



## Nephrotoxicity Evaluation of Methanolic Extracts of *Calliandra surinamensis* Seeds in Rodents

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### Abstract

*Calliandra surinamensis* seed has been used historically in folk medicine for various purposes. However, its safety profile, particularly its potential nephrotoxic effects, remains unknown. This study aimed to investigate the nephrotoxic effects of the methanolic extract of *Calliandra surinamensis* seed on kidney functions, along with histopathology assessment of the kidneys. Acute toxicity and sub-chronic toxicity methods were conducted in Wistar rats using modified Lörks' method, following the Organization of Economic Cooperation and Development (OECD) guidelines, with testing chemicals 423 and 407 to determine the nephrotoxic effects of the seed. The mean Lethal Dose (LD50) could not be determined as there was no mortality. The kidney function parameters indicated an increase in the serum urea in the group treated with 400 mg/kg and 800 mg/kg of the extract, with mean values of 25.58 and 29.54, respectively, suggesting reduced renal clearance or increased protein catabolism. The Creatinine levels remained relatively stable, despite a slight increase at 800 mg/kg (0.39). However, values across all groups are comparable, including the control (0.40), suggesting no glomerular filtration impairment, as creatinine is a more stable marker. There were disturbances in electrolyte balance with more pronounced effects at the highest dosage (800 mg/kg). The average organ weight of the group treated with the seed extract and the control showed statistically no significant difference ( $P>0.05$ ). These findings highlight that the methanolic extract of *Calliandra surinamensis* seed did not exhibit any nephrotoxic effects in Wistar rats, particularly at higher doses. However, further histopathological assessment and urine analysis would be beneficial to confirm and elaborate on the kidney parameters.

**Keywords:** *Calliandra Surinamensis* Seed, Wistar Rat, Kidney, Acute Toxicity, Sub-Chronic Toxicity

### Introduction

Kidneys are vital organs in the body, and they play a significant role in maintaining the overall health of the body by performing several critical functions: they are two bean-shaped organs located in the lower back, just below the ribcage. The kidneys help maintain healthy bones by regulating calcium and phosphate levels and producing hormones that help regulate bone metabolism (McKenna et al., 2024). They regulate the removal of waste, such as urea, creatinine, and other toxins, from the blood, maintain electrolyte balance by adjusting the amount excreted in the urine, and control fluid balance by adjusting the amount of water reabsorbed into the bloodstream or excreted in the urine. (Feraille et al., 2022). The kidneys play a vital role in maintaining healthy blood pressure by regulating fluid balance, electrolytes, and producing blood pressure-regulating hormones. (Pearce et al 2022; Castaneda-Bueno et al., 2022). Kidneys process everything that goes into the body, such that over time, a bad diet can put the kidneys at risk. So the need to keep the kidneys healthy cannot be overemphasized. Plant-based foods, like legumes, are linked to a decreased risk of inflammatory disease (Orlich & Fraser, 2016). Various plant-based foods, such as fruits, seeds, and vegetables, are rich in flavonoid compounds with antioxidant properties that help protect cells from damage caused by free radicals and have anti-inflammatory effects that reduce inflammation, which can contribute to chronic diseases. (Craddock et al., 2019). Kaempferol and quercetin are common flavonoid glycoside compounds found in many plants possessing multiple biological activities (Aghababaei & Hadidi, 2023; Khazdair et al., 2021).

Kaempferol and quercetin have been examined for their potential health benefits, including reducing the risk of oxidative stress and inflammation in the kidneys, inhibiting pro-inflammatory enzyme activity, and enhancing the activity of antioxidant enzymes. (Chunlian et al., 2021). *Calliandra surinamensis*, commonly known as Pink Powder Puff, a leguminous flowering plant native to South America, with widespread distribution in Nigeria, East, and South Africa (Irabor et al., 2023), has been shown by research to possess various bioactive compounds, including flavonoids, alkaloids, and glycosides, which may contribute to its potential nephroprotective effects (Falodun et al., 2010). Recent research has demonstrated that phytochemicals present in *Calliandra surinamensis* exhibit antioxidant and anti-inflammatory activities, which may help protect the kidneys from oxidative stress and inflammation (Mans et al., 2020; Falodun et al., 2010). Studies have highlighted the pharmacological therapeutic properties of *Calliandra surinamensis*, including its antimicrobial (Falodun et al., 2010), anti-inflammatory, and antioxidant activities (Deshmukh et al., 2024). The plant's potential uses in medicine have also been explored, with research suggesting its efficacy in treating various ailments, including fever, rheumatism, and skin conditions (Irabor et al., 2023).

