum albumin ($p<0.0001$) compared to GM-treated animals (Figure 5).

Effect of C. cajan on assessment of oxidative stress markers

Oxidative stress was observed in GM-treated animals, as MDA (nM/mg of protein), MPO (nM/mg protein), and catalase (U/mg of protein) were found to be increased, and GSH (U/mg protein) and SOD (U/mg protein) were found to be decreased when compared to the normal control group. Treatment with CC400 and CC600 significantly decreased MDA and MPO ($p<0.0001$) compared to the treated group; however, reduced catalase levels were found to be non-significant at both doses. Additionally, CC400 and CC600 increased GSH ($p<0.01$ and $p<0.0001$, respectively) and SOD ($p<0.05$ and $p<0.001$, respectively) levels compared to the GM-treated group (Figure 6).

Effect of C cajan on Assessment of proinflammatory markers

Serum proinflammatory IL-6, IL-8, TNF-α, and IL-1β levels were significantly increased in GM-induced kidney inflammation in rats. Treatment with CC400 and 600 significantly decreased the inflammatory IL-6, IL-8, TNF-α, and IL-1β levels ($p<0.0001$) compared to the GM-treated groups, as shown in Figure 7.

Histopathology of kidney

As shown in Figure 8, light microscopic examination of renal tissues in the normal control group showed normal structure of the glomerulus, renal cortex, and renal interstitium (black arrow). In the treated group, marked degenerative changes in the renal epithelium, vacuolization, hemorrhage, necrosis of tubular cells, protein cast, and severe congestion of vessels (red arrow) were observed. In the CC400 treated Group, the Histological section of renal tissue taken from treated rats showed improvement in the glomerulus and renal interstitium (black arrow), with minimal congestion, necrosis, and hemorrhage (red arrow). In the CC600 treated Group: Histological analysis of the renal sections from treated rats showed notable attenuation of renal tissue injury, with improved glomerulus and renal interstitium (black arrow).

IV. DISCUSSION

This study aimed to evaluate the kidney-protective potential of an ethanolic leaf extract of C. cajan. Initially, the leaves of C. Cajan was extracted using ethanol. GCMS analysis was further employed to study all the constituents of the ethanolic extract. So far, I have performed molecular docking. Of the 17 bioactive compounds isolated from the extract, 14 led to high docking scores. One molecule in the extract had a lower cilastatin dock score (-9.206). According to the

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

table, this molecule was identified as D-norandrostane-16-carboxylic acid, (5. alpha.,16.beta.)-(- 10.012). This investigation also yielded significant results for the dG binding values of the bioactive compounds in the extracts relative to the reference compounds. The values of bioactive compounds from the extract found in the present study were compared to those found in vivo. Furthermore, the hydrogen bond distance and its interaction with other amino acids of bioactive molecules are analogous to the amino acids present in the reference molecule, such as ARG294, HIE152, GLY291, ASH288, ARG230, ARG230, and TYR255.

The designed ADMET analysis approach as formulated development is good in predicting the therapeutic potential and safety of the formulation, regardless of whether it is based on synthesizing or bioactive compounds found in herbal extracts. When all these characteristics are present at the recommended dose, the drug concept should show the right ADMET and adequate action against the therapeutic target. The drug-like concept is a helpful framework in the early stages of drug development. Perhaps the most well-known and widely recognized rule-based filter for determining whether a molecule will likely suffer from poor oral absorption (not drug-like) is often called the "Rule of Five." In addition, it is limited to MW, $\leq$ 500; A log P, $\leq$ 5; HBDs $\leq$ 5; HBAs, $\leq$ 10.6. According to the Rule of Five, a molecule that does not satisfy two or more of these characteristics is not considered orally active [28].

Such medication candidates can be ingested at the right time, and their action throughout the body is directed and metabolized. Another aspect of this kind is toxicology, which affects how a medication is absorbed, distributed, metabolized, and excreted. Gastrointestinal absorption (GIA) is an essential attribute of a compound. Statistical models make it difficult to analyze the embedment of drug usage in the body. GIA, another important phase of compound (drug) transport to its target destination, results from many compound features, such as the membrane and receptors. The central nervous system vasculature is predictable based on a few parameters of the cerebral blood-brain barrier (BBB). CNS vessels are highly restrictive because of the absence of endothelial cell pores and other specific properties regulating the movement of ions, chemicals, and cells do not exist, which do not allow substances into the central nervous system. Carcinogenicity is defined as the ability of a chemical to cause cancer. The substance that uses the P-glycoprotein transporter for many fundamental functions, such as drug absorption or excretion, is a P-glycoprotein source. These attributes are often used to investigate the ADMET behavior of medications [29-31].

The extract was then evaluated for its protective effects against gentamicin-induced kidney damage in rats. The most commonly employed and studied aminoglycoside is GM. However, this antibiotic has one major downside: it can damage the ears and kidneys. The development of nephrotoxicity in rabbits, mice, and rats has been studied using this antibiotic. Different strategies have been attempted, and other agents have been tried to protect or reverse kidney damage, with varying success. The effects of various medicinal plant extracts, such as Gum Arabic, Nigella sativa, and Bauhinia purpurea, on GM-induced nephrotoxicity induced by GM was studied [32]. However, the exact mechanism by which these plant extracts provide protection is not specific, but is thought to be due to their antioxidant properties. In this case, the generation of reactive oxygen metabolites (ROM) can cause harmful effects, such as GM-induced nephrotoxicity. Studies have confirmed the localized effect of natural and synthetic antioxidant substances in protecting or diminishing the effects in experimental rats [33]. Consequently, the nephroprotective potential of C. sinensis Cajan was explored based on its chemical profile.

In our study, GM led to decreased body weight and increased kidney weight over the administration period. This low body weight is due to direct injury to the renal tubules, which prevents the tubular cells from retaining water (dehydration and loss of weight) or increased breakdown of substances within the body (acidosis, loss of appetite, and decreased food consumption). After GNT injection, kidney weight increases, and inflammation and edema could be the cause. Evidence shows that Previous studies have reported weight loss and elevated kidney weights [34].

