



EFFECT OF SUB-CHRONIC ORAL ADMINISTRATION OF HYDROMETHANOLIC STEM EXTRACT OF *COSTUS AFER* KER GAWL. (COSTACEAE) ON LIVER AND KIDNEY FUNCTIONS IN WISTAR RATS

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ABSTRACT

The liver and the kidneys are two very important organs in the body and they are responsible for the metabolism and excretion of drugs respectively amongst several other functions. Severe adverse effects on these organs can lead to organ dysfunction or failure and a consequential effect on wellbeing and can even be life-threatening. This study investigated the effects of hydromethanolic stem extract of *Costus afer* Ker Gawl. (Costaceae) on liver and kidney function indices and the histopathology of the organs in Wistar rats. Serum liver enzymes which include: alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP), total protein and albumin as well as serum urea, creatinine, sodium ions, potassium ions, chloride ions, bicarbonate ions were evaluated in biochemical studies. Sections of the liver and kidneys appropriately treated were examined microscopically for pathological lesions. There were decreased serum levels of ALT and ALP, but serum levels of AST increased at 500 and 1000 mg/kg doses. Serum levels of total protein (TP) and albumin concentration as well as urea and creatinine serum levels were not significantly ($p > 0.05$) affected. However, histological examination of the liver and kidneys revealed slight to moderate hepatic necrosis and slight tubular necrosis respectively especially at 500 and 100 mg/kg doses of the extract. The results showed that the extract may be harmful to the liver and to a lesser extent the kidneys on prolonged administration and therefore it should be used with caution in such instances.

Keywords: *Costus afer*, kidney function, Liver function, Wistar rats

INTRODUCTION

In sub-Saharan Africa, an estimated 60 - 80% of the population rely on traditional medicines and healers as the primary source of healthcare (James *et al.*, 2018). This is mainly due to easy accessibility and affordability of consulting with traditional healers as well as the perception of traditional medicine being natural and therefore safer than conventional medicine (Amira and Okubadejo, 2007; Adeyeye *et al.*, 2011; Nxumalo *et al.*, 2011; Adinma *et al.*, 2015; Ahwinahwi and Chukwudi, 2016). There has been an increased interest in the use of herbal plants in recent times in many countries of the world including Nigeria (Oreagba *et al.*, 2011; Chintamunnee and Mahomoodally, 2012; Ekor, 2013). Medicinal plants are believed to be the most abundant, affordable, reliable and well understood form of health care especially in African countries (Abalaka *et al.*, 2009). Numerous bioactive compounds that can ameliorate some diseases and also improve the body's resistance to cellular stress are present in herbal plants (Iwalewa *et al.*, 2007). Some of these bioactive compounds have been used as lead compounds in the manufacture of new drugs and out of the 877 novel medicines that were developed between 1981 and 2006, 6% were of natural products and 16% were synthetics (Newman *et al.*, 2008).

Costus afer Ker Gawl., commonly known as gingerlily or bush cane belongs to the family *Costaceae*. It is one of the 150 species of tall, perennial, and rhizomatous herbs of the genus *Costus* (Edeoga and Okoli, 2000). It can attain a height of up to 4 m. It is commonly found in moist and shady forest belt of Senegal, South Africa, Guinea, Niger, Sierra Leone,

Ghana, Cameroon and Nigeria (Burkill, 1985; Edeoga and Okoli, 2000). In Nigeria, *Costus afer* is known as *Ireke omode* in Yoruba, *Kakizuwa* in Hausa, *Okpete* or *Okpoto* in Igboland, *Mbritem* in Efik. In Ijaw it is called *Ogbodou*, Anglophone Cameroon calls it 'Monkey sugar cane' (Anaga *et al.*, 2004). *Costus afer* Ker Gawl. (Costaceae) is commonly used as a medicinal plant throughout Africa. Some of its ethnomedicinal use include anti-diabetic, anti-inflammatory and anti-arthritis (Soladoye and Oyesika, 2008), cough, malaria, venereal diseases and skin eruptions (Okoko, 2009). Although some studies on the toxicity of the stem extract on the liver (Ukpabi, *et al.*, 2012) and of the aqueous leaf extract of *Costus afer* on liver and kidney (Ezejiofor *et al.*, 2013) of Wistar rats have been reported in literature, there is need for more toxicity studies on this plant in view of the various uses to which it is employed in traditional medicine and due to possible variation in phytochemical constituents of various plant parts as a result of differences in geographical locations, soil type and or climatic conditions. Hence, the study investigated the effects of sub-chronic oral administration of hydromethanolic stem extract of *Costus afer* on liver and kidney function indices as well as on the histology of the liver and kidneys of Wistar rats.

