



EFFECT OF ETHANOL LEAF EXTRACT OF CASSIA ANGUSTIFOLIA EXTRACT ON KIDNEY OF WISTER RATS

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Abstract

Introduction/Aim: Plants are frequently consumed and abused for different purposes without consideration of the short and long time implications. *Cassia angustifolia* L. (senna) is traditionally used as a laxative. Its major components are sennosides that are responsible for the laxative effect. Senna is recommended for the short-term treatment of acute constipation. Nevertheless people use its preparations as self-medication, often for long periods, to treat chronic constipation thus exposing themselves to adverse reactions. The aim of this work is to evaluate the effect of this plant on kidney function.

Method: Animals of either sex were selected. Group 1 received distilled water (10 ml/kg), while group 2, 3 and 4 received *Cassia angustifolia* 50, 100 and 200 mg/kg respectively. Animals were kept in standard cages and given access to the extract, water and food orally for 28 days, after which they were weighed and sacrificed. Blood was collected by cardiac puncture and taken immediately for hematological and chemo pathological analysis. The histological toxic potential of the plant on the kidney was studied using haematotoxylin and eosin (H&E) staining technique.

Result: There was Significant ($P < 0.05$) decrease in RBC, HGB, MCV, while there was no change in the level of neutrophiles, basophiles, eosinophiles and platelets. *Cassia angustifolia*, slightly significantly ($p < 0.05$) increased Na level at 50 mg/kg and Creatinine level at 50 mg/kg dose levels respectively when compared to the control when compared to the control. Other parameters (K, CL and Urea levels) were not significantly affected. Histological study reveals slight tubular distortion.

Conclusion: The result of the study showed that at low dose the plant could have slight effect on the kidney which suggests that the plant should be use with caution when taken for a sustained period.

Keyword: *Cassia angustifolia*, rat, blood, kidney

Introduction

The therapeutic use of herbs is as old as human civilization and has evolved along with it¹. Local practitioners have used indigenous plants and herbs for centuries all over the world to treat a variety of ailments and these have exhibited clear pharmacological activities¹. Historically, herbal drugs were used as tinctures, poultices, powders and teas followed by formulations, and lastly as pure compounds^{1,2}. Across the cultures, knowledge about use of medicinal plants exists in the form of local folklore available with families, tribes and cultures, handed down from generation to generation. Medicinal plants or their extracts have been used by humans since time immemorial for different ailments and have provided valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardiotonics (digoxin), antineoplastics (vinblastine and taxol) and antimalarials (quinine and artemisinin)³. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, malaria, cardiovascular diseases and neurological disorders³. Plants have proven to be a novel source for bioactive natural products. They have evolved and adapted over millions of years to withstand bacteria, insects, fungi and weather to produce unique, structurally diverse secondary metabolites. Their ethnopharmacological properties have been used as a primary source of medicines for early drug discovery^{3,4}.

Nephrotoxicity is one of the most common kidney problems and occurs when your body is exposed to a drug or toxin that causes damage to your kidneys. When kidney damage occurs, you are unable to rid your body of excess urine, and wastes⁵. Your blood electrolytes (such as potassium, and magnesium) will all become elevated. Nephrotoxicity can be temporary with a temporary elevation of lab values (BUN and/or creatinine). If these levels are elevated, these may be due to a temporary condition such as dehydration or you may be developing renal (kidney failure). If the cause of the increased BUN and/or creatinine levels is determined early, and your healthcare provider implements the appropriate intervention, permanent kidney problems may be avoided⁶.

Cassia angustifolia, also known as Alexandrian Senna is a shrubby plant that reaches 0.5–1, rarely two, metres in height with a branched, pale-green erect stem and long spreading branches bearing four or five pairs of leaves. These leaves form complex, feathery, mutual pairs⁷. The leaflets vary from 4 to 6 pairs, fully edged, with a sharp top.