Administration of C. cajan extract restored both body and kidney weights to normal levels. The kidneys maintain the balance of body salt and water. An imbalance in salt and water is possible when renal function regulation is disrupted. In the present investigation, a serious disturbance ($p < 0.001$) in serum electrolytes was observed in rats treated with GM injections compared to control rats. A lower serum sodium value indicates that the kidneys may be unable to retain sodium and

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

chloride. Decreased sodium levels may be caused by excessive water intake and/or exogenous water production. This may result in a reverse rise in potassium levels because of decreased K+ excretion and passage of intracellular potassium into the bloodstream. Gentamicin-induced lesions in the renal tubular epithelium may exacerbate these effects [35, 36]. C. cajan orally administered significantly improves the depleted plasma level of K+, elevates the low Na+ level, and promotes the healing of injured kidneys. The antioxidant properties of C. cajan may be responsible for significantly enhancing these values. It is generally known that the overproduction of renal ROS in GEN-induced nephrotoxicity correlates with a decrease in renal antioxidant enzymes, including CAT, SOD, GPx, and GSH [37, 38]. Scott et al. [1996] proposed the primary defense mechanism of GSH in cells to scavenge hydroxyl radicals and singlet oxygen plays [39]. Superoxide dismutase (SOD) is an antioxidant enzyme present inside cells. Admittedly, its primary role is the efficient and selective conversion of superoxide to hydrogen peroxide. Additionally, two naturally occurring antioxidants, GPx and CAT, detoxify by converting hydrogen peroxide into water [40]. We quantified renal glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities to assess antioxidant activity. Our results confirmed those of previous studies in which GEN-treated rats showed decreased renal antioxidant enzymes, including CAT, SOD, GPx, and GSH [40]. Activation of proinflammatory cytokines after GM treatment in rats inhibits the NF-κB pathway. In normal cells, the interaction between NF-κB and IκB (inhibitor protein) complexes inactivates NF-κB. GM administration induces ROS production and IκB destruction, which in turn releases NF-κB into the nucleus. NF-κB activates the transcription of inflammatory cytokines, TNF-α and IL-1β [41]. Impaired kidney function is caused by decreased blood flow, narrowing of blood vessels, and an increase in the number of leukocytes at the site of damage by TNF-α [42]. Rats exposed to GM showed significantly higher TNF—α and IL—1β levels than control rats. However, the rats treated with C. cajan had lower amounts of IL-6, IL-8, TNF-α, and IL-1β than the untreated rats. Moreover, C. cajan also inhibited the generation of TNF-α, a proinflammatory cytokine involved in inflammation, fibrotic tissue remodeling, and the pathogenesis of renal fibrosis [43].

Histopathological studies have shown structural changes in GM-treated renal tissue, and specific scientists have mentioned that aminoglycoside antibiotics, such as GM, also cause structural changes in renal tissue. Kidney tissues obtained from rats treated with GM were examined histologically. It showed evidence of extensive and widespread kidney damage, including dilatation and death of renal tubules, glomerular hardening, swelling, and fluid accumulation in cells. These findings are consistent with previous research on this topic. On the other hand, the production of highly reactive free radicals is a potential source of this type of harm induced by oxidative stress produced by the administration of GM therapy in rats [44].

Histologically, the typical kidney structure in NC rats was restored, whereas renal tubule necrosis was more extensive and visible in GM-treated rats. Regardless, GM + treated rats developed slight glomerular sclerosis and increased numbers of inflammatory cells (neutrophils), but their kidney histological architecture was normalized. Ultimately, 14 bioactive components were confirmed to be present in the crude C. cajan extract and comprised of compounds, including 9,12-octadecadienoic acid (z, z), meso-4,5-octanediol, and 1,4-dioxane, all of which have antioxidant properties. The extract also contains 2’ -2’ methyl cajanone, 2’ -hydroxy genistein, isoflavones, cajanin, and cahanones, among others. The antioxidant activity of the extract is attributed to these compounds. The pharmacological activities of herbal compounds have been reported [45]. Therefore, the extract is ascribed to antioxidant compounds that could protect the kidneys from gentamicin-induced nephrotoxicity.

V. CONCLUSION

The ethanolic extract of C. cajan was studied using GC-MS, molecular docking, and ADMET analyses to determine its nephroprotective effect against gentamicin-induced nephrotoxicity in experimental rats. The results indicate the process response and the presence of antioxidant compounds (elucidated via GC-MS and molecular docking) in the extract. ADMET analysis showed that the compounds found in the extract had the best pharmacokinetics and were the least toxic. Furthermore, the ethanolic extract of C. cajan is safe and nephroprotective. This finding might be attributed to the different antioxidant compounds present in the extract.

AUTHOR CONTRIBUTIONS

Riya Thapa, M Arockia Babu, Piyush Mittal, Arcot Rekha, Imam Shaik contributed to the development of the research design.

Deepthi Kuturu, Saumya Das, Mohit Rana, Kavita Goyal, Lakshmi Thangavelu, Lakshmana Bendre, Gaurav Gupta contributed to writing the manuscript and reviewing the results.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Animal Ethics Committee of the Deccan College of Pharmacy, Hyderabad (protocol number DC-24- 211), approved all protocols involving animals in this study.

HUMAN AND ANIMAL RIGHTS

All procedures performed in this study involving animals were conducted in accordance with the national ethical standards for the protection of animals used for scientific purposes.

CONFLICTS OF INTEREST

The authors report no financial or other conflicts of interest in this study.

ETHICAL APPROVALS

This study did not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this study.

REFERENCE:

[1]. Pillans, P., et al., Outcomes in patients with gram-negative sepsis treated with gentamicin. Ther Adv Drug Saf, 2012. **3**(3): p. 109-13.

[2]. Basile, D.P., M.D. Anderson, and T.A. Sutton, Pathophysiology of acute kidney injury. Compr Physiol, 2012. **2**(2): p. 1303-53.

[3]. Nix, A., C.A. Paull, and M. Colgrave, The flavonoid profile of pigeonpea, Cajanus cajan: a review. Springerplus, 2015. **4**: p. 125.

[4]. Alomar, M.Y., Physiological and histopathological study on the influence of Ocimum basilicum leaves extract on thioacetamide-induced nephrotoxicity in male rats. Saudi J Biol Sci, 2020. **27**(7): p. 1843-1849.