**Table 1: scientific classification of *Calliandra surinamensis***

Binomial Name	<i>Calliandra surinamensis</i>
Common Name	Pink Powder Puff
Kingdom	Plantae
Order	Fabales
Family	Febaceae
Genus	<i>Calliandra</i>
Species	<i>Surinamensis</i>



Plate 1: *Calliandra surinamensis* Tree



Fig 1. Leaves of *C. Surinamensis*



Fig 2. Flowers of *C. Surinamensis*



Plate 2. Seeds of *C. Surinamensis*

Furthermore, *Calliandra surinamensis* has been reported to possess antiviral (Sarker et al., 2018) and antidiabetic properties (Kumar et al., 2020), making it a promising candidate for developing novel therapeutic agents. Research is needed to elucidate the nephroprotective effects of *Calliandra surinamensis* and explore its potential as a therapeutic agent for preventing and treating kidney diseases. This study evaluates the effects of *Calliandra surinamensis* seed consumption on renal function in rats, with a focus on its physiological implications.

## Materials and Methods

### Plant Collection and Authentication

*Calliandra surinamensis* pods containing dry seeds were collected from their natural habitat. A botanist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Edo State, confirmed and verified the Plant's identity.

### Sample Preparation

The pod was shelled to extract the dry seeds, which were then finely powdered using a mechanical blender. Methanol served as the solvent for obtaining the crude components, after which the extract was concentrated using a rotary evaporator and then refrigerated at 4°C for subsequent analysis.

### Animal Model

This study utilized Swiss mice and Albino Wistar rats obtained from the Department of Pharmacology's Animal House at the University of Benin. The animals underwent a 14-day acclimatization period before being used in the experiment. Ethical clearance for the study was provided by the Institutional Ethics Committee of the Faculty of Life Sciences, University of Benin (Reference No. LS19018).

### Experimental Design

The study consists of two phases: an acute toxicity phase and a chronic toxicity phase.

#### Acute Toxicity Phase

Acute toxicity evaluations were conducted on mice using a modified version of Lörke's method, adhering to the guidelines provided by the Organisation for Economic Co-operation and Development (OECD) for testing chemicals (No. 423 OECD, 2001). A total of 24 male Swiss mice, weighing between 26.9 and 31.0 g, were randomly grouped into four groups of three mice each. In the initial phase, 12 mice were utilized, with Groups 1, 2, and 3 receiving a single dose of the methanolic crude extract at concentrations of 10, 100, and 1000 mg/kg body weight, respectively. Group 4 served as the control group. The mice were observed for 24 hours. The first phase results then guided the dosing for the second phase, with 12 mice organized into four groups of three mice each. This time, Groups 1, 2, and 3 received a single oral dose of 1600, 2900, and 5000 mg/kg of the methanolic seed extract, respectively, while Group 4 continued as the control. No mortality was recorded in the mice over a 14-day monitoring period. The oral administration of the seed extract to mice did not result in any fatalities during the acute toxicity evaluations in either phase.

#### Chronic Toxicity Study

The oral chronic toxicity study was carried out in compliance with the OECD Guideline for the Testing of Chemicals No.407. 16 Albino Wistar rats were randomly assigned to 4 groups of 4 rats each. Group 1, 2, and 3 received a daily dose of the seed extract orally at a dose level of 200, 400, and 800 mg/kg body weight, respectively, for 28 days, while Group 4 served as the control for the 28 days. The weights of the animals for the 28 days were recorded. On day 29, the animals were sacrificed under chloroform anesthesia. Blood samples collected in dry tubes were allowed to completely clot and then centrifuged at 3,500 rpm at 4 °C for 15 minutes. The kidney organs were isolated and weighed for renal histopathological examination.

### Observations and Measurements

Daily observations monitored mortality, clinical signs, and symptoms pre-and post-dosing. Body and organ weights were recorded.

