Science World Publishing

Open Access Journal | Volume: 1.1

ADVANCED CLINICAL PHARMACOLOGY AND TOXICOLOGY, THERAPEUTICS

Acute and Sub-Chronic Nephrotoxicity Study on Orally Administered Crude *Costus afer* Stem Juice in Male Wistar Albino Rat

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Article Information

Article Type: Research Article
Journal Type: Open Access
Volume: 1 **Issue: 1**
Manuscript ID: ACPTT-1-110
Publisher: Science World Publishing
Received Date: 25 May 2019
Accepted Date: 6 June 2019
Published Date: 10 June 2019

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Citation: Nwaogwugwu CJ (2019) Acute and Sub-Chronic Nephrotoxicity Study on Orally Administered Crude Costus afer stem juice in Male Wistar Albino rat. Adv Clin Pharmacol Toxicol Ther, 1(1);1-7

ABSTRACT

Aim: To study Acute and Sub-Chronic Nephrotoxicity on Orally Administered Crude *Costus afer* stem juice in Male Wistar Albino rat.

Method: Forty (40) healthy male albino rats aged about 7 weeks weighing between 91 g-155 g were used, 15 albino rats were used for the acute toxicity test. The albino rats were randomly divided into five groups of five animals each. Group I which received food and water *ad libitium* served as the control group while group II, III, IV and V received crude stem juice 250 mg/kg body weight, 500 mg/kg body weight, 750 mg/kg body weight, and 1000 mg/kg body weight (b.wt) respectively for 30 days. The animals were sacrificed under ether anesthesia and the hepatic biomarkers were analyzed for Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT); Alkaline Phosphatase (ALP); Triglyceride (TG); Total Bilirubin (TB); Conjugated Bilirubin (CB); Albumin (ALB) and kidney biomarkers urea, creatinine, sodium, potassium and bicarbonate were investigated.

Statistical Analysis: The statistical analysis of result was done using statistical package for social sciences (SPSS) version, Means were separated using Least Significant Difference. Group means were compared for significance at $P<0.05$. Data were represented as mean ± standard deviation.

Result: The acute motility was recorded during the study. There was progressive increase in body weight at doses of 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg of rats during 28 days oral administration of the extract($p<0.05$). There were significant increases in kidney function biomarkers such as creatinine, urea and electrolytes at 750 mg/kg and 1000 mg/kg ($p<0.05$). There was a significant reduction in levels of RBC, HB, PCV MCHC, platelet, MCH and MCV in all groups from the control ($p<0.05$).

Conclusion: The study revealed that *C. afer* crude stem juice exhibits nephrotoxicity at high doses of 750 mg/kg and 1000 mg/kg, therefore it is time dependent and dose dependent toxicity.

KEYWORDS : Nephrotoxicity, Kidney, Liver, Hepatoxic, Sub-Chronic, *Costus afer*

INTRODUCTION

The use of plants for medicinal purposes is the oldest form of health care known to mankind (Atsukwei et al., 2015). Medicinal plants are various plants used in herbalism because they possess therapeutic properties (Ogunlesi et al., 2008). Some medicinal plants includes; *Vernonia amygdalina* which possesses cytotoxic effects towards human carcinoma cells of the nasopharynx (Farombi and Owoeye, 2011), *Aloe vera* has anti-inflammatory and anti-tumour activities (Bawankar et al., 2014) and *Costus afer* which possesses many pharmacological activities including antinociceptive, anti-anthritic and anti-inflammatory properties (Ijioma et al., 2014; Soladoye and Oyesika, 2008).

C. afer (Ker Gawl) is commonly called Ginger lily or Bush cane in English, Kaki zuwa by the Hausas, Okpete or Okpoto by the Igbo's and Tete Ogun by the Yoruba's, all of Nigeria (Anaga et al., 2004; Ukpabi et al., 2012; Ijioma et al., 2014). It is a medicinal plant in that it contains substances that can be used for therapeutic purposes. These substances are precursors for chemopharmaceutical synthesis as such this plant

has been used traditionally in cases of cough, measles and malaria. Numerous works have shown the efficacy of *C. afer* plant extracts, stem juice of *C. afer* is used as a remedy for stomach ache, cough and rheumatic pains (Akpan et al., 2012). Also, antimicrobial activity of the ethanolic extract of its leaves has been assessed (Moke et al., 2015). Ijioma et al., (2014) reported antinociceptive properties of ethanol leaf extract and stem juice of *C. afer*. Several researchers (Akaninwor et al., 2014; Emeh et al., 2014; Uchegbu et al., 2016) among others have reported the medicinal potentials of this plant.