[5]. Al-Naimi, M.S., et al., Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharm Technol Res, 2019. **10**(3): p. 95-99.

[6]. Campbell, R.E., C.H. Chen, and C.L. Edelstein, Overview of Antibiotic-Induced Nephrotoxicity. Kidney Int Rep, 2023. **8**(11): p. 2211-2225.

[7]. Krause, K.M., et al., Aminoglycosides: An Overview. Cold Spring Harb Perspect Med, 2016. **6**(6).

[8]. Thy, M., J.-F. Timsit, and E. de Montmollin, Aminoglycosides for the Treatment of Severe Infection Due to Resistant Gram-Negative Pathogens. Antibiotics, 2023. **12**(5): p. 860.

[9]. Tienda-Vázquez, M.A., et al., Nephroprotective Plants: A Review on the Use in Pre-Renal and Post-Renal Diseases. Plants (Basel), 2022. **11**(6).

[10]. Pal, D., et al., Biological activities and medicinal properties of Cajanus cajan (L) Millsp. J Adv Pharm Technol Res, 2011. **2**(4): p. 207-14.

[11]. Zhao, Y., et al., Stilbenoids and Flavonoids from Cajanus cajan (L.) Millsp. and Their α-Glucosidase Inhibitory Activities. Molecules, 2023. **28**(9).

[12]. Kong, Y., et al., Cajanuslactone, a new coumarin with anti-bacterial activity from pigeon pea [Cajanus cajan (L.) Millsp.] leaves. Food Chemistry, 2010. **121**: p. 1150-1155.

[13]. Gargi, B., et al., Revisiting the Nutritional, Chemical and Biological Potential of Cajanus cajan (L.) Millsp. Molecules, 2022. **27**(20).

[14]. Pal, D., et al., Biological activities and medicinal properties of Cajanus cajan (L)Millsp. Journal of advanced pharmaceutical technology & research, 2011. **2**: p. 207-14.

[15]. Vineeta, et al., Species richness and folk therapeutic uses of ethnomedicinal plants in West Bengal, India – A meta-analysis. Phytomedicine Plus, 2022. **2**(1): p. 100158.

[16]. Tungmunnithum, D., et al., Green Extraction of Antioxidant Flavonoids from Pigeon Pea (Cajanus cajan (L.) Millsp.) Seeds and Its Antioxidant Potentials Using Ultrasound-Assisted Methodology. Molecules, 2021. **26**(24).

[17]. Monrroy, M. and J.R. García, Phytochemical Characterization and Antioxidant Activity of

Cajanus cajan Leaf Extracts for Nutraceutical Applications. Molecules, 2025. **30**(8).
[18]. Arumugam, S., Evaluation of diuretic activity on methanolic extract of Cajanus cajan (Linn.). 2010: p. 3004-3005.
[19]. Bafrani, H.H., et al., Biochemical and Pathological Study of Hydroalcoholic Extract of Achillea millefolium L. on Ethylene Glycol-Induced Nephrolithiasis in Laboratory Rats. N Am J Med Sci, 2014. **6**(12): p. 638-42.
[20]. Abubakar, A.R. and M. Haque, Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci, 2020. **12**(1): p. 1-10.
[21]. Tlili, H., et al., Biochemical profile and in vitro biological activities of extracts from seven folk medicinal plants growing wild in southern Tunisia. PLoS One, 2019. **14**(9): p. e0213049.
[22]. Adetuyi, A.R., et al., Protective effect of Spondias mombin leaf extracts against aluminum chloride-induced brain oxidative stress, inflammation and apoptosis in rats. Future Journal of Pharmaceutical Sciences, 2025. **11**(1): p. 49.
[23]. Syed, R.U., et al., Rhodiola rosea L. extract ameliorates ethanol-induced gastric ulcer in rats by alleviating oxidative stress and inflammation via NF-κB pathway inhibition. Current Plant Biology, 2024. **40**: p. 100421.
[24]. Boye, A.T., et al., Histopathological Evaluation of Ethanolic Leaf Extract of Lippia adoensis on Liver, Kidney, and Biochemical Parameters in Swiss Albino Mice. Hepat Med, 2022. **14**: p. 123-133.
[25]. Olayode, O.A., M.O. Daniyan, and G. Olayiwola, Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of Stachytarpheta cayennensis in rats. Journal of Traditional and Complementary Medicine, 2020. **10**(6): p. 544-554.
[26]. Madushanka, A., et al., Quantum Mechanical Assessment of Protein-Ligand Hydrogen Bond Strength Patterns: Insights from Semiempirical Tight-Binding and Local Vibrational Mode Theory. Int J Mol Sci, 2023. **24**(7).
[27]. Daina, A., O. Michielin, and V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep, 2017. **7**: p. 42717.
[28]. Schapin, N., et al., Machine learning small molecule properties in drug discovery. Artificial Intelligence Chemistry, 2023. **1**(2): p. 100020.
[29]. Lin, J.H. and M. Yamazaki, Role of P-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinet, 2003. **42**(1): p. 59-98.
[30]. Fromm, M.F., P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. Int J Clin Pharmacol Ther, 2000. **38**(2): p. 69-74.
[31]. Veiga-Matos, J., et al., Study Models of Drug–Drug Interactions Involving P-Glycoprotein: The Potential Benefit of P-Glycoprotein Modulation at the Kidney and Intestinal Levels. Molecules, 2023. **28**(22): p. 7532.
[32]. Lakshmi, B.V., et al., Protective Effect of Bauhinia purpurea on Gentamicin-induced Nephrotoxicity in Rats. Indian J Pharm Sci, 2009. **71**(5): p. 551-4.
[33]. Jomova, K., et al., Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol, 2023. **97**(10): p. 2499-2574.
[34]. Trivic, T., et al., Rapid weight loss can increase the risk of acute kidney injury in wrestlers. BMJ Open Sport Exerc Med, 2023. **9**(2): p. e001617.
[35]. Lehnhardt, A. and M.J. Kemper, Pathogenesis, diagnosis and management of hyperkalemia. Pediatr Nephrol, 2011. **26**(3): p. 377-84.
[36]. Bankir, L., et al., Relationship between Sodium Intake and Water Intake: The False and the True. Ann Nutr Metab, 2017. **70 Suppl 1**: p. 51-61.
[37]. Vida, C., et al., Oxidative Stress in Patients with Advanced CKD and Renal Replacement Therapy: The Key Role of Peripheral Blood Leukocytes. Antioxidants (Basel), 2021. **10**(7).
[38]. Podkowińska, A. and D. Formanowicz, Chronic Kidney Disease as Oxidative Stress- and Inflammatory-Mediated Cardiovascular Disease. Antioxidants (Basel), 2020. **9**(8).
[39]. Powers, S.K., et al., Reactive oxygen species: impact on skeletal muscle. Compr Physiol, 2011. **1**(2): p. 941-69.
[40]. Ighodaro, O.M. and O.A. Akinloye, First line defence antioxidants-superoxide