#### Determination of body and organ weight

The weight of Wistar rats across the groups was recorded using the Weigh 7001DX Multi-purpose Digital Scale on days 0, 7, 14, 21, and 28. The net change of body weight (difference between final body weight and initial body weight) was recorded for all animals. The visceral organ weight of the kidney was determined using the formula:

$$\text{Relative organ weight} = \frac{\text{Organ weight} \times 100}{\text{Body weight}} \text{----- (1)}$$

### Biochemical Analysis

**Renal Function test;** the assay for urea, creatinine, and electrolytes was carried out.

**Assay for urea:** This test exploits the hydrolysis of urea into ammonia by the urease enzyme. 100 µl of reagent 1 was added to 10 µl (0.01 cm<sup>3</sup>) of serum sample in a test tube. 100 µl of the reagent 1 was added to 10 µl water as a blank. Solutions in test tubes were thoroughly mixed for incubation for 10 minutes at 37 °C. Similarly, 2.5 mL of reagents 2 and 3 were added to the test tubes. Solutions in test tubes were properly mixed for incubation for 10 minutes at 37 °C. Sample solution absorbance and standard against blank with a 546 nm wavelength. Urea concentration calculation is thus: (Van Skye & Neil, 1924).

$$\text{Urea concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 80 \text{----- (2)}$$

**Assay for creatinine:** This test exploits the reaction of creatinine present in an alkaline solution containing picric acid, producing a complex coloration. The quantity of complex colour synthesized directly relative to creatinine concentration. The sample of macro and semi-micro of 0.2 ml was added to the test tube. 0.2 ml of standard macro and 0.1 ml of semi-micro were added to the standard solution test tube. Two (2) ml of standard macro, 1 ml of semi-micro, 2 ml of sample macro, and 1 ml of semi-micro were added to the working reagent test tube.

The solutions in the test tubes were properly mixed. Absorbances of samples and standards are being read at 492 nm. Creatinine values were calculated as illustrated by Tietz (1995).

$$\text{Creatinine level unit (mg/dl)} = \text{Absorbance of sample} / \text{Absorbance of standard} \times 2 \dots \dots \dots (14)$$

**Assay for electrolytes (sodium, chloride, bicarbonate, and potassium).** The assay for bicarbonate exploits the reaction between bicarbonate ions and dilute hydrochloric acid to yield carbon dioxide. The excess acid is titrated with dilute sodium hydroxide using phenol red as an indicator. Solution of 0.01 N HCl was added to 200 µl of the serum sample. The solution was mixed, and 1 drop of phenol red indicator was added. The mixture was titrated using 0.01 N sodium hydroxide to acquire a brick red colour, which serves as the endpoint (Van Skye & Neil, 1924). Bicarbonate was calculated as follows:

$$\text{Bicarbonate } (\mu\text{mol/l}) = 50 - \text{Titre } 5 \dots \dots \dots (3)$$

$$\text{Titre} = \text{Endpoint} \times 100 \dots \dots \dots (4)$$

The chloride assay involves the formation of a chloride precipitate in a sample by using mercuric nitrate. Titration of chloride with mercuric ions yields stable, undissociated mercuric chloride. Any excess mercuric chloride nitrate reacts with diphenylcarbazone, resulting in a violet hue. The analysis involved mixing 2 mL of deionized water with 200 µL of serum sample, followed by the addition of diphenylcarbazone indicator and nitric acid. The mixture was titrated with mercuric nitrate until a persistent violet color appeared, signaling the endpoint of the titration. The procedure was replicated under identical conditions using a chloride standard solution (Schales & Schales, 1941). The calculation of chloride concentration was determined using the equation:

$$\text{Chloride } (\mu\text{mol/l}) = \text{Titre of the sample} / \text{Titre of the standard} \times 100 \dots \dots \dots 5$$

The technique used to measure sodium and potassium involves aspirating solutions containing these elements into a flame, resulting in the formation of solid salt that dissociates to reach a neutral ground state. The atoms are excited by the flame, causing them to move to a higher energy level. As the excited atoms return to their ground state, they emit light at characteristic wavelengths (590 nm for sodium and 770 nm for potassium). This light then passes through an appropriate filter to a photosensitive component, with the measured current being proportional to the levels of sodium and potassium present in the serum sample (Magoshes, 1956).

### Statistical Analysis

The data obtained from the rats from each group for four weeks were statistically expressed as standard error of mean (SEM). The data was further analysed using ANOVA with Turkey's Post hoc test to compare the levels of significance between control and experimental groups.

All statistical analysis were evaluated using the IBM SPSS version 20 software and Microsoft excel. The values  $P < 0.05$  were considered statistically significant.

