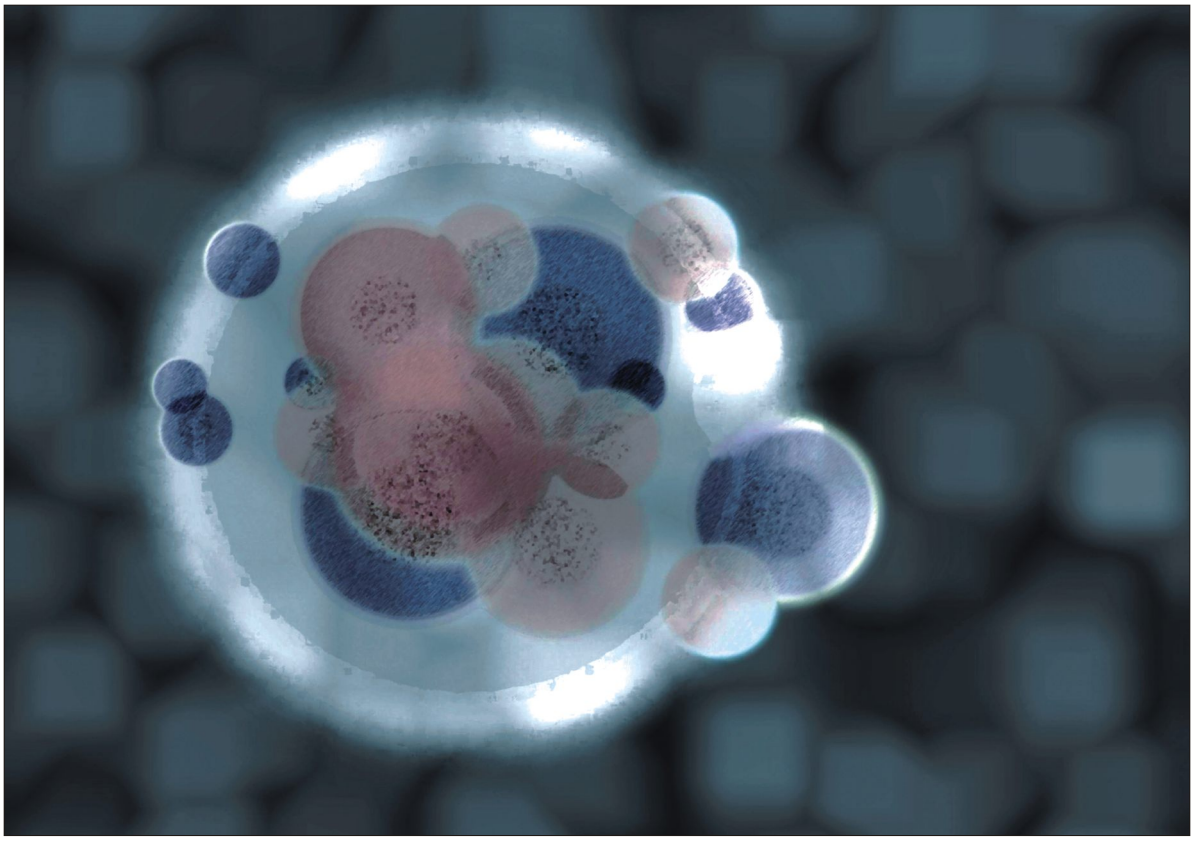

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Research Article
Cajanus cajan (L) Mill Sp. Leaf Extract Exacerbates Acute Renal Injury Induced by Acetaminophen in Albino Rats