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria Journal of Medicine, 2018. **54**(4): p. 287-293.

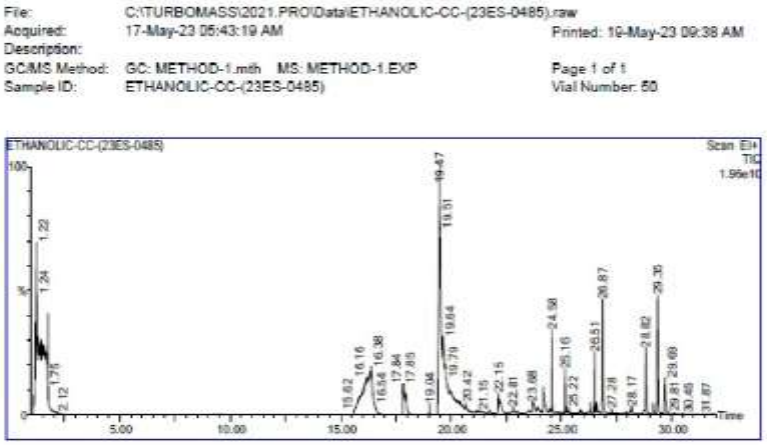
[41]. Lingappan, K., NF-κB in Oxidative Stress. Curr Opin Toxicol, 2018. **7**: p. 81-86.

[42]. Ramseyer, V.D. and J.L. Garvin, Tumor necrosis factor-α: regulation of renal function and blood pressure. Am J Physiol Renal Physiol, 2013. **304**(10): p. F1231-42.

[43]. Qin, M. and Z. Qiu, Changes in TNF-α, IL-6, IL-10 and VEGF in rats with ARDS and the effects of dexamethasone. Exp Ther Med, 2019. **17**(1): p. 383-387.

[44]. Hassan, N.H., D.M. Yousef, and A.E. Alsemeh, Hesperidin protects against aluminum-induced renal injury in rats via modulating MMP-9 and apoptosis: biochemical, histological, and ultrastructural study. Environ Sci Pollut Res Int, 2023. **30**(13): p. 36208-36227.

[45]. Patel, K. and D.K. Patel, Medicinal importance, pharmacological activities, and analytical aspects of hispidulin: A concise report. Journal of Traditional and Complementary Medicine, 2017. **7**(3): p. 360-366.



#	RT	Scan	Height	Area	Area %	Norm %
1	16.380	3075	3,637,389,056	1,671,184,384.0	21.511	76.45
2	17.855	3370	2,388,724,992	203,942,144.0	2.625	9.57
3	17.955	3390	1,636,994,816	106,099,016.0	1.366	4.98
4	19.481	3696	19,515,322,368	2,130,190,720.0	27.419	100.00
5	19.641	3727	5,992,622,592	1,402,045,056.0	18.047	65.82
6	20.326	3864	752,960,480	149,243,536.0	1.921	7.01
7	22.147	4226	1,619,964,160	119,465,808.0	1.538	5.61
8	22.252	4249	906,036,288	77,268,352.0	0.995	3.63
9	23.688	4536	847,127,424	74,527,496.0	0.959	3.50
10	24.193	4636	2,031,017,216	142,973,696.0	1.840	6.71
11	24.578	4714	6,557,466,600	157,200,864.0	2.023	7.38
12	25.153	4831	3,474,569,744	91,664,520.0	1.180	4.30
13	26.514	5101	4,812,002,944	152,405,120.0	1.962	7.15
14	26.874	5173	8,988,973,056	314,692,288.0	4.051	14.77
15	28.825	5563	5,104,706,560	191,702,240.0	2.468	9.00
16	29.356	5669	9,375,486,000	617,289,536.0	7.946	28.98
17	29.690	5736	2,783,071,488	167,028,976.0	2.150	7.84