### Results

**Table 2: Average Organ Body Weight**

Organs	200mg/kg	400mg/kg	800mg/kg	Control
Kidney	0.006±0.000	0.006±0.001	0.006±0.000	0.007±0.000

### Results of Kidney function test

The kidney functions by removing metabolic waste, controlling the amount of water needed, and maintaining electrolyte balance in the body.

**Table 3: showing an average level of each kidney function parameter in each rat group**

Groups	Urea (mg/dl)	Creatinine (mg/dl)	Bicarbonate (mg/dl)	Sodium (mg/dl)	Potassium (mg/dl)	Chlorine (mg/dl)
200mg/kg	23.66±0.17	0.35±0.02	12.31±1.10	98.41±3.16	26.61±0.96	65.75±2.68
400mg/kg	25.58±0.27	0.36±0.01	12.93±1.00	99.18±0.47	25.88±0.25	69.26±0.40
800mg/kg	29.54±0.07	0.39±0.01	16.31±0.35	106.06±1.73	26.21±0.94	75.85±1.26
Control	24.24±0.08	0.40±0.02	13.02±0.93	101.09±1.19	25.30±0.74	71.13±0.43



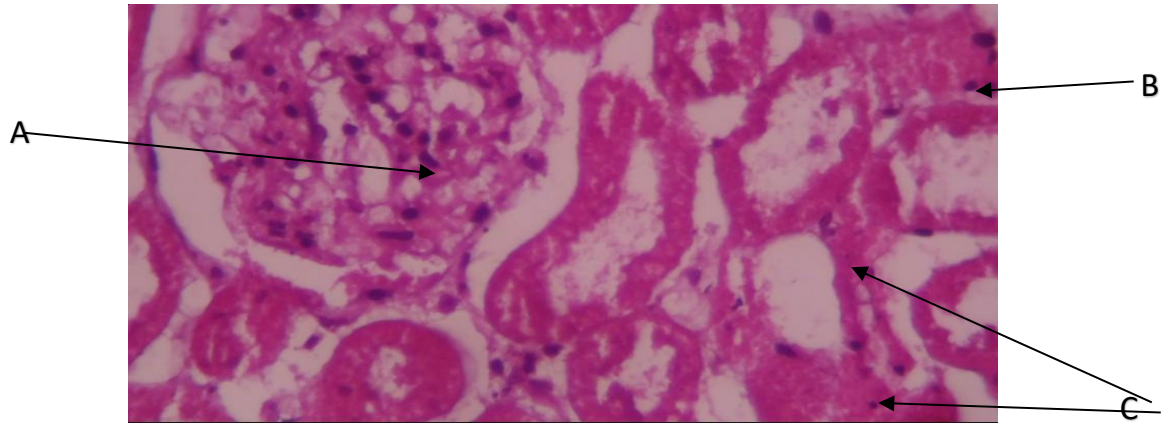


Plate 1. Rat kidney. Control. Composed of A: glomerulus, B, interstitial space and C, tubules (H&E x 400)

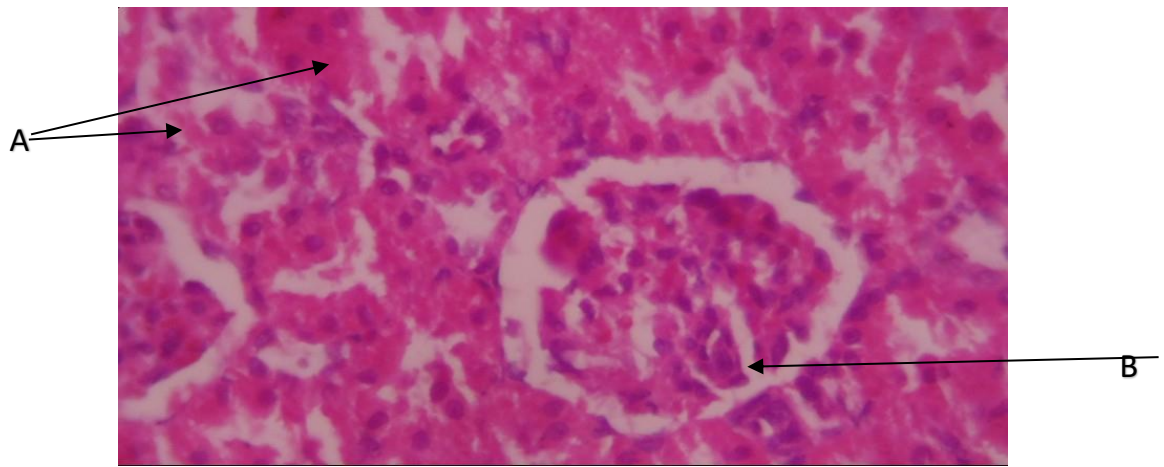


Plate 2. Kidney of rat given 200mg/kg *C. surinamensis* showing: A, normal tubular and B, glomerular architecture (H&E x 400).