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Abstract

Background and Objective: *Cajanus cajan* is traditionally used for the treatment of various ailments due to its antioxidant properties. The present study investigated the effect *C. cajan* crude aqueous leaf extract (*CCLE*) in rats with acetaminophen (APAP)-induced acute renal injury. **Materials and Methods:** Preliminary acute toxicity testing of the extract was conducted to determine the oral median lethal dose (LD_{50}). Twenty rats were divided into five experimental groups labelled (A-E) of 4 rats per cage. Group A served as the normal control group and was given only water and feed. Rats in groups C, D and E received 100 mg kg^{-1} b.wt., of ascorbic acid, 200 and 500 mg kg^{-1} of *CCLE*, respectively once daily for 7 days via oral route using an oral cannula. Thirty min after the daily treatments, 600 mg kg^{-1} b.wt., of acetaminophen (APAP) was administered orally to rats in groups B-E. At the end of the treatments, blood samples were collected from the animals via retro-orbital puncture for the estimation of serum urea, creatinine and uric acid levels. The rats were sacrificed under anaesthesia and the kidneys were excised for histopathological analysis. **Results:** Acute toxicity testing showed that *CCLE* has an oral LD_{50} of 8000 mg kg^{-1} b.wt. Serum biochemistry showed a statistically significant increase ($p>0.05$) in creatinine and uric acid levels in all treatment groups when compared to normal control. Histopathological findings revealed the observable renal injury, with *CCLE*-treated groups showing more severe histomorphological alterations when compared to the APAP (negative) control. **Conclusion:** Data obtained suggest that acute toxicity was not observed with *C. cajan* administration up to 8 g kg^{-1}, however, its co-administration with a high dose of acetaminophen does not protect the kidneys but rather aggravates the acute renal injury.

Key words: *Cajanus cajan*, acetaminophen, nephrotoxicity, kidney, histopathology, serum biochemistry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acute renal injury is a serious medical condition and is commonly caused by medications especially in patients who are exposed to many agents during treatment[1]. In developing countries like Nigeria, the unavailability of advanced surgical procedures and the difficulty in accessing dialyzing facilities, pose a great challenge to its management[2]. Cases of drug-induced acute renal injury (ARI) have been attributed to many drugs including an overdose of acetaminophen[3,4]. Toxic effects of medications on the kidneys often induce changes in renal function and structure[5]. The development of drug-induced ARI is dependent on several mechanisms including the inherent nephrotoxicity of the drug, its transport and metabolism by the kidney and an individual's underlying susceptibility to the toxicity of the drug[1].

Acetaminophen (Paracetamol) is a common drug prescribed in clinical practice either as an analgesic or for antipyretic purposes. Acute ingestion of acetaminophen at 150 mg kg^{-1} or 7.5-10 g/day could be fatal[6], resulting in centrilobular necrosis in the liver tissue which may usually be associated with acute renal tubular necrosis[7]. A previous report has documented renal damage resulting from chronic consumption of doses of acetaminophen without hepatic damage[8]. Conventionally, the drug is used to induce hepato-renal damage in experimental animal models in search of potent hepatoprotective and renoprotective agents[9-12].

Medicinal plants have a role to play in modern medicine and a large number of these plants are yet to be explored for their potential uses. *Cajanus cajan* (L) mill sp., commonly known as pigeon pea, have shown profound biological activities. It is a tropical leguminous cover crop belonging to the family of the Fabaceae[13]. It is a perennial plant that sometimes grows into an erect hairy shrub and may reach a height of 10 feet[14]. It has been widely cultivated in all tropical and semitropical regions but India contributes up to 90% of production in the world[15]. Previous researchers have documented the antioxidant, antidiabetic, hypolipidaemic, anticancer, hepatoprotective and antimicrobial effects of *C. cajan* extracts[13,15]. Due to its antioxidant properties, it has been used as a therapeutic agent against tissue damage resulting from oxidative stress[16,17]. Traditionally, it is also claimed to be beneficial in the treatment of kidney problems. Because of these, the present study was conducted to evaluate the effect of the crude aqueous leaf extract of *Cajanus cajan* on acetaminophen-induced renal injury in Albino rats.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Medical Laboratory Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria, Enugu Campus, Nigeria. This research work was conducted from 2012-2013.

Laboratory animals: Thirty-two Albino Wistar rats were procured and housed in the old site of College of Medicine Animal House, University of Nigeria, Enugu Campus. The animals were placed under standard conditions of temperature (25±3°C) and relative humidity (40-70%) and lighting of 12 hrs light/dark cycle. They were fed with standard commercial rat feed and water *ad libitum* and were left to acclimatize for 2 weeks before the commencement of the study. All the animal experiments were approved and performed in conformity with institutional protocols and the International guidelines for care and use of animals for scientific research.

Collection of plant materials: Fresh leaves of *Cajanus cajan* (*CCLE*) were obtained from the premises of Government Technical College, Nsukka, Enugu State. A sample of the plant material was identified and authenticated by comparison with a voucher specimen at the Herbarium section, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Preparation of plant extract: The leaves were shade-dried for 2 weeks after which 500 g was pulverized into a fine powder and subsequently extracted with 700 mL of distilled water using standard methods. The extractive value obtained after extraction was 120 mg mL^{-1}.