The fact that many health problems have been solved using medicinal plants cannot be over emphasised, but a number of them are toxic to human when not properly prepared or dispensed. As such a scientific approach needs to be applied towards the use of plant extracts in managing ailments, especially in the developing countries where the level of literacy is low and the status of health management is poor, and about 80% of the population patronize herbal drugs. The toxicity potentials of the extracts of some medicinal plants including crude *C. afer* stem juice have not been documented. Therefore this work was designed to evaluate the toxicity potential of *C. afer* on kidney. Also a study of the effect of a drug extract on the kidney is essential because of the prime role the organ plays in plasma clearance, some detoxification, homeostasis and excretion of xenobiotics.

MATERIALS AND METHODS

Plant Material and Extract Preparation

The whole plant of *Costus afer* was harvested at Amuke, Isiala Ngwa south L.G.A. in Abia state. The plant was identified at the Department of Plant Science and Biotechnology, Abia State University, Uturu and voucher specimen deposited at the herbarium of the Department.

The leaves were removed from the stem and then were debarked. The debarked stems were introduced into a clean manual blender and were crushed to squeeze out their juice. The resulting juice was filtered using a sterile Muslin cloth and Whatman No. 1 filter paper. The juice was concentrated by lyophilizing in a freeze dryer (Edwards, USA) and the resultant volume was 850 ml. 1 ml was pipetted into a pre-weighed 100 cm^3 beaker and evaporated to dryness on a boiling water bath. The beaker was cooled and weighed again and the concentration was determined to be 12.0 mg/ml by calculation. The juice was stored in the refrigerator until required.

Phytochemical Screening Tests

Desirable amount of *C. afer* stem juice was used for phytochemical tests. The stem juice was tested for alkaloid, flavonoid, phenol, tannin, Anthraquinones, Cardiac Glycosides and terpenoid according to the procedure described by Harbone (1973) and Sofowora (1982).

Animals and Treatments

Forty (40) healthy male albino rats aged about 7 weeks weighing between 91 g-155 g purchased from the animal house of Department of Veterinary Medicine, University of Nigeria, Nsukka were used for this study. The animals were maintained in cages under standard environmental conditions. They were allowed to acclimatize for two weeks at the experimental site with access to food (standard rat feed) and water *ad libitum.* 15 albino rats were used for the acute toxicity test while 25 Wistar rats were divided into five groups of five animals in each group. The albino rats were randomly divided into five groups of five animals each. Group I which received food and water *ad libitium* served as the control group while group II, III, IV and V received crude stem juice 250 mg/kg body weight, 500 mg/kg body weight, 750 mg/kg body weight and 1000 mg/kg body weight (b.wt) respectively for 30 days. In the test groups, juice was administered through oral route using gavage intubation. The animal groups were then placed in individual cages lined with filter paper at the bottom. Animals were treated humanely; Veterinary care and supervision were provided throughout the period of study.

Measurement of Body Weight

Body weight was measured on days 0, 7, 14, 21, and 28. Body weight noted was expressed as mean body weight (g).