METHODS

Preparation of the extract: Three hundred grams (300g) of the powdered stem was mercerated with 1.8L 70% v/v methanol (JHD, China) for 72 hours at room temperature with occasional shaking. The mixture was filtered using muslin cloth, followed by Whatman No 1 filter paper to ensure all

debris was filtered out. The filtrate obtained was then concentrated at 45° C using a Rotary Evaporator (Searchtech Instruments, England. RE 52-3), under reduced pressure. The residue was dried in an oven at 45°C. The dried extract was weighed and the percentage yield was calculated as follows:

% Yield = Weight of dried extract (g) / Weight of powdered plant material (g) X 100

One gramme (1 g) of the dried extract was weighed and dissolved in 10 ml distilled water to get a stock solution of 100 mg/ml and other concentrations were prepared by serial dilution of the stock solution on each day of the experiment.

Sub-chronic Toxicity Studies: The study was carried out according to the OECD (1995) Guideline 407 for repeated dose 28 days oral toxicity study of chemicals in Wistar rats. Forty (40) rats of both sexes (20 males and 20 females) were weighed (160g -220g) and randomized into 4 groups of 10 rats each (5 males and 5 females kept in separate cages based on sex). Group I (Control) rats were administered distilled water 1ml/kg body weight, Groups II, III and IV rats received 250, 500 and 1000 mg/kg body weight (LD₅₀ > 5000 mg/kg, Jimoh *et al.*, 2019) of the hydromethanolic stem extract of *Costus afer* orally daily respectively, for 28 days. The rats were allowed free access to food and water during the duration of the study and they were observed daily for general symptoms of toxicity (hyperactivity, sedation and salivation) and mortality. The rats were weighed once weekly and the average change in weight calculated. The rats were starved of food (and not water) overnight on Day 28 and on the 29th day, they were first weighed and then sacrificed under light halothane anaesthesia. Blood samples were then collected through cardiac puncture for evaluation of biochemical parameters. The livers and the kidneys were harvested, weighed and examined macroscopically and were then preserved in 10% formalin prior to histological examination.

Biochemical studies: Blood samples were collected from the sacrificed rats into plain bottles, allowed to clot and centrifuged (Biobase, China. Model C200) at 3,500 rpm for 10 minutes. The separated sera were stored at - 4°C, and used for the evaluation of serum liver enzymes which include: alanine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP), total protein and albumin. Serum urea, creatinine, sodium ions, potassium ions, chloride ions, bicarbonate ions were also estimated using reagent kits (Randox Laboratories Limited, U.K) and a Colorimeter (Mediguard, China, Model 110)

Histological studies: These studies were carried out according to the methods described by Bancroft *et al.* (2018) in the Histology Unit, Department of Anatomy, Ahmadu Bello University, Zaria, Nigeria. Briefly: The livers and kidneys, after harvesting from the animals, were immediately

fixed in 10% formalin for 48 hours. After the fixing process was completed, the tissues were processed by passing them through ascending grades of methanol from 70% to 90% and 100% for 12 hours to properly dehydrate them. The tissues were then cleared in xylene for 2 hours and then infiltrated and embedded in liquid paraffin. The tissues were then cut using Rotary Microtome (RM 2125, Leica Microsystem, Germany) at 5 micron thickness and the sections were stained using haematoxylin and eosin staining technique. They were examined microscopically for pathological lesions. The lesions were observed for the following: infiltration of lymphocytes into portal and central veins, mucosal atrophy, presence of inflammatory cells on the wall, eosinophils, lymphocytes and plasma cells.

Photomicrographs were taken at x 250 magnification.

Ethical Approval: All experiments were carried out in accordance with the guidelines and principles of the Ahmadu Bello University Committee on Animal Use and Care (Approval Number: ABUCAUC/2018/015).