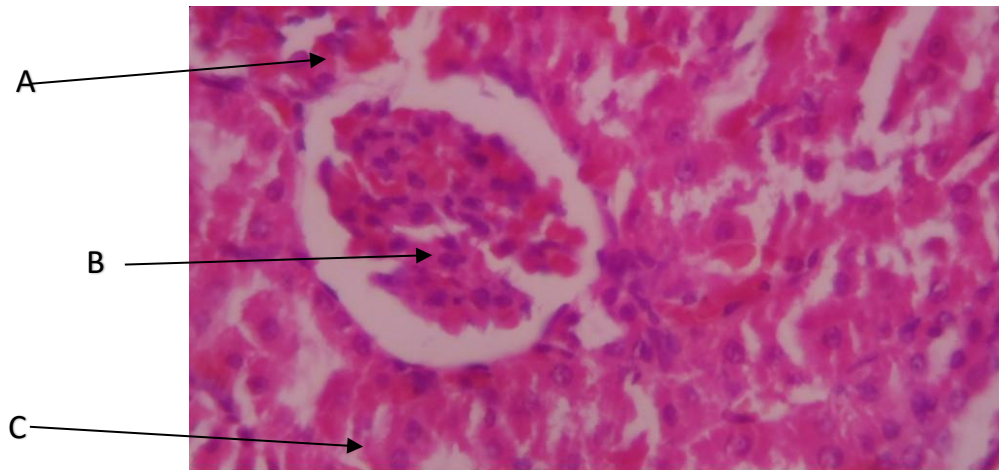


Plate 3. Kidney of rat given 400mg/kg *C. surinamensis* showing: A, active interstitial congestion, B, normal glomerular and C, tubular architecture (H&E x

400)

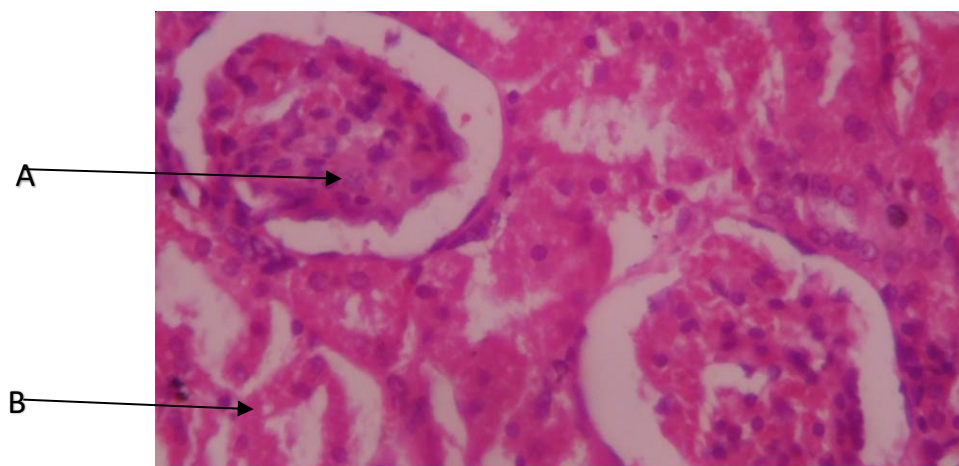


Plate 4. Kidney of rat given 800mg/kg *C. surinamensis* showing: A, normal glomerular and B, tubular architecture (H&E x 400)

**Blood Urea Nitrogen level;** From Table 3, the level of the Blood urea nitrogen (BUN) (mg/dl) increased progressively with dose, peaking at 800 mg/kg. The 800 mg/kg group (29.54 mg/dl) showed a notable rise compared to the control (24.24 mg/dl), suggesting potential decreased renal clearance at higher dosages. Statistical analysis showed  $P < 0.05$  across all the treated groups, indicating that there was a significant difference between the treated groups and the control. The increased concentration of urea was obviously expected from the breakdown of the protein consumed by the rats. The kidneys normally excrete urea, a by-product of protein metabolism, so elevated levels suggest filtration or excretion problems. Excessive protein consumption can raise urea and if prolonged, it can lead to uremic syndrome, cardiovascular disease, and immune impairment (Adeyomoye et al., 2022). However, the crude extract did not have a toxic effect on the kidney parameters.