The midribs are equally divided at the base of the leaflets. The flowers are in a raceme interior^[1] blossoms, big in size, coloured yellow that tends to brown. Its legume fruit are horned, broadly oblong, compressed and flat and contain about six seeds. When cultivated, the plants are cut down semi-annually, dried in the sun, stripped and packed in palm-leaf bags. It also serves as a fungicide⁸.

Modern medicine has used extracts since at least the 1950s as a laxative^{9,10}. If accidentally ingested by infants, it can cause side effects such as severe diaper rash¹¹. The active ingredients has several senna glycosides which interact with immune cells in the colon. This work was intended to investigate the effect of ethanol extract of *Cassia angustifolia* on the kidney of rats.

Materials and Method

Animals

Male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

Plant collection

Leaves of *Cassia angustifolia* were collected from its natural habitat from nearby Karu village, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State Nigeria.

Plant extraction

The leaves were shadow dried for two weeks. The dried plant material was further reduced into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40°C *in vacuum* using rotary evaporator. The ethanol extract was stored at -4°C until used.

Animal study

Twenty four (24) rats of either sex (159-283g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10ml/kg)

while the rats in groups 2, 3 and 4 were giving 50, 100, and 200 mg/kg of extract respectively. The weights of the rats were recorded at the beginning of the experiment and at weekly intervals. The first day of dosing was taken as D₀ while the day of sacrifice was designated as D₂₉.

Haematological analysis

The rats were sacrificed on the 29th day of experiment. Blood samples were collected via cardiac puncture. One portion of the blood was collected into sample bottles containing EDTA for hematological analysis such as Hemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocyte and monocyte), red blood cell count (RBC), platelets and hemoglobin (Hb) concentration using automated Haematology machine (Cell-Dyn, Abbott, USA).

Kidney Function Test

The following biochemical parameters were assayed as markers of kidney function using diagnostic kits; Level of electrolytes (Na⁺, K⁺, Cl⁻, and HCO₃⁻), creatinine and blood urea. The above parameters were determined at the Chemical Pathology Department of University of Jos Teaching Hospital.

Kidney harvested were preserved in 10% formal saline solution, processed, sectioned and stained with Heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Jos Teaching Hospital, Jos.

Statistical analysis

Data were expressed as the Mean \pm Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

Result

Effect of 28 days oral administration of *Cassia angustifolia* on hematological parameters in rats.

Cassia angustifolia caused significant ($p < 0.05$) decrease in the level of red blood cell, hemoglobin, platelet etc. and significantly ($p < 0.05$) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 50 mg/kg compared to the control. The level of basophiles, neutrophils, eosinophils and lymphocytes were however not significantly ($p < 0.05$) affected by mean corpuscular hemoglobin concentration

Effect of 28 days oral administration of *Cassia angustifolia* on renal indices and electrolytes in Wistar rats.

Cassia angustifolia significantly ($p < 0.05$) increased Na and creatinine level at 50 mg/kg when compared to the control. Other parameters (K, CL, and Urea levels) were not significantly affected.

Histopathological Investigations of the effect of 28 days oral administration of *Cassia angustifolia* on renal indices and electrolytes in Wistar rats.

The kidney showed very slight tubular distortion and glomerular necrosis at 50 mg/kg. There was also, Slight tubular necrosis with lymphocyte hyperplasia at 100 mg/kg. Normal renal histological features were observed in the control group.

Table 1: Effect of 28 days oral administration of ethanol leaf extract of *Cassia angustifolia* on hematological parameters in wistar rats.