Determination of Acute Toxicity (Median Lethal Dose, Ld_{50})

The method employed was the two phase method described by Lorke (1983). The study was conducted in two phases using a total of sixteen male rats. In the first phase, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given 10, 100 and 1000 mg/kg body weight (b.w.) of the juice, respectively, to possibly establish the range of doses producing any toxic effect. Each rat was given a single dose after two weeks of acclimatization. In addition, a fourth group of three rats was set up as control group and animals in the group were not given the juice. In the second phase, further specific doses (1500, 2500 and 5000 mg/kg b.w.) of the juice were administered to three rats (one rat per dose) to further determine the correct LD_{50} value

Biochemical Analysis

Whole blood samples were collected and allowed to clot. The sera samples collected were analyzed for creatinine, urea, potassium, sosdium, bicarbonate and chloride based on the principles outlined by (Bartels et al., 1972), (Weatherburn, 1967), (Terri and Sesin, 1958), (Maruna, 1958; Trinder, 1951), (Forrester et al., 1976) and (skeegs and Hochestrasser, 1964) respectively, using standard biochemical reagent kits (Randox Laboratories Ltd., UK). The first part was dispensed in heparinised tubes (EDTA) bottles for haematological analysis. Part of the whole blood was dispensed in heparinised tubes (EDTA) bottles for haematological analysis. The haematological parameters analyses were carried out automatically using the Sysmex® Automated Haematology Analyser KX-21N by the method described by Oladimeji et al., 2014).

Histopathological Studies

The kidneys were embebbed in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin (Drury et al., 1993). Detailed microscopic examination was conducted on the kidneys from both control and treatment groups.

Statistical Analysis

The statistical analysis of result was done using Statistical Package for Social Sciences (SPSS) version 20 computer software and data collected were analyzed using Analysis of Variance (ANOVA). Means were separated using Least Significant Difference. Group means were compared for significance at $P<0.05$. Data were represented as mean ± standard deviation.

RESULTS

Table 1: Qualitative phytochemical screening of *Costus afer* stem

Phytochemical	Presence
Alkaloids	++
Flavonoids	++
Saponins	_
Phenols	++
Tannins	+
Anthraquinones	+
Cardiac Glycosides	+
Phlobatannins	_
Terpenoids	++
Cardenolides	_
Cyanogenic Glycosides	_
Oxalates	_

Key: + = present ++=Abundant - = absent

Table 2: GC-MS Phytochemical analysis of *Costus afer* stem juice

S. NO	RT	Compound Name	Formula	MW	Area, %
1	5.417	2-Furancaboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	70.357
2	5.649	Maltol	$C_6H_6O_3$	126	3.428
3	5.863	dl-Malic disodium salt	$C_4H_6O_5$	134	3.041
4	5.904	Malic acid	$C_4H_6O_5$	134	2.288
5	6.002	Butanedioic acid, hydroxyl-, diethyl ester, (±)-	$C_8H_{14}O_5$	190	0.399
6	6.024	1,6;3,4-Dianhydro-2-O-acetyl-β-d-galactopyranose	$C_8H_{10}O_5$	186	0.788
7	6.369	Dihydro-3-methylene-5-methyl-2-furanone	$C_6H_8O_2$	112	0.149
8	6.466	Furazan-3-ol, 4-amino-	$C_2H_3N_3O_2$	101	0.228
9	6.616	Conhydrin	$C_8H_{17}NO$	143	0.515
10	7.381	D-Allose	$C_6H_{12}O_6$	180	0.163
11	7.711	3-Hydroxy-4-methoxybenzoic acid	$C_8H_8O_4$	168	0.178
12	7.827	1,6-Anhydro-β-D-glucofuranose	$C_6H_{10}O_5$	162	0.153
13	8.266	Ethyl β-d-riboside	$C_7H_{14}O_5$	178	1.433
14	8.869	B-D-Glucopyranoside, methyl 3,6-anhydro-	$C_7H_{12}O_5$	176	5.39
15	9.848	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.293
16	10.27	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.32
17	10.61	Cyclohexane, 1-butyl-	$C_{10}H_{18}$	138	0.22
18	10.76	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	0.185
19	11.32	2-Naphthalenol, decahydro-, (2α,4aα,8aβ)-	$C_{10}H_{18}O$	154	0.616
20	11.51	Urea, ethyl-	$C_3H_8N_2O$	88	0.222
21	15.01	Silicic acid, diethyl bis(trimethylsilyl) ester	$C_{10}H_{28}O_4Si_3$	296	0.354
22	15.53	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}C_{10}$	298	1.872
23	17.58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_20H_40_0$	296	0.173
24	18.09	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	268	1.553
25	18.19	I-Gala-I-ido-octose	$C_8H_{16}O_8$	240	0.399
26	18.54	Farnesyl bromide	$C_{15}H_{25}Br$	284	0.19
27	27.1	6-epi-shyobunol	$C_{15}H_{26}O$	222	0.258
28	27.12	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	0.375
29	31.95	Squalene	$C_{30}H_{50}$	410	0.184
30	34.35	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.276