Blood urea nitrogen, also known as BUN, is a waste from dietary protein metabolism. It is a way of removing ammonia, a waste product of protein. It is the nature of nitrogen in the blood in the form of urea. High BUN value is a marker of kidney damage or kidney disease (Salazar, 2014).

**Creatinine level;** From Table 3, the level of creatinine in the control was  $(0.40 \pm 0.02)$ . Serum creatinine is another sensitive marker of kidney function. Interestingly, the control group had the highest creatinine level (0.40 mg/dl), while the treated groups showed slightly lower levels, even at the highest dose, suggesting that Kidney function remained relatively normal. This may also reflect variations in muscle metabolism or limitations in detection sensitivity. However, Data analysis showed ( $P > 0.05$ ), indicating that there was no significant difference in the creatinine level across all groups treated with the crude extract, therefore suggesting no potential nephrotoxicity. Creatinine is a waste product originating from the breakdown of muscle. Creatinine the body through the kidneys through urine, such that low creatinine is a marker for low muscle mass or low protein diet, while on the other hand, high creatinine level is a prediction of kidney failure (Salazar, 2014).

**Bicarbonate level;** From Table 3, the bicarbonate level in control ( $13.02 \pm 0.93$ ), 200mg/kg ( $12.31 \pm 1.10$ ), and 400mg/kg ( $12.93 \pm 1.00$ ) body weight of the treated group are relatively similar, indicating no significant acid-base disturbance at lower dose. The statistical analysis showed  $P > 0.05$ , also indicating that there was no significant difference between them. However, the 800mg/kg body weight of the treated group ( $16.31 \pm 0.35$ ) in comparison with the control group had the highest bicarbonate level, which may suggest a compensatory metabolic alkalosis or altered acid-base handling at higher doses.

Bicarbonate is a key component in acid-base regulation and a byproduct of the body's metabolism. The bicarbonate is transported to the lungs, where the carbon dioxide is released. A high or low level of bicarbonate in the blood is an indication of either an accumulation of carbon dioxide in the lungs, or alternatively, an inability of the body to maintain its acid-base balance, or an electrolyte imbalance as a result of a deficiency of potassium. A high level of bicarbonate in the blood increases the pH of the tissues, a condition known as metabolic alkalosis, as a result of vomiting or dehydration (Geor & Coenen, 2013).

**Sodium:** According to Table 3, the sodium level peaked at 106.06 in the 800 mg/kg body weight group. In contrast, the 200 and 400 mg/kg groups had sodium levels slightly below the control (101.09), suggesting dose-dependent alterations in sodium homeostasis. The statistical analysis showed ( $P > 0.05$ ) across all treated groups,

indicating no significant difference between the control and the treated groups. This means that the administration of doses of the extract did not induce disruption in cell electrolytes, suggesting no potential nephrotoxicity. Sodium, in the blood, is a form of electrolytes in the body that help maintain fluid levels and balance acid and base. Sodium also helps the muscles and nerves function adequately. Hence, low levels of sodium in the blood, a condition known as hyponatremia, which is caused by the consumption of excessive fluid, can result in brain dysfunction. While a high level of sodium in the blood, hypernatremia can result from dehydration or loss of body fluids (Pohl et al., 2013; Gradual et al., 2014).

**Potassium levels;** From Table 3, the Potassium levels were quite comparable to the control. Quantitative analysis revealed no significant difference in potassium levels ( $p > 0.05$ ). Alternatively, various concentrations of *C. surinamensis* seed extracts did not influence any significant changes in the serum electrolytes of the treated animals, suggesting there was no potential nephrotoxicity.