Figure 1: GC-MS chromatogram of ethanolic leaves extract C.cajan

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

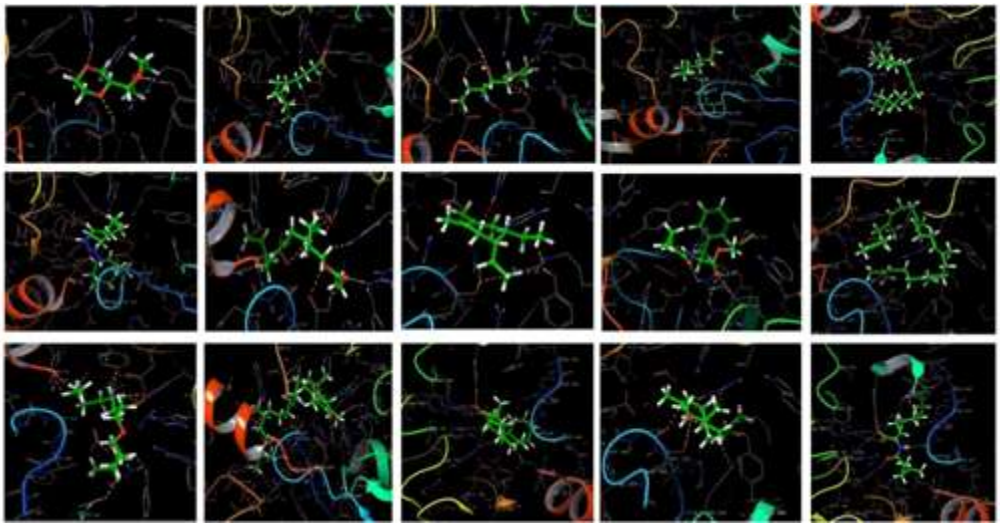


Figure 2 (3D) : **Top row L to R:** 2,2'-BI-1,4-Dioxane, Undecanoic acid, Meso-4,5-Octanediol, 9,12-octadecadienoic Acid (z,z)-, 9-Eicosyn.**Middle row L to R:**9-azabicyclo[6.1.0]nonane, 9,9'-azobis-, [1.Alpha.,8.Alpha.,9[e(1'r*,8's*)]], E-7-Dodecen-1-ol Acetate, Cyclohexene, 4,5-diethyl-1,2-dimethyl-, Cis-L-phenylalanine, N-cyclopropylcarbonyl-, Methyl ester, Squalene**Last row L to R :** Hexyl Octyl Ether, DL-.Alpha.-Tocopherol, D-norandrostane-16-carboxylic Acid, (5.Alpha.,16.Beta.), 2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene, Cilastatin.

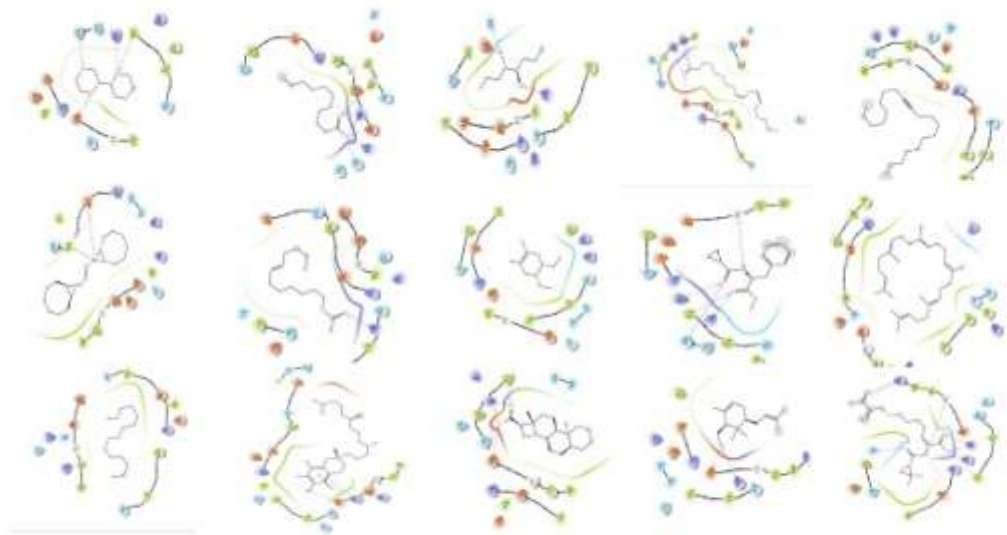


Figure 3 (2D) : Top row L to R: 2,2'-BI-1,4-Dioxane, Undecanoic acid, Meso-4,5-Octanediol, 9,12-octadecadienoic Acid (z,z)-, 9-Eicosyne.**Middle row L to R:** 9-azabicyclo[6.1.0]nonane, 9,9'-azobis-, [1.Alpha.,8.Alpha.,9[e(1'r*,8's*)]], E-7-Dodecen-1-ol Acetate, Cyclohexene, 4,5-diethyl-1,2-dimethyl-, Cis-L-phenylalanine, N-cyclopropylcarbonyl-, Methyl ester, Squalene. **Last row L to R :** Hexyl Octyl Ether, DL-.Alpha.-Tocopherol, D-norandrostane-16-carboxylic Acid, (5.Alpha.,16.Beta.), 2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene, Cilastatin.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

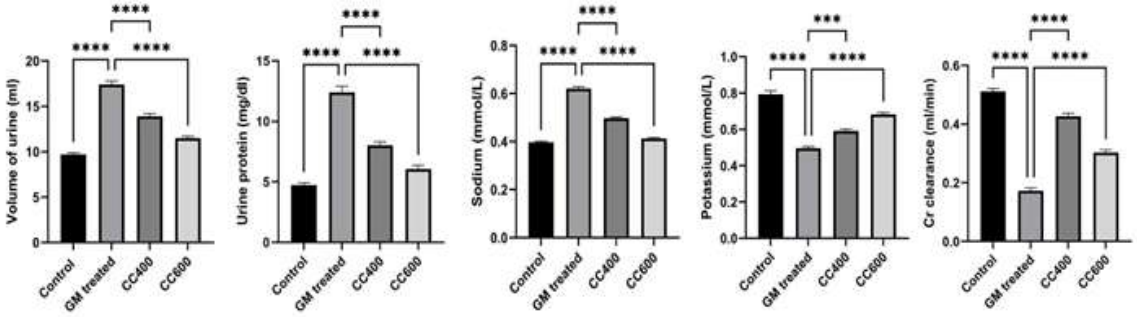


Figure 4: Effect of C cajan ethanolic leaves extract on urine biochemical parameters against.

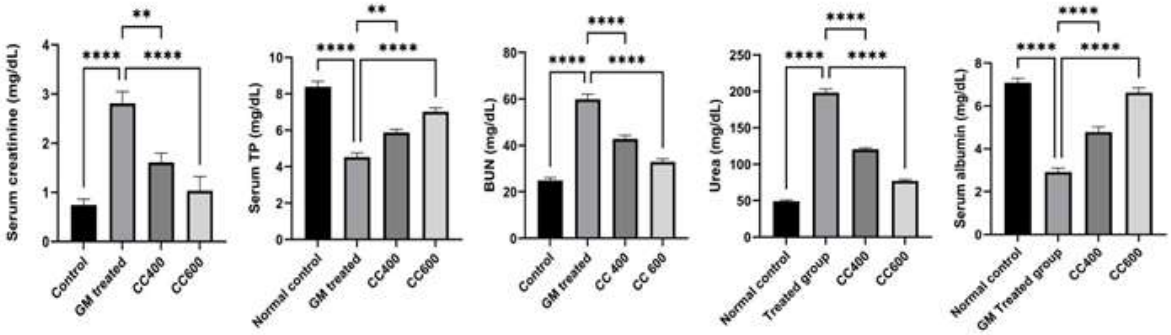


Figure 5: Effect of C. cajan ethanolic leaves extract on serum biochemical parameters against GM induced nephrotoxicity.