Key: RT: Retention Time; MW: Molecular weight

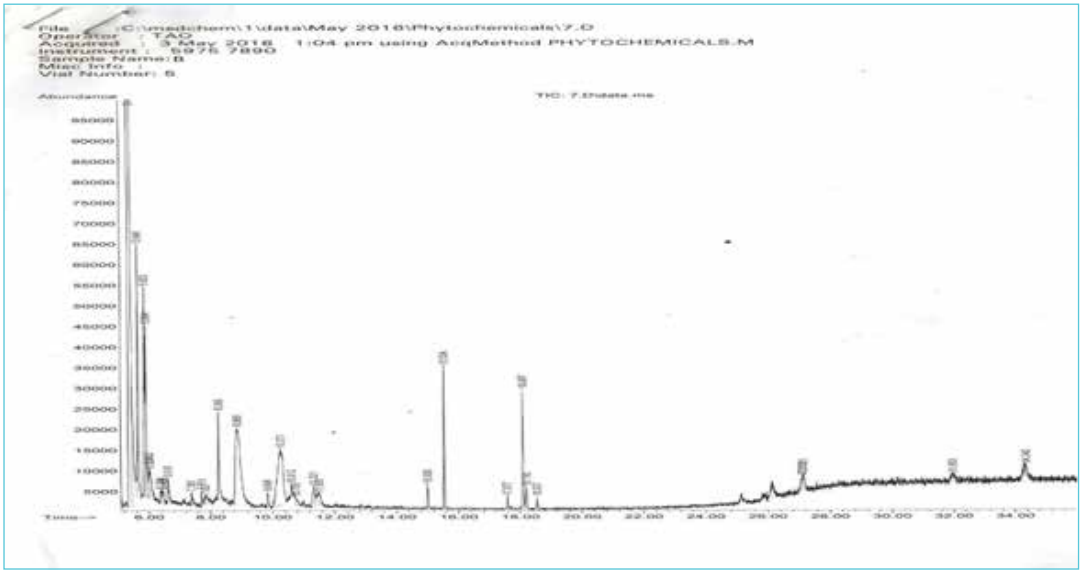

Figure 1: Chromatogram of GC-MS analysis of *Costus afer* crude stem juice

Table 3: Acute Toxicity (LD_{50}) Result of the *Costus afer* Stem Juice

Group of Rats	Dose of Juice (mg/kg)	Number of Death Recorded
I	10	Nil
II	100	Nil
III	1000	Nil
IV	1500	Nil
V	2500	Nil
VI	5000	Nil

There was no motility

Table 4: Growth Profile of Animals Administered with *Costus afer* Stem Juice Post 28 Days

Groups	Week 1	Week 2	Week 3	Week 4
I	111.67 ± 12.71^{b}	110.77 ± 14.24^{b}	112.90 ± 14.44^{b}	132.88 ± 28.20^{b}
II	95.50 ± 10.41^{a}	95.52 ± 11.72^{a}	98.12 ± 12.11^{a}	115.93 ± 16.43^{a}
III	88.90 ± 8.56^{a}	87.97 ± 8.03^{a}	89.28 ± 8.15^{a}	101.78 ± 8.71^{a}
IV	91.57 ± 14.83^{a}	116.73 ± 17.56^{b}	120 ± 17.50^{c}	138.77 ± 16.65^{b}
V	155.95 ± 28.92^{c}	167.10 ± 29.92^{c}	170.48 ± 31.86^{c}	166.00 ± 27.36^{c}

Values are mean + Standard Deviation (SD) for n=5 and mean along the column with different alphabetical superscripts indicates a significant different ($P<0.05$)