Acute toxicity testing: Preliminary acute toxicity testing was done according to a previously described method[18] using 12 rats. Clinical signs of toxicity were assessed hourly for 24 hrs after the drug administration. The oral median lethal dose (LD_{50}) was thereafter determined.

Experimental design: Twenty rats were divided into five groups of four rats each, labelled A to E. Group A and B received water and served as the normal and negative control groups, respectively. Groups C, D and E received 100 mg kg^{-1} b.wt., of vitamin C, 200 mg kg^{-1} b.wt., of *CCLE* and 500 mg kg^{-1} b.wt., of *CCLE*, respectively daily for

7 days by oral gavage using an oral cannula. A dose of 600 mg kg^{-1} b.wt., Acetaminophen (APAP) was administered to rats in groups B to E once daily, 30 min after treatments with vitamin C and *CCLE* treatments for 7 days.

Biochemical analysis: The rats were bled via retro-orbital puncture on day 8 into plain tubes for the determination of serum urea, uric acid and creatinine levels using standard methods.

Animal sacrifice and necropsy: The rats were thereafter sacrificed under light chloroform anaesthesia and the kidneys were excised, examined macroscopically and immediately fixed in 10% formal saline for further tissue processing for light microscopical examination.

Histopathological studies: The fixed kidney tissues were processed through the stages of dehydration, clearing, infiltration and embedding with paraffin wax in plastic tissue cassettes using the automatic tissue processor. Microtomy was performed on the tissue blocks and 5 µm sections were obtained. The sections were eventually picked with slides over a water bath, dewaxed with xylene and rehydrated before staining with Hematoxylin and Eosin staining procedure[19]. Semi-quantitative scoring of the lesions was carried as follows: - for Nil, 1+ for mild, 2+ for moderate and 3+ for severe.

Microscopy and photomicrography: The stained sections were viewed microscopically using 10 and 40x objectives of Olympus™ Binocular microscope with the inbuilt lighting system. Areas of interest were photomicrographed using the AmScope® digital microscope camera (MU300 Model) attached to the eyepiece of the microscope.

Statistical analysis: Data obtained from the present study were analyzed using the Statistical Package for Social Sciences (SPSS) Analytical Software (SPSS, Chicago, IL, version 20.0). One way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* tests was used to determine differences between means and multiple comparisons, respectively. The results were expressed as Mean±Standard Error of Mean (SEM). Significant values were considered at $p<0.05$.

RESULTS

Evaluation of clinical signs of acute toxicity: No clinical sign of toxicity including mortality was observed in the rats used for the acute toxicity testing up to the dose of 8 g kg^{-1} b.wt.

Determination of oral LD_{50}: Since there was no death of any animal in all the test doses administered, the oral median lethal dose (LD_{50}) of *C. cajan* leaves aqueous extract is, therefore, greater than (>) 8 g kg^{-1} b.wt.

Effects of treatment on serum biochemical parameters: Results of serum creatinine, urea and uric levels of control and treatment groups are represented in Table 1. Statistically significantly increased values ($p<0.05$) were observed in groups B, C, D and E for serum uric acid levels (0.24±0.03, 0.26±0.02, 0.27±0.03 and 0.25±0.01 mmol L^{-1}, respectively) and serum creatinine levels (36.75±2.59, 53.50±2.60, 43.75±2.32 and 49.75±2.25 µmol L^{-1}, respectively) when compared with the normal control values of 0.17±0.01 mmol L^{-1} and 33.50±2.25 µmol L^{-1} for uric acid and creatinine, respectively. Treatments with vitamin C and 500 mg kg^{-1} APAP also increased creatinine levels (53.50±2.60, 43.75±2.32 and 49.75±2.25 µmol L^{-1}, respectively) compared to the APAP control group (36.75±2.59 µmol L^{-1}) ($p<0.05$).

Necropsy findings: Macroscopical examination of the kidney tissues from control and treatment groups did not reveal any obvious changes.