Potassium in the blood is an electrolyte vital for the proper functioning of the nerves and muscles in the body. Potassium is vital in regulating nerve signals, muscle contractions, and fluid balance within body cells. Sufficient potassium levels help lower blood pressure, reduce water retention, and protect against stroke. This also prevents osteoporosis and kidney stones, on the other hand, potassium deficiency can lead to muscle weakness, cramps, and abnormal heart rhythms can cause muscle weakness, cramps, and abnormal heart rhythms (Elia et al., 2011).

**Chloride levels;** From Table 2, the serum chloride levels increased progressively with dosage, peaking at the 800mg/kg (75.85). Statistical analysis showed  $P < 0.05$ , indicating a significant difference in the serum chloride level across all the treated groups compared to the control group. The mean chloride levels across groups fell within normal reference ranges. Chloride plays a crucial role in maintaining acid-base balance, fluid balance, blood pressure, and pH levels, with the kidneys tightly regulating its levels. High chloride levels can lead to hyperchloremia, indicating dehydration or kidney injury (Renaud et al., 2023).

## Discussion

Table 2 above shows the average organ body weight index of the various rat groups. The kidney ratio values are nearly the same across all doses (0.006), and only slightly higher in the control group (0.007). The standard deviations ( $\pm$ ) are very small or zero, indicating low variability in the data. There is no significant difference in the change in kidney weight relative to body weight across increasing doses (200, 400, 800 mg/kg), suggesting no potential nephrotoxic effects at these doses. Serum creatinine is a more accurate indicator than blood urea for assessing kidney function, as it remains unaffected by dietary intake. It is noted that a rise of 50% or more from the initial creatinine level by 2 mg/dL is regarded as an early sign of acute renal failure (Al-Naimi et al., 2019). Interestingly, the control group exhibited the highest creatinine concentration (0.40 mg/kg), whereas the treated groups presented somewhat lower levels, even at the maximum dosage. Therefore, there were no potential nephrotoxic effects. Potassium levels were relatively consistent across all groups, with a slight increase in the 200 mg/kg and 800 mg/kg groups compared to the control. These variations fall within a narrow range and may not indicate significant potassium abnormalities.

The kidney consists of the glomerulus, capillaries surrounded by a clear space, the Bowman space, bound by walls, the tubules called the Bowman's capsule, which are well defined. The glomeruli are so tough and resilient that the only thing that can damage them will be something that can cause a dysfunction in the immune system (Meijer-Schaap et al., 2018). Most glomeruli pathologists are immune to dysfunctional pathologies. So if that agent can affect the immune mechanism, it can damage the glomerulus; otherwise, the injuries will appear in the tubules. The tubules constitute about seventy-five percent of the renal mass. So, where there is renal failure, it then means that over seventy-five percent of the tubules are damaged, and only less than twenty-five percent are left (Al-Naimi et al., 2019).

**200mg/kg** body weight; in plate 2, the glomeruli were beautiful, appear more compact and normal in structure with preserved capillary loops and no apparent hyper-cellularity or sclerosis. The tubules are uniform and less disrupted, maintaining typical architecture and cytoplasmic detail, suggesting no potential nephrotoxic effects.

**400mg/kg** body weight; in plate 3, the two glomeruli were still beautiful and clearly defined. There was increased blood flow in the interstitial spaces. The tubules appeared normal and regular in appearance suggesting no potential tubular dysfunction.

**800mg/kg** body weight showed two glomeruli which are appear relatively normal with good tubular architecture. The interstitial area shows no significant inflammation, indicating no nephrotoxicity.

## Conclusion



The biochemical parameters (urea, creatinine, bicarbonate, sodium, potassium, and chloride) across all treated groups show values that are within relatively normal physiological ranges. However, there are minor fluctuations, especially with the 800 mg/kg group showing slightly elevated urea and chloride levels, without renal dysfunction. Creatinine levels remain low and consistent across all groups, including control (ranging from 0.35-0.40 mg/dl), a strong indicator of preserved glomerular filtration rate (GFR). Urea levels were slightly elevated in the 800 mg/kg group but remained below the threshold for significant renal impairment. Bicarbonate and electrolyte levels (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>) are stable, suggesting normal acid-base and electrolyte handling by the kidneys. These findings align with the histological appearance in plates 2-4, where the kidney architecture appears well preserved, with clearly defined glomeruli and tubular structures. There is therefore no histological evidence of nephrotoxicity.

### Recommendation

The slight rise in urea and chloride at 800 mg/kg suggests the need for further monitoring of kidney parameters, especially over longer durations.

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