Statistical Analysis: All quantitative data were expressed as mean ± standard error of mean and presented as Tables or Plates. Data were analysed using One Way Analysis of Variance (ANOVA) followed by Bonferroni post hoc multiple comparisons test. Significant differences between means were assessed at 95% level of significance ie p-values less than or equal to 0.05 ($p \leq 0.05$) were considered significant. SPSS Version 20 (2011) software package was used to carry out the statistical analysis of the data.

RESULTS

Mortality and Behavioural Effects

There were no obvious signs of toxicity in the rats in the extract treated groups, but a total of five rats died within the 28 day period of the study in both the control and extract treated groups (Week 1: 1 rat in 500 mg/kg extract group, Week 2: 2 rats in control (distilled water) group and 1 rat in 1000 mg/kg extract group, Week 3: 1 rat in 1000 mg/kg extract group).

Effects on Biochemical Parameters

The hydromethanolic stem extract of *Costus afer* insignificantly ($p > 0.05$) reduced serum levels of ALT at the tested doses of 250, 500 and 1000 mg/kg but not in a dose-dependent way, and produced significant ($p \leq 0.05$) and dose-dependent reductions in serum levels of ALP when compared with the control (distilled water) (Table 1). The effect on AST was not in a particular manner (Table 1)

The extract at doses of 250, 500 and 1000 mg/kg had no significant effect ($p > 0.05$) on serum total protein (TP) and serum albumin concentration when compared with the control (Table 1).

Table 1: Effect of 28 days repeated oral administration of the hydromethanolic stem extract of *Costus afer* on liver function indices in Wistar rats

Liver Indices	Function	Distilled water	1	HMECA 250 mg/kg	HMECA 500 mg/kg	HMECA 1000 mg/kg
ALT (iu/L)		19.33 ± 1.02		17.67 ± 0.99	12.33 ± 2.17 ^{*b}	16.00 ± 3.14
AST (iu/L)		36.50 ± 1.48		30.67 ± 3.21 ^{abc}	47.33 ± 3.90 ^{ab}	40.17 ± 3.65 ^{ac}
ALP (iu/L)		34.05 ± 8.21		12.64 ± 1.34 ^{*a}	9.73 ± 0.50 ^{*b}	9.66 ± 0.65 ^{*c}
TP (g/dL)		5.85 ± 0.12		5.96 ± 0.44	5.75 ± 0.16	6.01 ± 0.51
ALB (g/dL)		2.80 ± 0.08		2.67 ± 0.08	2.88 ± 0.15	2.77 ± 0.11

Values are means ± S.E.M, n = 10, One Way ANOVA + Bonferroni post hoc test, *p ≤ 0.05 = significant difference between test groups and control and common alphabets in superscripts = significant difference between treatment groups at p ≤ 0.05

HMECA = Hydromethanolic stem extract of *Costus afer*

ALT = Alanine aminotransferase

AST = Aspartate aminotransferase

ALP = Alkaline phosphatase

TP = Total protein

ALB = Albumin concentration

Effect of 28 days repeated oral administration of hydromethanolic stem extract of *Costus afer* on kidney function indices in Wistar rats

There were no significant differences (p > 0.05) in all the kidney function indices between the extract treated doses of 250, 500 and 1000 mg/kg and the control (distilled water) except for potassium ions where the dose of 500 mg/kg of the extract showed a significant decrease (p ≤ 0.05) in serum level of potassium ions when compared with the control (Table 2). Bonferroni post hoc test showed no consistent pattern in changes in serum potassium ions levels in the extract treated groups (Table 2).

Table 2: Effect of 28 days repeated oral administration of the hydromethanolic stem extract of *Costus afer* on kidney function indices in Wistar rats