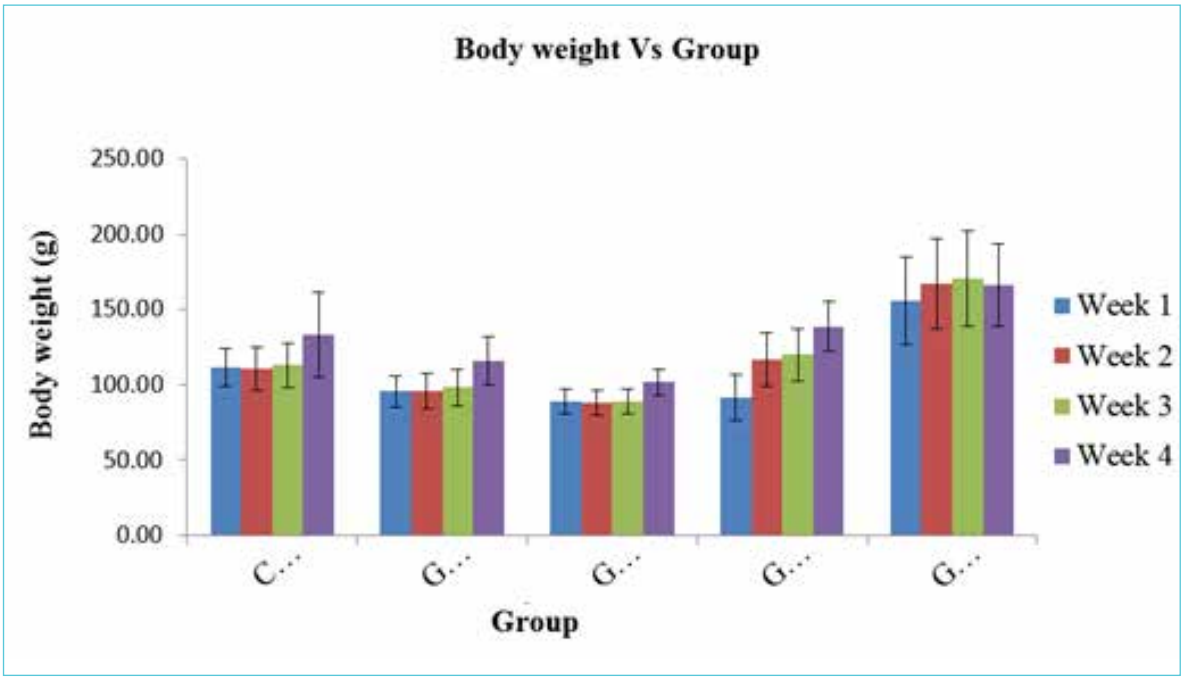

Figure 2: Growth profile of animals administered with crude *Costus afer* Stem juice post 28 days

Table 5: Levels of Kidney function Status Biomarkers in Wistar albino rats administered with crude *Costus afer* stem juice post 28 days

Groups	Na^{+} (mEq/L)	Cl^{-} (mEq/L)	K^{+} (mEq/L)	$HCO3^{-}$ (mEq/L)	Creatinine (mg/dl)	Urea (mg/L)
I	139.94 ± 0.34^{e}	98.567 ± 0.72^{a}	4.20 ± 0.90^{c}	24.10 ± 0.09^{a}	0.80 ± 0.01^{a}	40.76 ± 1.85^{a}
II	138.93 ± 0.41^{d}	100.75 ± 0.52^{b}	4.00 ± 0.02^{c}	25.99 ± 0.09^{a}	0.84 ± 0.01^{b}	46.79 ± 2.13^{b}
III	135.98 ± 0.43^{c}	104.17 ± 0.47^{c}	3.70 ± 0.021^{b}	28.01 ± 0.08^{b}	0.89 ± 0.02^{c}	51.32 ± 2.82^{c}
IV	133.16 ± 0.36^{b}	105.67 ± 0.26^{d}	3.49 ± 0.02^{b}	30.02 ± 0.12^{b}	0.94 ± 0.01^{d}	61.89 ± 2.39^{d}
V	129.97 ± 0.58^{a}	108.585 ± 0.38^{e}	2.80 ± 0.44^{a}	25.07 ± 5.47^{a}	0.98 ± 0.01^{e}	69.43 ± 2.39^{e}