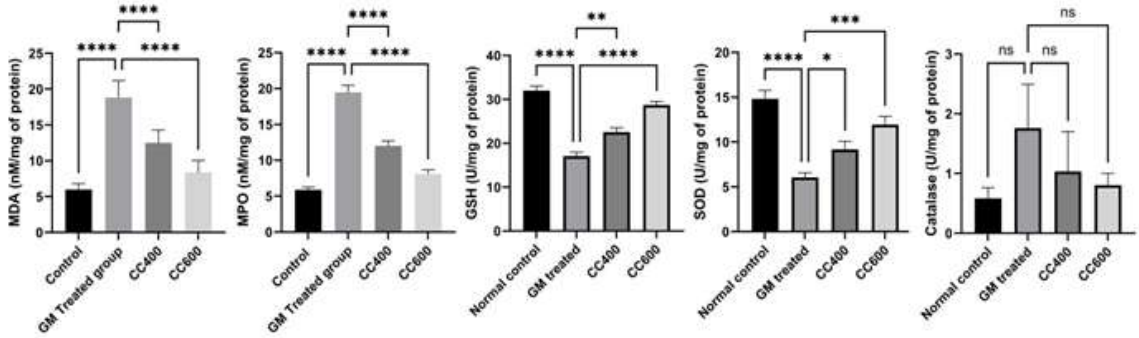


Figure 6: Effect of C. cajan ethanolic leaves extract on oxidative stress parameters against GM induced nephrotoxicity.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

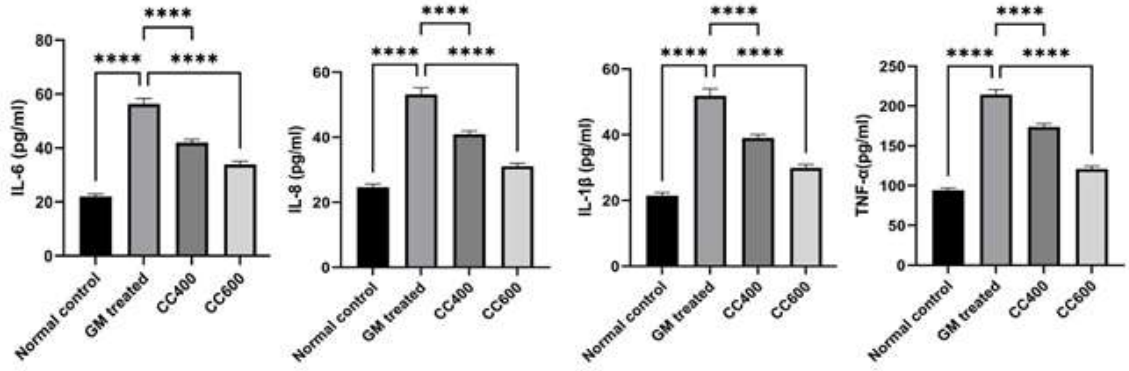


Figure 7: Effect of C. cajan ethanolic leaves extract on pro- inflammatory markers against GM induced nephrotoxicity.

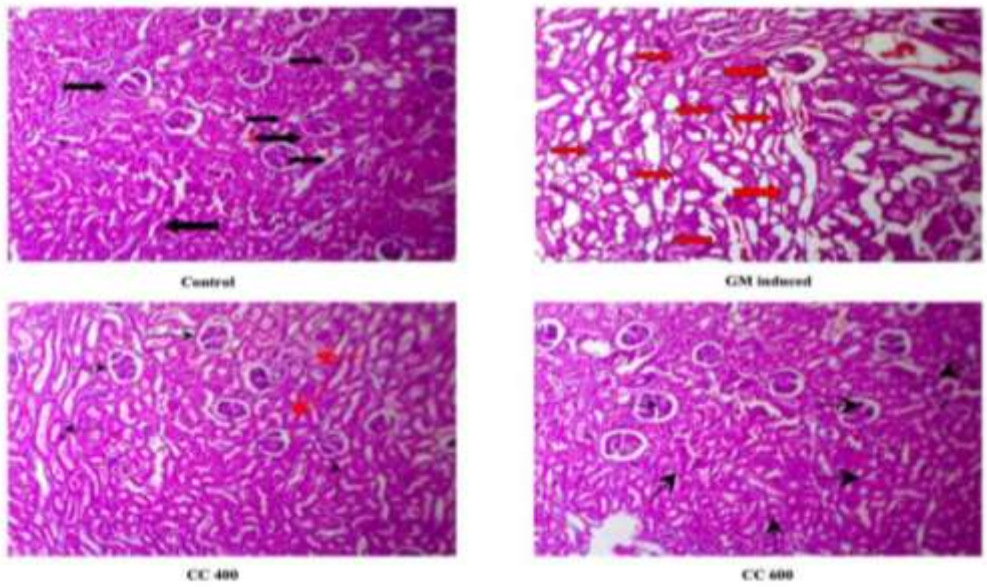


Figure 8: H & E-staining histology of renal tissues (10x) of various treatment groups against GM induced nephrotoxicity.