Kidney Indices	Function	Distilled water	1	HMECA 250 mg/kg/day	HMECA 500 mg/kg/day	HMECA 1000 mg/kg/day
Urea (mg/dL)		72.63 ± 20.48		108.88 ± 4.91	97.05 ± 5.98	83.96 ± 16.62
Creatinine (meq/L)		1.42 ± 0.30		1.68 ± 0.31	1.10 ± 0.15	1.33 ± 0.18
Sodium (mmol/L)		129.23 ± 4.01		129.32 ± 4.17	131.32 ± 4.84	133.49 ± 3.55
Potassium (mmol/L)		16.19 ± 0.47		16.58 ± 0.74 ^{ab}	13.70 ± 0.59 ^{*abc}	17.05 ± 1.04 ^{bc}
Chloride (mg/dL)		31.17 ± 3.39		29.50 ± 0.56	33.83 ± 1.66	30.50 ± 3.76
Bicarbonate (mg/dL)		91.17 ± 6.04		101.83 ± 3.83	98.17 ± 6.41	88.67 ± 2.16

values are means ± S.E.M, n = 10, One Way ANOVA + Bonferroni post hoc test, *p ≤ 0.05 = significant difference between test groups and control group and common alphabets in superscripts = significant difference between treatment groups (extract doses) at p ≤ 0.05

HMECA = Hydromethanolic stem extract of *Costus afer*

Effects on Histology of Organs

Effect on body weights and Relative Organ Weights (R.O.W)

Although there were increases in the mean body weights of rats in all the groups, percentage increases in the extract treated groups at all the doses tested were higher (p > 0.05) than that of the mean body weights of rats in the control (distilled) group throughout the 28-day period of the study (Table 3).

The hydromethanolic stem extract of *Costus afer* at the doses of 250, 500 and 1000 mg/kg did not cause any significant changes (p > 0.05) in relative weights of the livers and kidneys in the rats when compared with control group (distilled water) (Table 4).

Table 3: Changes in body weights of rats after 28 days repeated oral administration of the hydromethanolic stem extract of *Costus afer*

Time	Distilled water	1	HMECA 250 mg/kg/day	HMECA 500 mg/kg/day	HSECA 1000 mg/kg/day
Week 0	177.80 ± 8.06		178.90 ± 8.34	176.10 ± 8.55	181.30 ± 9.36
Week 1	177.30 ± 9.06		186.90 ± 9.95	169.40 ± 9.09	174.40 ± 10.99
Week 2	197.6 ± 10.03		189.80 ± 11.15	183.10 ± 10.79	191.56 ± 12.49
Week 3	199.28 ± 13.00		189.80 ± 11.33	197.11 ± 7.71	189.11 ± 14.86
Week 4	186.17 ± 2.75		201.78 ± 11.04	214.00 ± 8.74	191.89 ± 17.26
% Change in Weight	+ 4.7	+ 12.4	+ 21.5	+ 5.8	

Values are means \pm S.E.M, n = 10, One Way ANOVA, p > 0.05 = No significant difference between test group and control (Distilled water).

HMECA = Hydromethanolic stem extract of *Costus afer*.

Table 4: Changes in relative organ weights (R.O.W) in rats after 28 days repeated oral administration of the hydromethanolic stem extract of *Costus afer*

ORGAN	Distilled water ml/kg/day	HMECA mg/kg/day	250 mg/kg/day	HMECA mg/kg/day	500 mg/kg/day	HMECA mg/kg/day	1000
Liver (g)	3.87 \pm 0.25	3.81 \pm 0.09	4.08 \pm 0.27	4.02 \pm 0.33			
Kidney (g)	0.60 \pm 0.06	0.64 \pm 0.03	0.68 \pm 0.06	0.71 \pm 0.08			

Values are means \pm S.E.M, n = 10, One Way ANOVA, p > 0.05 = No significant difference between test groups and control (distilled water),

HMECA = Hydromethanolic stem extract of *Costus afer*.

Effects on the liver

The hydromethanolic stem extract of *Costus afer* at the doses of 250 and 500 mg/kg showed vascular congestion (VC) with lymphocyte hyperplasia (LH) and slight hepatic necrosis (HN). At the higher dose of 1000 mg/kg, the liver showed moderate hepatic necrosis (HN) as compared to rats in the control group (distilled water) which showed normal hepatocytes (Plate I).