Table 1: Effect of *C. cajan* leaf extract and vitamin C on serum urea, uric acid and creatinine in acetaminophen-induced nephrotoxicity in Albino rats

	Biochemical parameters		
Treatment groups	Urea (mmol L^{-1})	Uric acid (mmol L^{-1})	Creatinine (µmol L^{-1})
A (control)	4.50±0.17	0.17±0.01#	33.50±2.25#
B (APAP only)	5.20±0.58	0.24±0.03*	36.75±2.59*
C (vitamin C+APAP)	5.73±0.86	0.26±0.02*	53.50±2.60*#
D (200 mg kg^{-1} *CCLE*+APAP)	4.78±0.27	0.27±0.03*	43.75±2.32*
E (500 mg kg^{-1} *CCLE*+APAP)	5.50±0.00	0.25±0.01*	49.75±2.25*#
F-ratio	1.082	3.371	12.262
p-value	>0.05	<0.05	<0.05

*$p<0.05$ when compared with the control (group A) and #$p<0.05$ when compared with the negative control (group B)

Histopathological findings: Changes were observed upon microscopical examination of all treatment groups (B-E) whereas no structural alteration was shown in the Control group. Figure 1-5 represents the photomicrographs of the cortical and medullary regions of the kidney tissues sections from all groups. Normal control rats' kidney tissue section shows normal parenchymal morphology revealing normal glomeruli (G) and tubules (T) in the cortex in Fig. 1a and normal morphology of the medullary structures in Fig. 1b. However, glomerular shrinkage and segmentation (eG), increased Bowman's capsular space (thick arrows), eosinophilic tubular casts (e) and tubular degeneration (thin arrows) were noted in the cortex of the APAP-only treatment group in Fig. 2a, whereas mildly degenerating features (arrows) were noted in the corresponding medulla in Fig. 2b.

Vitamin C and *CCLE* treatments did not show any obvious preservation of the tissues but rather more deleterious changes were observed. For vitamin C-treatment group, glomerular shrinkage (eG) and eosinophilic tubular casts (e) were noted in the cortical region in Fig. 3a while the medullary structures appear degenerating (arrows) as depicted in Fig. 3b. For *CCLE* 200 mg kg^{-1} b.wt., treated group, glomerular

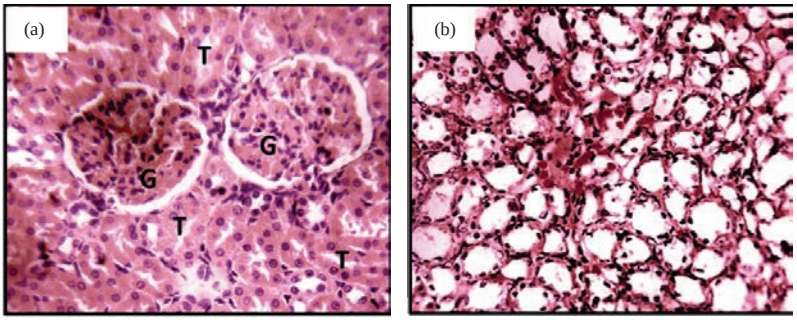

Fig. 1(a-b): Light photomicrographs of the kidney sections from the normal control

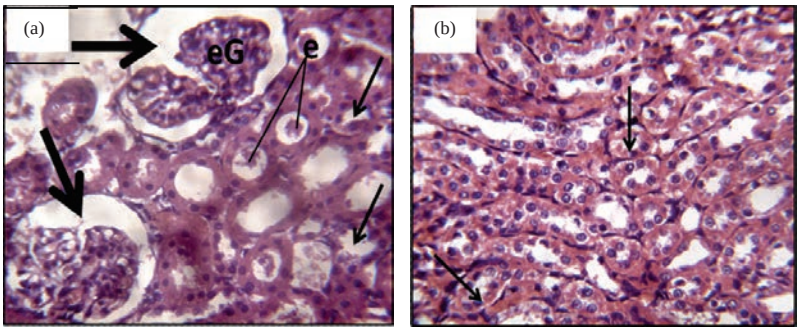

Fig. 2(a-b): Light photomicrographs of the kidney sections from the control and APAP-(NEG. control)

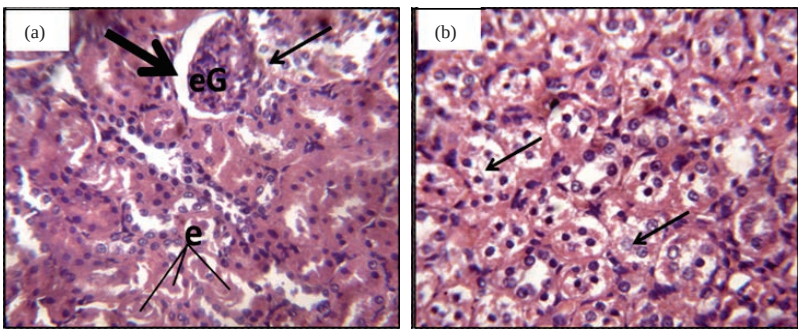

Fig. 3(a-b): Light photomicrographs of the kidney sections from the control and vitamin C+APAP