Hematological parameters	DW(1ml/kg)	Treatment (mg/kg)		
		50 mg/kg	100 mg/kg	200 mg/kg
WBC ($\times 10^9/L$)	7.67 \pm 0.772	7.74 \pm 1.419	3.700 \pm 0.657*	6.420 \pm 1.085
RBC ($\times 10^{12}/L$)	8.21 \pm 0.37	8.65 \pm 0.20	6.11 \pm 0.35*	7.91 \pm 0.27
HGB (g/dL)	14.92 \pm 0.66	14.24 \pm 0.46	10.93 \pm 0.76*	13.58 \pm 0.77
HCT (g/dL)	54.27 \pm 2.13	55.60 \pm 2.75	34.67 \pm 2.28*	52.41 \pm 2.73
MCV (fL)	66.62 \pm 0.93	65.40 \pm 1.44	57.17 \pm 0.31*	69.60 \pm 1.72
MCH (pg)	19.17 \pm 0.17	17.80 \pm 1.02	18.83 \pm 0.37	18.80 \pm 0.20
MCHC (g/dL)	34.17 \pm 0.16	32.30 \pm 0.72	35.50 \pm 0.53*	30.62 \pm 0.74
PLT ($\times 10^9/L$)	626.86 \pm 46.71	577.20 \pm 93.32	242.13 \pm 45.28*	688.40 \pm 45.26
LYM (%)	76.45 \pm 5.16	74.00 \pm 5.19	75.83 \pm 6.33	75.40 \pm 5.23
NEUT ($\times 10^9/L$)	20.86 \pm 4.57	21.83 \pm 2.69	25.33 \pm 5.66	21.36 \pm 4.17
EOSI ($\times 10^9/L$)	2.55 \pm 0.24	3.44 \pm 0.65	2.87 \pm 0.36	2.32 \pm 0.51
BASO ($\times 10^9/L$)	2.12 \pm 0.19	2.60 \pm 0.85	2.44 \pm 1.77	3.45 \pm 1.64

Data presented as Mean \pm SEM: n = 6, One way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at p<0.05. DW = distilled water

(WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils).

Table 2: Effect of 28 days oral administration ethanol leaf extract *Cassia angustifolia* on renal indices and electrolytes in wistar rats.

Renal indices and electrolytes	DW(10ml/kg)	Treatment (mg/kg)		
		50 mg/kg	100 mg/kg	200 mg/kg
Potassium (mmol/L)	5.88±0.52	6.53±0.69	5.45±0.49	5.38±0.27
Sodium (mmol/L)	131.0±20±2.90	148.26±2.82*	133.00±1.55	136.34±1.76
Chloride (mmol/L)	950.00±5.66	94.22±6.78	100.20±2.28	100.43±2.52
Urea (mmol/L)	5.55±0.36	6.34±0.39	6.56±0.29	6.23±0.45
Creatinine (µmol/L)	70.35±8.52	93.84±19.32*	64.38±15.65	75.75±5.56

Data presented as Mean ± SEM: n = 6, One Way ANOVA, followed by Dunnett's post hoc for multiple comparison *significantly different from the distilled water (DW) control at p <0.05. ethanol leaf extract *Cassia angustifolia*, DW = distilled water.