Values are mean ± Standard Deviation (SD) for n=5 and mean along the column with different alphabetical superscripts indicates a significant different ($P<0.05$). Na^{+} = Sodium ion; K^{+} = Potassium ion; Cl^{-} = Chloride ion; HCO_3^{-} = Bicarbonate. Group 1: Control, Group II: 250 mg/kg, Group III: 500 mg/kg; Group IV: 750 mg/kg Group V: 1000 mg/kg of *Costus afer* stem juice.

Table 6: Levels of Heamatological Parameters of Wistar albino Rat administered with *Costus afer* Stem Juice Post 28 days

Group	HB (gldl)	PCV (%)	WBC ($X10^{12}$/L)	RBC ($X10^{12}$/l)	MCHC (gldl)	Platelet (X10/L)	MCH (Pg)	MCV (Fl)
Control-I	13.26 ± 0.28^{d}	40.20 ± 0.84^{d}	9.38 ± 0.08^{e}	4.24 ± 0.09^{c}	31.64 ± 0.10^{d}	188.80 ± 0.47^{b}	30.16 ± 0.05^{c}	95.18 ± 0.16^{b}
II	13.27 ± 0.16^{d}	44.60 ± 0.54^{e}	9.36 ± 0.09^{c}	4.26 ± 0.05^{c}	31.66 ± 0.05^{e}	189.60 ± 0.55^{b}	31.17 ± 0.05^{d}	95.56 ± 0.05^{a}
III	10.32 ± 0.08^{a}	32.60 ± 0.55^{a}	7.34 ± 0.09^{d}	3.36 ± 0.21^{a}	31.46 ± 0.05^{c}	169.40 ± 0.89^{c}	29.70 ± 0.07^{c}	94.28 ± 0.04^{a}
IV	11.18 ± 0.08^{b}	36.80 ± 1.30^{b}	4.60 ± 0.31^{b}	4.06 ± 0.09^{b}	29.34 ± 0.09^{a}	200.20 ± 0.84^{e}	28.06 ± 0.09^{a}	94.82 ± 0.46^{a}
V	11.50 ± 0.10^{c}	38.40 ± 0.89^{c}	2.72 ± 0.13^{a}	4.04 ± 0.09^{b}	29.64 ± 0.09^{b}	198.00 ± 0.71^{d}	28.26 ± 0.05^{b}	95.06 ± 0.05^{b}

Values are mean ± Standard Deviation (SD) for n=5 and mean along the column with different alphabetical superscripts indicates a significant different ($P<0.05$). Group 1: Control; Group II: 250 mg/kg; Group III: 500 mg/kg; Group IV: 750 mg/kg; Group V: 1000 mg/kg of *Costus afer* stem juice. HB, Haemoglobin; PCV, Packed Cell Volume; WBC, White Blood Cell; RBC, Red Blood Cell; MCHC, Mean Corpuscular Haemoglobin Concentration; MCH, Mean Corpuscular Haemoglobin; MCV, Mean Corpuscular Volume.