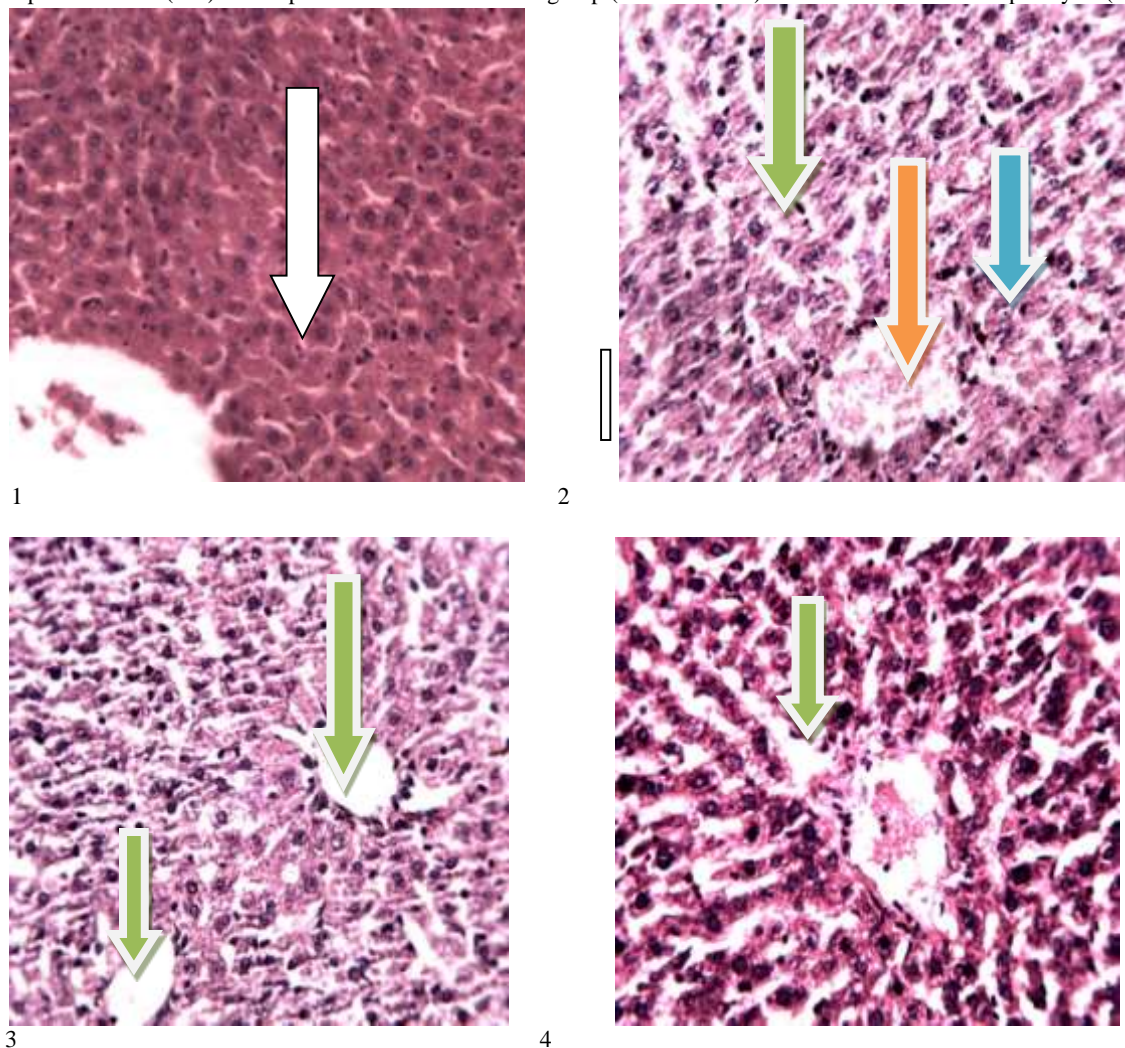


Plate I: Photomicrographs of the liver of rats administered with 250, 500 and 1000 mg/kg of the hydromethanolic stem extract of *Costus afer* compared with control (Distilled water) H and E stain (x 250 magnification)

1 = Distilled water showing normal hepatocytes (White arrow)
 2 = 250 mg/kg extract showing vascular congestion (Blue arrow) with lymphocyte hyperplasia (Orange arrow) and slight hepatic necrosis (Green arrow), 3 = 500 mg/kg extract showing slight hepatic necrosis (Green arrow), 4 = 1000 mg/kg extract showing moderate hepatic necrosis (Green arrow)

Effect on the kidneys

The hydromethanolic stem extract of *Costus afer* at the doses of 250, 500 and 1000 mg/kg produced slight tubular necrosis as compared to the normal glomeruli and normal tubules in the control group (Plate II).