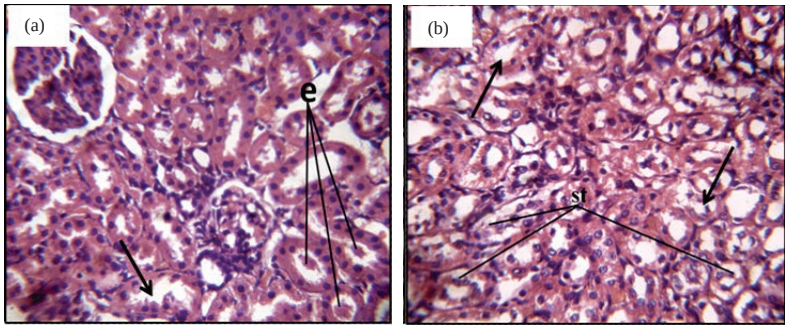

Fig. 4(a-b): Light photomicrographs of the kidney sections from the control and *CCLE* 200 mg kg^{-1}+APAP

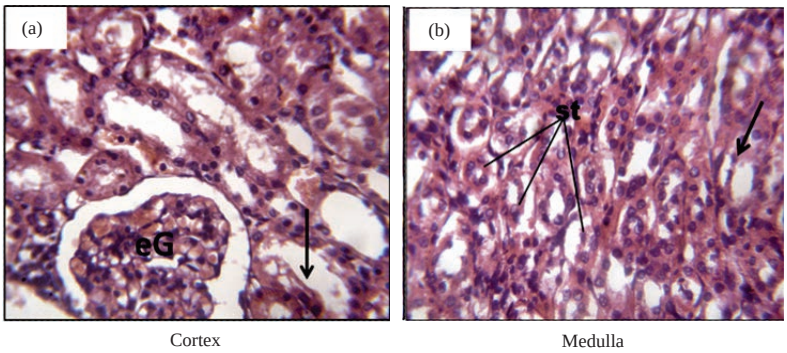

Fig. 5(a-b): Light photomicrographs of the kidney sections from the control and *CCLE* 500 mg kg^{-1}+APAP

Table 2: Semi-quantitative histological grading showing the effects of *C. cajan* leaf extract and vitamin C on the kidney histoarchitecture of rats with acute renal injury induced with acetaminophen

	Histopathological findings					
Treatment groups	Glomerular injury	Increased bowman's capsular space	Tubular degeneration, erosion and dilation	Tubular casts	Sloughing of medullary structures	Leukocytes infiltration
A (control)	-	-	-	-	-	-
B (APAP only)	+++	+++	++	+	-	-
C (vitamin C+APAP)		++	++	+++	+	++
D (200 mg kg^{-1} *CCLE*+APAP)	++	++	+	+	+++	++
E (500 mg kg^{-1} *CCLE*+APAP)	++	+++	+++	++	+++	++

-: Nil, +: Mild, ++: Moderate and +++: Severe

segmentation, presence of eosinophilic tubular casts (e) and tubular dilation (arrow) are observed within the cortex in Fig. 4a, whereas sloughing of medullary tubules (st) is noted in the medulla in Fig. 4b. More so, *CCLE* 500 mg kg^{-1} b.wt., treated group showed more severe lesions including glomerular shrinkage (eG), mild cellular infiltration at the peri-glomerular region, tubular degeneration and dilation (arrow) within the cortex in Fig. 5a, while the medulla in Fig. 5b also revealed sloughing of medullary tubules (st).

The semi-quantitative grading of the lesions is as represented in Table 2. The grading shows that most severe changes (+++) were observed upon treatment with 500 mg kg^{-1} b.wt., of *CCLE* particularly in the following lesions: Increased Bowman's capsular space, sloughing of medullary structures, tubular degeneration, erosion and dilation.