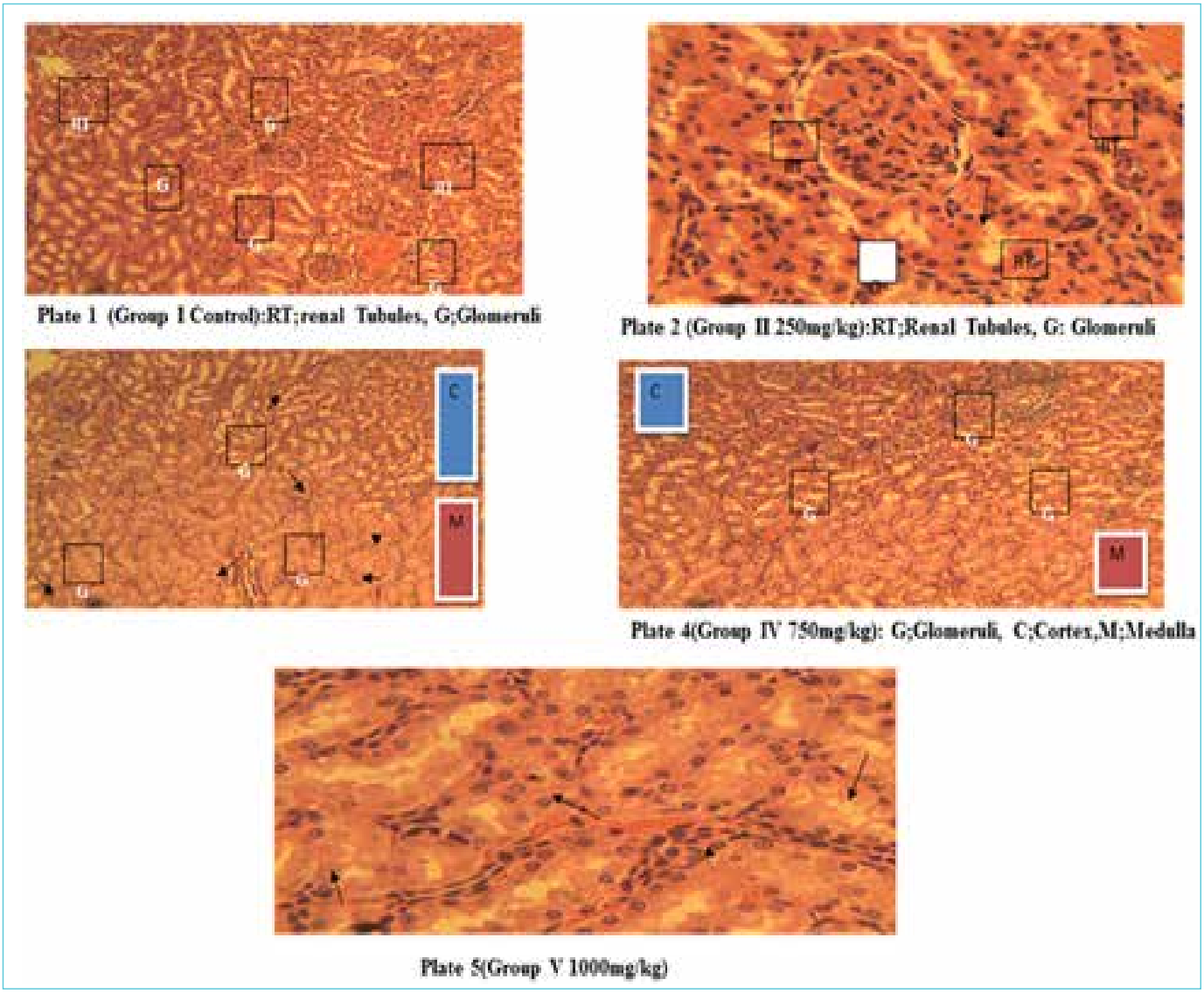

Figure 3: Histopathological Examination of the kidney

DISCUSSIONS

The qualitative phytochemical analysis of *C. afer* stem revealed the presence alkaloid, flavonoid, phenol, tannin, anthraquinones, cardiac glycosides and terpenoid. *C. afer* crude stem juice contains rich phytochemical constituents. Thirty bioactive compounds which give credence to the numerous medicinal uses of the plant were identified in its GC/MS analysis.

Increase in final body weight when compared to initial body weight was found to be significant ($P<0.05$), even in the control animal. The progressive increase in body weight at doses of 250 mg/kg, 500 mg/kg, 750 mg/kg and 1000 mg/kg of rats during 28 days oral administration of the extract probably reflect the nutritional status of the animals, which probably means that the extract did not affect the nutritional state of the treated animals.

The Kidney is highly susceptible to toxicants because, a high volume of blood flows through it and its ability to filter large amounts of toxins which can concentrate in the kidney tubules. It can result in systemic toxicity causing: Decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones. Blood urea nitrogen is derived in the liver protein /amino acid from diet or tissue sources and is normally excreted in the urine. In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance (Mayne and Mayne, 1994).