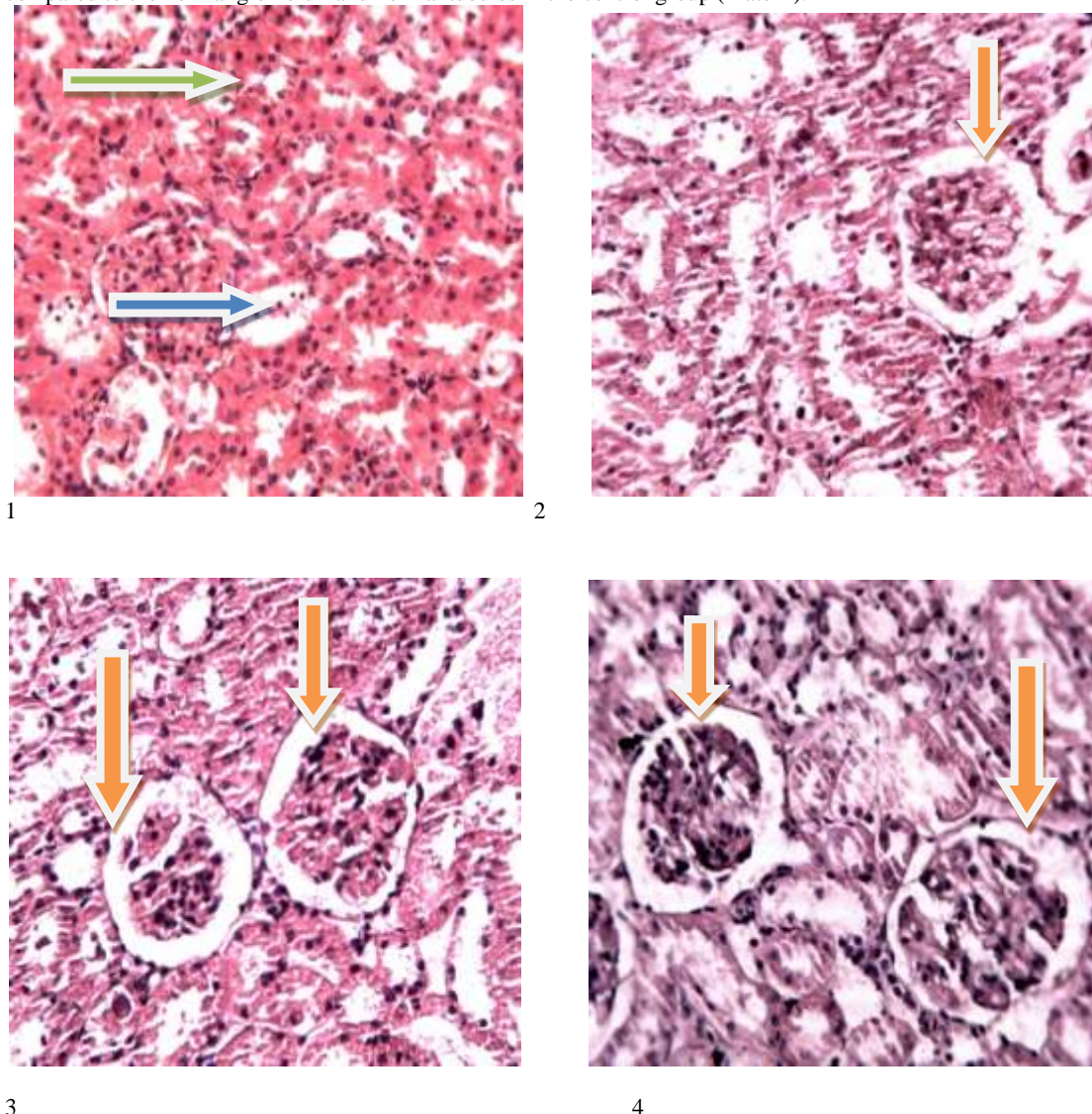


Plate II: Photomicrographs of the kidneys of rats administered with 250, 500 and 1000 mg/kg of the hydromethanolic stem extract of *Costus afer* compared with control (Distilled water) H and E stain (x 250 magnification)