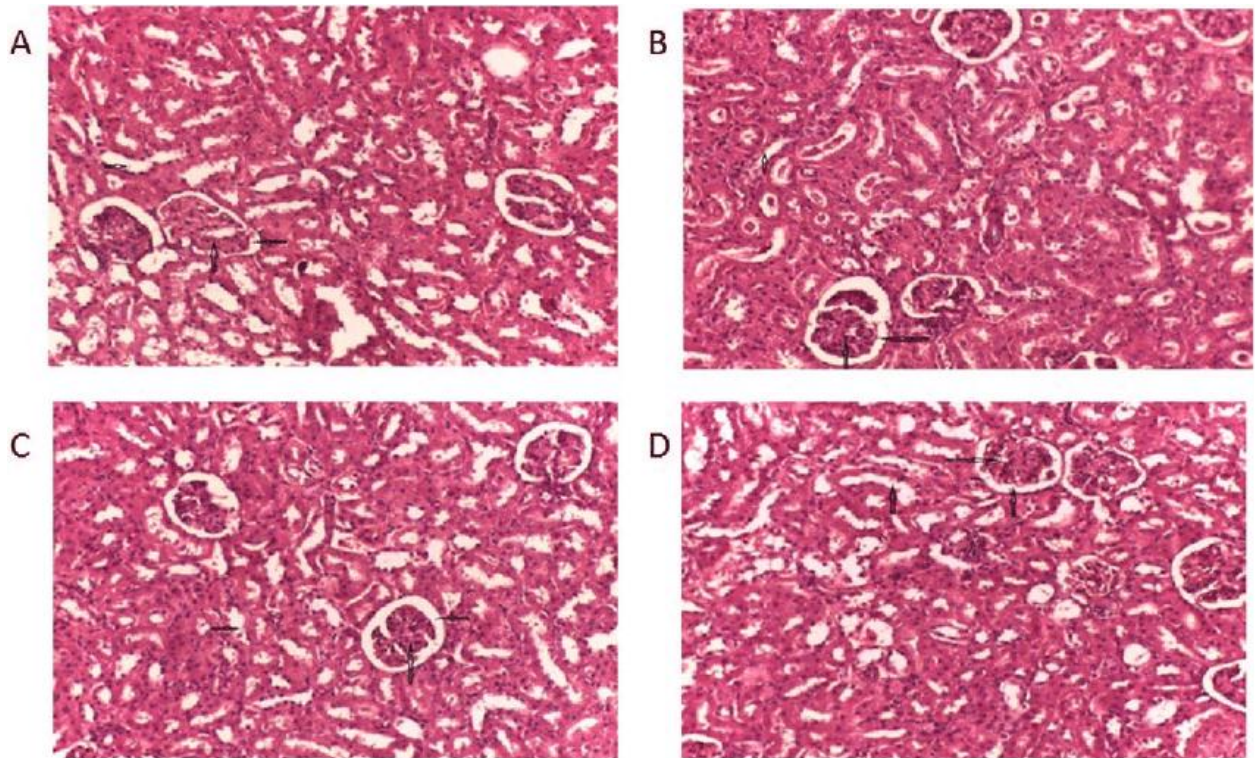


Plate 1: Histological sections of Kidneys of a) rats treated with Normal saline 10 ml/kg, (b) *Cassia angustifolia* 50 mg/kg (c), *Cassia angustifolia* 100 mg/kg bw (d) and *Cassia angustifolia* 200 mg/kg stained with H&E Technique.

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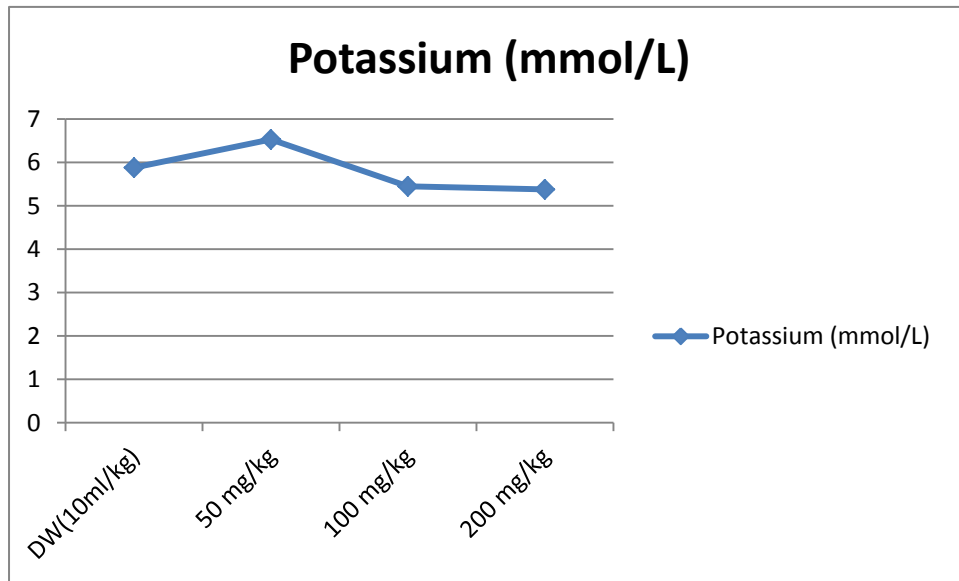