DISCUSSION

The present study sought to investigate the effects of orally administered crude aqueous leaf extract of *Cajanus cajan* on the acetaminophen-induced renal injury. Acetaminophen (APAP), a commonly prescribed analgesic and antipyretic drug, is considered safe at therapeutic doses but can produce toxic effects in both the liver and the kidney when high doses are consumed. Large quantities can be

purchased over the counter either as a single compound or in combination with other medications. Most drug oxidations in tissues are controlled by a terminal oxygenase, cytochrome P_{450}. At normal doses, APAP is metabolized to its highly reactive species, N-acetyl-p-benzoquinone imine (NABQI). When a large dose of acetaminophen is consumed, it leads to rapid depletion of renal glutathione stores to a critical point and acute tubular injury results in the kidneys[20]. This mechanism of action explains the nephrotoxic effect that acetaminophen exerted on the renal tissues as evidenced in the present study.

The increased serum levels of some kidney function markers (urea, uric and creatinine) in all treatment groups following APAP administration suggest an interaction of the toxic metabolite with the cellular membrane, leading to altered permeability and loss of the renal tissue functional integrity. The biochemical findings also corroborate with the histopathological reports. Microscopical examination of the renal tissues revealed that APAP treatment showed marked histomorphological alterations on the renal tubular epithelium with somewhat milder effects on the glomeruli. APAP treatment has been shown to result in tubular degeneration, glomerular changes and interstitial congestion[9,21,22]. The tubular epithelial damage (degeneration) observed in the present work could be as a result of the direct toxic action of the drug or due to impaired oxygen delivery. The presence of eosinophilic granular casts in the lumen of the renal tubules is consistent with acute tubular necrosis observed in acetaminophen overdose.

Many plant extracts with antioxidant activities have demonstrated potent nephroprotective abilities. They do this by their scavenging capability on reactive oxygen species (ROS) which is a major factor contributing to tissue injury. However, the present study showed that *C. cajan* leaf extract did not offer any observable protection to the renal tissue upon oxidative stress induced by acetaminophen treatment of the animals. Conversely, upon treatment with crude aqueous extract of *C. cajan* on acetaminophen toxicity, the severity of lesions on the renal tissue was increased by increasing doses of the extract as evidenced by histopathological findings, increased serum biochemical levels of renal function markers especially at the highest dose of 500 mg kg^{-1} b.wt. It thus suggests that co-administration of both substances (APAP and *C. cajan* leaves extract) in doses explored in the present study, exerts deleterious effects on the kidneys and hence consequently lead to organ dysfunction. Contrarily, proteins from the seeds of *C. cajan* have demonstrated profound nephroprotective effects in a previous study by minimizing renal damage and delaying disease progression against acetaminophen overdose-induced nephrotoxicity[23]. The plant material when administered up to a dose of 6 g kg^{-1} daily in mice for four weeks did not produce any obvious histopathological effects[24]. More so, acute toxicity testing in the present study suggests that the leaves may be considered safe for consumption according to OECD Standards as previously documented[25] since its LD_{50} was observed to be greater than 8000 mg kg^{-1} b.wt. Thus, it may be inferred that the extract does not exert any toxic effect when consumed alone. Since the exact mechanism at which the exacerbation of renal damage occurred in the present study cannot be explained, it may, however, be suggested that a single or combination of the active components present in *C. cajan* leaf extract may have interacted with acetaminophen to enhance nephrotoxicity.

Similarly, vitamin C did not produce a protective effect in the present study against APAP-induced damage. Previous researchers have documented that its prevention of APAP-induced renal injury could be complete or partial depending on the dose and time administered following intoxication with APAP[26,27]. Furthermore, administration of mega doses of vitamin C via routes for enhanced absorption other than oral route, has been considered more beneficial in the treatment of paracetamol-induced renal injury[26].

CONCLUSION

From the findings of this study, it can be deduced that acute toxicity effects were not observed upon oral treatments with *Cajanus cajan* leaf extract at the tested doses. Interestingly, however, treatment with the extract did not proffer any protection on the kidney tissues of rats upon acetaminophen-induced acute renal injury but enhanced nephrotoxicity was rather observed. Further studies to elucidate the mechanism of action and the bioactive compound(s) in the leaf extract responsible for the observed effects are underway.

SIGNIFICANCE STATEMENT

The present study discovered that the crude aqueous leaf extract of *Cajanus cajan* administered orally is though safe and devoid of any observable acute toxicity effects but, conversely, when co-administered with high doses of acetaminophen, it exacerbates acute renal injury. This study will therefore help researchers to better elucidate the effects of arbitrary use of herbal-based products in the treatment or prevention of renal injury induced by acetaminophen that many researchers were not able to explore.

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