1 = Distilled water showing normal glomeruli (Blue arrow) and normal tubules (Green arrow)
 2 = 250 mg/kg extract, 3 = 500 mg/kg extract and 4 = 1000 mg/kg extract showing slight tubular necrosis (Orange arrow)

DISCUSSION

Changes in body weights are markers of adverse effects of drugs and it is considered statistically significant if a body weight loss is more than 10% (Tepongning *et al.*, 2018). In this study there were moderate increases in body weights of rats in all the extract treated groups but these increases were not significantly ($p > 0.05$) different from the increases in mean weights of rats in the control group. This result is important as it is an indication that feeding of the rats was not adversely affected by the administration of the extract and it

can be inferred that there were no adverse effects of the extract on the body weights of rats. There were no obvious signs of behavioural toxicity in the study. However, a total of five rats died during the course of the study but this mortality involved rats in the treated groups as well as the control group and this deaths were, therefore, not likely due to the effect of the extract. The result showed that this plant is relatively non-toxic and may be safe for use in traditional medicine. Elevated serum levels of AST in the extract treated groups observed in the study as well as the vascular congestion and lymphocyte hyperplasia due to infiltration of inflammatory cells seen on

histological examination of sections of the liver may have led to the necrosis of the hepatocytes. This result is similar to that of Ezejiofor *et al.* (2013) who had earlier reported likelihood of hepatotoxicity with aqueous extracts of *Costus afer* leaves in Wistar rats. High levels of liver enzymes could be associated with liver necrosis and other conditions that promote abnormal liver cell membrane permeability (Cameron and Greger, 1998). Cytoplasmic enzymes are only found in high concentrations in mild liver injury, while severe damage results in the release of both mitochondrial and cytoplasmic enzymes (Cameron and Greger, 1998). The liver damage in this study was mild to moderate and it was likely due to the elevated serum levels of AST. Alkaloids and especially pyrrolizide alkaloids (PAs) induce hepatocyte necrosis that may progress to liver failure (Diaz, 2015). Hydrolyzable tannins or products of their degradation such as pyrogallol are hepatotoxic (Reed, 1995) and the tannins and alkaloids in the hydromethanolic extract of *Costus afer* may have caused the hepatic necrosis observed on histological examination of the liver of Wistar rats in this research.

Assessment of the kidney function is very important in toxicity evaluation of drugs and plant extracts as they are both necessary for the survival of the organism (Ezejiofor *et al.*, 2013). Measurements of serum levels of urea and creatinine are usually performed to evaluate kidney function (Arsad *et al.*, 2013). Both serum creatinine and urea levels are elevated in patients with renal malfunction, especially decreased glomerular filtration. In the early stage of kidney damage, increase in serum urea level usually precedes the increase in serum creatinine that is observed in chronic kidney damage (Craig, 2007). Serum creatinine is a significantly more reliable renal function screening test than serum urea because serum urea levels may be affected by dehydration, diet and protein metabolism, which do not affect serum creatinine levels. In this study there were increases ($p > 0.05$) in the serum levels of urea, creatinine, sodium ions, chloride ions and bicarbonate ions in all extract treated groups when compared with the control group (distilled water). The elevated serum urea levels may be an indication of early kidney damage and this is supported by the result of histological examination of the kidneys which showed slight tubular necrosis at all the extract treated doses and this will likely affect the efficient reabsorption of minerals and fluids from urine as it forms. The toxic effect on the liver and kidneys may be due to the presence of alkaloids in the extracts which can interfere with membrane permeability, membrane proteins (ion channels and receptors), enzymes and other proteins, DNA, RNA and corresponding proteins, electron chain and the cytoskeleton (Wink, 2016). The result of this study is in contrast to that of Ezejiofor *et al.*, 2013 who reported non-toxicity of the aqueous leaf extract of *Costus afer* on the kidneys of male albino Wistar rats in both biochemical and histological examination of the kidneys and this may be due to differences in phytochemical constituents.

CONCLUSION

The result of this study showed that the hydromethanolic stem extract of *Costus afer* Ker Gawl. (Costaceae) may be toxic to the liver and kidney on prolonged administration at the doses used in this study and therefore caution should be exercised when the plant is used to treat chronic disease conditions in traditional medicine.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

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