Creatinine is derived from creatinine and creatine phosphate in muscle tissues and may be defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass. Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The serum level of creatinine is independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of serum creatinine is an indicative of under-excretion, suggesting kidney impairment (Adekola et al., 2006). In this study, there were significant changes in the level of creatinine between control and test groups which suggests kidney impairment. Also in the case of serum urea, there was also significant difference $P<0.05$ between the values of control group and test groups, which implies that there was elevation of serum urea due to administration of the plant extract. Creatinine is more reliable than urea to assess kidney function, a significant increase in both creatinine and urea indicated that the plant extract is nephrotoxic.

The significant decrease in the serum sodium ion concentration following the administration of extract suggests a possible effect on the pump that maintains the constancy of its extracellular concentration (Low level of sodium MI. Hyponatraemia is defined as a serum sodium level of less than 135 mEq/L and is considered severe when the level is below 120 mEq/L, normal serum sodium levels are 135 - 145 mEq/liter (135-145 mmol/L) (Williams et al., 2016). This indicates that the animals in group IV (133.16 ± 0.360 mEq/l) and V (129.97 ± 0.581 mEq/l) had hyponatraemia, though it wasn't severe, this may be attributed to the diuretic property of the plant extract. Normal concentration of sodium and/or potassium in serum can affect the osmotic pressure of the body fluid which is related to blood pressure (Healy, 1995; Cheesbrough, 2002; Abubakar and Sule, 2010). The relationship between sodium and hypertension is based on the capacity of this mineral to "attract" water. When the sodium level decreases, the body loses water, thereby increasing sodium level. Blood pressure which depends in part on blood volume decreases as water loss increases. This is in agreement with the finding of Uchegbu et al., (2016) which proved that *C. afer* has anti-hypertensive activity.

Normal serum potassium levels are considered to be between 3.6 mmol/L and 5.4 mmol/L. Levels above 5.4 mmol/L indicate hyperkalemia, and those below 3.6 indicate hypokalemia (Williams et al., 2016). Also a significant decrease was observed in the test groups when compared with control in Serum potassium ion concentration which was dose dependent because it was still within the normal range. But in group IV and V its levels were below 3.6 mmol/L suggesting hypokalemia which is in consonance with the work of Osadolo and Emokpae, (2010).

Losses of Bicarbonate (HCO_3^-) values were significant in this study. Bicarbonate buffer system is the most important amongst blood buffers when the pH of the blood is considered (Fisher, 1969). Reduction in serum bicarbonates implies that the pH of the blood was lowered. This reduction can be due to two mechanisms: Excessive respiratory excretion via hyperventilation, or increased renal excretion of bicarbonates (Saba et al., 2009). There was no hyperventilation observed in the experimental rats, the renal route of excretion appears as the factor for the probable loss of bicarbonate.

It has been reported, that reduction in RBC, Hb and PCV is an indication of either the destruction of RBC or their decreased production, which may lead to anaemia. On the contrary an increase in the count of RBC, Hb, PCV, MCHC, platelet, MCH and MCV and is suggestive of polycythaemia and positive erythropoiesis (Arhoghro et al., 2014). There was a significant reduction in levels of RBC, HB, PCV MCHC, platelet, MCH and MCV in all groups from control indicating anaemia. Although platelet level increased beyond control in group IV and V indicating increased blood clothing property. General reduction in level of haematological parameters in this study is an indication of adverse effect.

The lowest dose of the test extract administered did not show any adverse effect on the renal histo-architecture. Groups 3 to 5 showed changes consistent with renal toxicity. The restriction of the histopathological changes to the renal tubules in the medulla suggests a possibility of bio-activation. It is possible that as the glomerular filtrate (renal excretion) travels down the renal tubules, bio-activation of the bi-products of the extract to a reno-toxic form occurs, which resulted in the observed histopathological changes

CONCLUSION

The study revealed that *C. afer* crude stem juice exhibits nephrotoxicity at high doses of 750 mg/kg and 1000 mg/kg, therefore it is time dependent and dose dependent toxicity.

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