Fig 1: graph showing effect of the ethanol leaf extract of *Cassia angustifolia* on serum potassium level in rats

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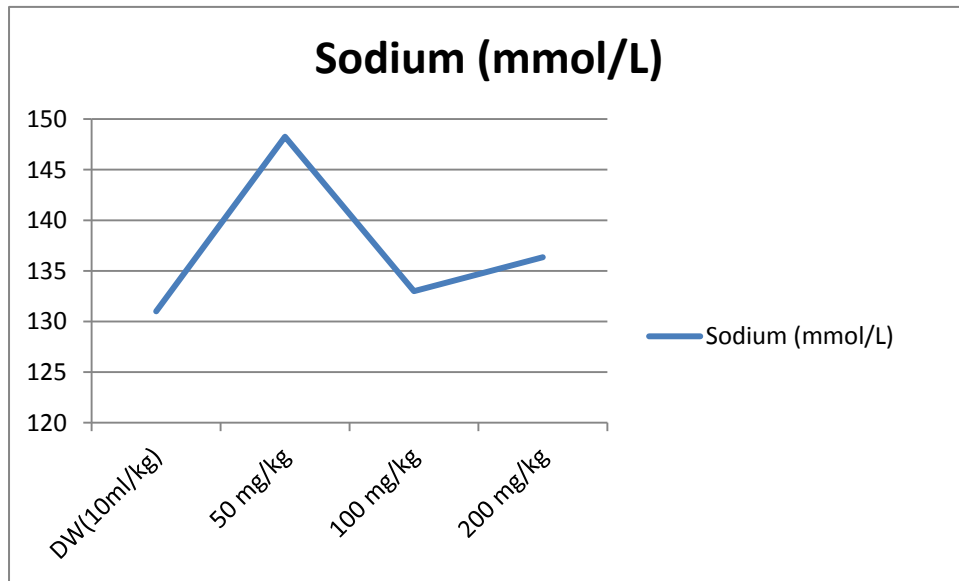


Fig 2: graph showing effect of the ethanol leaf extract of *Cassia angustifolia* on serum sodium level in rats

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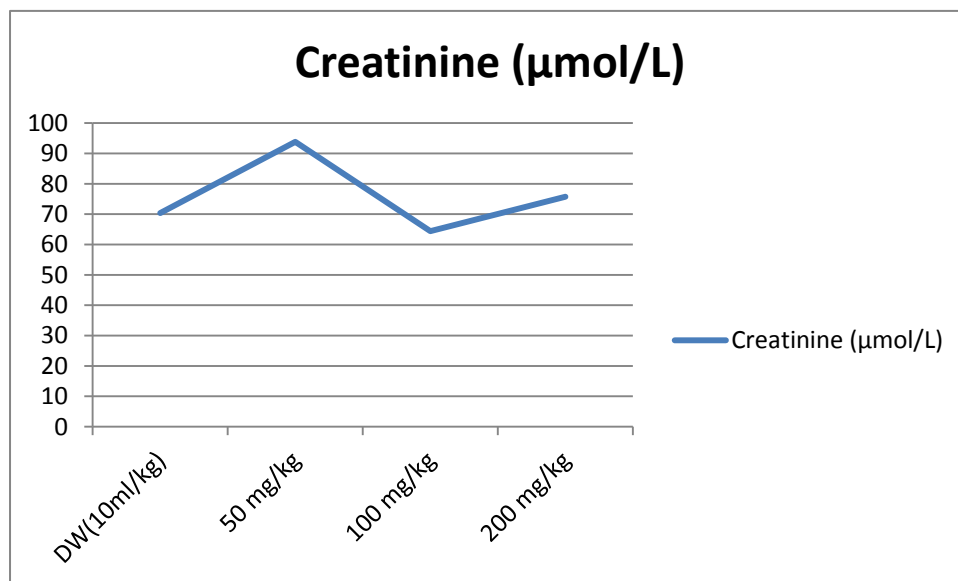


Fig 3: graph showing effect of the ethanol leaf extract of *Cassia angustifolia* on serum creatinine level in rats