Table 1: GC–MS bio active compounds of ethanolic leaves extract C. cajan

S.No.	Peak Name	Molecular Formula	Molecular Weight	Retention Time	% Area
1.	2,2'-Bi-1,4-Dioxane	$C_8H_{14}O_4$	174	16.380	21.511
2.	Undecanoic Acid	$C_{11}H_{22}O_2$	186	17.855	2.625
3.	Meso-4,5-Octanediol	$C_8H_{18}O_2$	146	17.955	1.366
4.	9,12-Octadecadienoic Acid (Z,Z)	$C_{18}H_{32}O_2$	280	19.481	27.419
5.	9-Eicosyne	$C_{20}H_{38}$	278	19.641	18.047
6.	9-Azabicyclo[6.1.0]Nonane, 9,9'-Azobis-,	$C_{16}H_{28}N_4$	276	20.326	1.921

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

	[1.Alpha.,8.Alpha.,9[E(1'r*, 8's*)]]				
7.	E-7-Dodecen-1-Ol Acetate	$C_{14}H_{26}O_2$	226	22.147	1.538
8.	Cyclohexene, 4,5-Diethyl-1,2-Dimethyl	$C_{12}H_{22}$	166	22.252	0.995
9.	L-Phenylalanine, N-Cyclopropylcarbonyl-, Methyl Ester	$C_{14}H_{17}O_3N$	274	23.688	0.959
10.	Squalene	$C_{14}H_{30}O$	410	24.183	1.840
11.	Hexyl Octyl Ether	$C_{30}H_{50}$	214	24.578	2.023
12.	Silane, Trichlorodocosyl-	$C_{22}H_{45}Cl_3Si$	442	25.163	1.180
13.	Dl-.Alpha.-Tocopherol	$C_{29}H_{50}O_2$	430	26.514	1.962
14.	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,	$C_{30}H_{48}O$	424	26.874	4.051
15.	D-Norandrostane-16-Carboxylic Acid, (5.Alpha.,16.Beta.)-	$C_{19}H_{30}O_2$	290	28.875	2.468
16.	2,4,4-Trimethyl-3-Hydroxymethyl-5a-(3-Methyl-But-2-Enyl)-Cyclohexen	$C_{15}H_{26}O$	222	29.355	7.946
17.	2-Hexanone, 3-Methyl-4-Methylene-	$C_8H_{14}O$	126	29.690	2.150

Table 2: Dock score and dG binding values of extract bioactive compounds.

S.No.	Compound Name	2D Structure	Dock Score	dG Binding
1	2,2'-BI-1,4-DIOXANE		-3.441	-38.54
2	UNDECANOIC ACID		-5.090	-33.76
3	MESO-4,5-OCTANEDIOL		-4.351	-43.14
4	9,12-OCTADECADIENOIC ACID (Z,Z)-		-6.566	-41.16
5	9-EICOSYNE		0.180	-41.83

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

6	9-AZABICYCLO[6.1.0]NONANE, 9,9'-AZOBIS-, [1.ALPHA.,8.ALPHA.,9[E(1'R*,8'S*)]]-		-4.859	-41.50
7	E-7-DODECEN-1-OL ACETATE		-0.754	-44.06
8	CYCLOHEXENE, 4,5-DIETHYL-1,2-DIMETHYL-, CIS-		-1.398	-25.54
9	L-PHENYLALANINE, N-CYCLOPROPYLCARBONYL-, METHYL ESTER		-3.299	-42.18
10	SQUALENE		-1.727	-43.40
11	HEXYL OCTYL ETHER		-0.128	-31.01
12	DL-. ALPHA.-TOCOPHEROL		-3.761	-36.24
13	D-NORANDROSTANE-16-CARBOXYLIC ACID, (5.ALPHA.,16.BETA.)-		-10.012	-34.25
14	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXENE		-2.808	-42.84
15	Reference Cilastatin		-9.206	-65.12

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

Table 3: Hydrogen bonding 2D interaction and bond length distance of extract molecule.

Compound Name	2D INTERACTION	DISTANCE (A^0)
2,2'-BI-1,4-DIOXANE	H-Bond: HIE152, ASH288, ARG230, TYR255	2.20,2.09, 2.74,1.85
UNDECANOIC ACID	H-Bond: ASH288, ARG230, TYR255, ASN250 Salt-Bridge: HIP198, ARG230	2.21,1.77,1.66, 2.72 4.87,4.72
MESO-4,5-OCTANEDIOL	H-Bond: HIE152, GLU125, ARG230	1.77,1.91,2.68
9,12-OCTADECADIENOIC ACID (Z,Z)-	H-Bond: ASH288, ARG230, TYR255 Salt-Bridge: HIP198, ARG230	1.81,2.24,1.87 3.81,4.73
9-EICOSYNE	NO INTERACTION	NO DISTANCE
9-AZABICYCLO[6.1.0]NONANE, 9,9'-AZOBIS-, [1.ALPHA.,8.ALPHA.,9[E(1'R*,8'S*)]]-	Salt-Bridge:ASP22 Pi-Cation: TRP25	4.30 4.72
E-7-DODECEN-1-OL ACETATE	H-Bond: ARG230	2.01
CYCLOHEXENE, 4,5-DIETHYL-1,2-DIMETHYL-, CIS-	NO INTERACTION	NO DISTANCE
L-PHENYLALANINE, N-CYCLOPROPYLCARBONYL-, METHYL ESTER	H-Bond: HIE152, GLY291, ARG230, TYR255, HIP198	2.49,1.89,2.14, 1.91,2.21
SQUALENE	NO INTERACTION	NO DISTANCE
HEXYL OCTYL ETHER	NO INTERACTION	NO DISTANCE
DL-.ALPHA.-TOCOPHEROL	H-Bond: ASH288 Pi-Pi Stacking: HIP198 Pi-Cation: HIP198	2.10 5.27 5.63
D-NORANDROSTANE-16-CARBOXYLIC ACID, (5.ALPHA.,16.BETA.)-	H-Bond: ASH288, ARG230, TYR255, ASN250 Salt-Bridge: ARG230	1.85,1.67,1.83 2.61 4.91
2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXENE	H-Bond: ASH288, ARG230	2.05,2.62
Reference Cilastatin	H-Bond: ARG294, HIE152, GLY291, ASH288, ARG230, ARG230, TYR255 Salt-Bridge: ARG294, HIP198	2.07,1.89,2.01, 2.10,1.86,2.26,1.68 4.24,4.26

Table 4: ADME prediction Swiss ADME http://www.swissadme.ch/index.php

Sr.No.	Compound Name	MW	H-bond Acceptor	H-bond Donors	Log P	GI Absorption	BBB Permeant	Bioavailability Score	Lipinskin Violation	Synthetic Accessibility

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

1	2,2'-BI-1,4-DIOXANE	174.19 g/mol	4	0	2.13	**High**	No	0.55	0	3.39
2	UNDECANOIC ACID	186.29 g/mol	2	1	2.74	High	Yes	0.85	0	1.17
3	MESO-4,5-OCTANEDIOL	146.23 g/mol	2	2	2.10	High	Yes	0.55	0	2.28
4	9,12-OCTADECADIENOIC ACID (Z,Z)-	280.45 g/mol	2	1	5.8845	-	-	-	-	-
5	9-EICOSYNE	278.52 g/mol	0	0	5.43	Low	No	0.55	1	4.94
6	9-AZABICYCLO[6.1.0]NONANE, 9,9'-AZOBIS-, [1.ALPHA.,8.ALPHA.,9[E(1'R*,8'S*)]]-	276.42 g/mol	2	0	3.78	High	Yes	0.55	0	4.44
7	E-7-DODECEN-1-OL ACETATE	226.36 g/mol	2	0	3.53	High	Yes	0.55	0	2.60
8	CYCLOHEXENE, 4,5-DIETHYL-1,2-DIMETHYL-, CIS-	166.30 g/mol	2	0	3.14	Low	Yes	0.55	1	3.83
9	L-PHENYLALANINE, N-CYCLOPROPYLCARBONYL-, METHYL ESTER	247.29 g/mol	3	1	2.39	High	Yes	0.55	0	2.24
10	SQUALENE	410.72 g/mol	0	0	10.605	-	-	-	-	-

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

11	HEXYL OCTYL ETHER	214.39 g/mol	1	0	4.18	High	Yes	0.55	0	2.62
12	SILANE, TRICHLORODOCOSYL-	444.04 g/mol	0	0	6.51	Low	No	0.55	1	4.67
13	DL-.ALPHA.-TOCOPHEROL	430.71 g/mol	2	1	6.04	Low	No	0.55	1	5.17
14	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A,14B-OCTADECAHYDRO-2	-	-	-	-	-	-	-	-	-
15	D-NORANDROSTANE-16-CARBOXYLIC ACID, (5.ALPHA.,16.BETA.)-	290.44 g/mol	2	1	3.04	High	Yes	0.85	1	4.05
16	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXENE	222.37 g/mol	1	1	3.21	High	Yes	0.55	0	3.98
17	Reference Cilastatin	358.45 g/mol	6	4	1.79	Low	No	0.11	0	4.22

Table 5: Protox=II TOXICITY PREDICTION (https://biosig.lab.uq.edu.au/pkcsm/)

Sr.no.	**Compound Name**	**AMES tox**	**Max. tolerated dose in**	**hERG I inhibitor**	**hERG II inhibito**	**Oral rat acute toxicit**	**Oral rat chronic toxicity (LOAEL**	**Hepatotoxici**	**Skin sensitization**	**T. pyriformis toxicity**	**Minnow toxicity (log**

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

		icity (yes/no)	human (log mg/kg/day)	(yes/no)	r (yes/no)	y (LD50) (mol/kg)	) (log mg/kg_bw/day)	ty (yes/no)	(yes/no)	(log µg/L)	mM)
1	2,2'-BI-1,4-DIOXANE	Yes	0.838	No	No	**2.025**	1.906	Yes	Yes	-0.727	2.713
2	UNDECANOIC ACID	No	-0.168	No	No	1.569	2.812	No	Yes	0.246	0.192
3	MESO-4,5-OCTANEDIOL	No	0.998	No	No	1.845	2.818	No	Yes	1.845	1.721
4	9,12-OCTADECADIENOIC ACID (Z,Z)-	No	-0.957	No	No	1.614	3.179	Yes	Yes	0.37	-1.31
5	9-EICOSYNE	No	-0.199	No	Yes	1.53	1.2	No	Yes	1.114	-2
6	9-AZABICYCLO[6.1.0]NONANE, 9,9'-AZOBIS-, [1.ALPHA., 8.ALPHA.,9[E(1'R*,8'S*)]]-	No	-0.242	No	No	2.87	0.467	No	Yes	0.868	1.437
7	E-7-DODECEN-1-OL ACETATE	No	0.21	No	No	1.565	2.638	No	Yes	2.084	-0.129
8	CYCLOHEXENE, 4,5-	No	0.511	No	No	1.692	2.531	No	Yes	1.134	0.547

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

	DIETHYL-1,2-DIMETHYL-, CIS-										
9	L-PHENYLALANINE, N-CYCLOPROPYLCARBONYL-, METHYL ESTER	Yes	0.116	No	No	2.13	1.31	No	No	1.286	1.116
10	SQUALENE	No	-0.191	No	Yes	1.819	0.962	No	No	0.484	-3.62
11	HEXYL OCTYL ETHER	No	0.442	No	No	1.473	1.154	No	Yes	2.33	-0.334
12	SILANE, TRICHLORODOCOSYL-	No	-0.16	No	Yes	2.472	0.621	No	Yes	0.434	-3.911
13	DL-.ALPHA.-TOCOPHEROL	No	0.595	No	Yes	1.965	2.365	No	No	0.979	-3.281
14	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,1	-	-	-	-	-	-	-	-	-	-

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 155-176 www.ijprajournal.com ISSN: 2456-4494

	4A,14 B-OCTA DECA HYDR O-2										
15	D-NORA NDRO STAN E-16-CARB OXYL IC ACID, (5.AL PHA., 16.BE TA.)-	NO	-0.644	No	Yes	2.301	2.411	No	No	0.399	-0.641
16	2,4,4-TRIM ETHY L-3-HYDR OXY METH YL-5A-(3-METH YL-BUT-2-ENYL)-CYCL OHEX ENE	Yes	0.367	No	No	1.758	1.382	No	Yes	1.842	0.592
17	Refere nce Cilasta tin	No	-0.852	No	No	2.172	1.548	No	No	0.279	1.938

Table 6: Effect of C. cajan ethanolic extract on body weight and kidney weight in GM induced nephrotoxicity.

Groups	**Initial Body weight(gm)**	**Final Body weight(gm)**	**Kidney weight(gms)**
Normal Control	175±4.98	183±4.06	1.013±0.03
GM treated	173±3.91	148±3.98	1.865±0.1
C cajan 400	175±4.23	165±3.66**	1.502±0.03**
C cajan 600	176±4.01	167±3.45**	1.293±0.02**