Discussion

There is increasing evidence for the nephroprotective role of phytochemical substances from vegetables, fruits and some herbs. *Cassia angustifolia* is a medicinal plant with lots of pharmacological potential. Medicinal properties of the plant would be due to the presence of alkaloids, flavonoids, phenolic, tannins, and other phytoconstituents¹². Several reports have demonstrated that secondary plant metabolites exert diverse medicinal biological effects^{9,12,13,14}. In the study, Wister rats were used to screen the effect of *Cassia angustifolia* at various dose level of the plant extract with hematological and biochemical estimation from blood and histopathology of kidney for 28 days.

Serum creatinine, urea, uric acid and serum electrolytes are renal biochemical markers that are perturbed with the advent of nephrotoxicity¹⁵, therefore, alterations in their levels connote impairment in the functional capacity of the kidney¹⁶. In the current study, the functional capacity of the kidney was not significantly affected in rat administered ethanol leaf extract of *Cassia angustifolia* due to no change in the level of serum levels of urea, uric acid, K⁺ Na⁺ Cl⁻ and HCO₃⁻, particularly, at higher doses.

Ethanol extract of leaves of *C. angustifolia* resulted in significant ($*p<0.05$) decrease in the red blood cell, hemoglobin and platelet when compared to the control group of rats. This indicated that the plant may either suppress the production of red blood cells, decrease the lifespan of red blood cells or causes problems with how the body uses iron. Anemia is a condition that develops when the blood lacks enough healthy red blood cells or hemoglobin¹⁷. Hemoglobin is a main part of red blood cells and binds oxygen. If the level of RBC too few or there is abnormal red blood cells, or hemoglobin is abnormal or low, the cells in the body will not get enough oxygen¹⁸. Also, the level of basophiles, neutrophils, eosinophils and lymphocytes were not affected by the extract. This indicates that the plant may not affect the body immune. It could also suggest that the plant may have immunomodulatory property. The study also showed that *C. angustifolia* caused slight increase in serum Na and creatinine level at the lowest dose administered. This may be due to deleterious and oxidative activity of some of the chemical constituent plant that seems to be more potent than the antioxidant chemical at lower dose. An increase creatinine level can be observe in some kidney diseases, due to loss of normal excretory function of the creatinine¹⁹, when there is a muscular cells damage or following an incompatible medication interfering with the normal functioning of the kidney²⁰. Creatinine, is mostly derived from endogenous sources by tissue creatinine breakdown²¹. The serum creatinine concentration of the group that received 50 mg/kg of the extract was significantly higher than the control group. Atangwho *et al.*²² reported elevated serum creatinine level as an indicator of possible kidney dysfunction. Gross *et al.*²³ in a study indicated that a rise in serum creatinine level could suggest a possible damage to the functioning nephrons of the kidney. The measurement of creatinine concentration in serum was a useful index for the diagnosis of chronic kidney disease and when serum creatinine level was higher than the normal value, renal failure was most likely a possible outcome²². Increased serum creatinine concentration has been considered a marker of assessing nephrotoxicity as reported by Anwar *et al.*²⁴ and Ali *et al.*²². it is also possible that at high dose the antioxidant activity of *Cassia angustifolia* becomes conspicuous, negating and possibly providing protective property to cells. In this study, serum urea was unaffected suggesting that the plant may cause slight damage to the kidney. Thus serum urea concentration is often considered a more reliable renal function predictor than serum creatinine^{21,22,26,27}.

The histopathological analysis, showed that in all groups after 28 days administration of ethanol extract of *Ocimum canum* the kidney there was slight changes at the cellular level in

comparison to control. This resonates with other parameters that the leaves of the plant slightly have nephrotoxic effect over a long period of time.

Conclusion

Result from biochemical parameters and histological study shows that the plant possesses chemical constituent that at low dose slight nephrotoxic potential precipitates suggesting that caution should be taken while consumed for sustained period. Further study can be carried out on the antioxidant property of the plant at higher doses.

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Referee

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