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TOXICITY PROFILES OF METHANOL EXTRACT OF CROSSOPTERYX FEBRIFUGA LEAF (AFZEL. EX G. DON) BENTH

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ABSTRACT

Crossopteryx febrifuga is one of the plants used for the local treatment of some disease conditions by the people of Nigeria via its leaf, root and bark without knowing the toxic nature of the plant recipes. This research was designed to ascertain the toxicity profiles of the plant leaf. Acute toxicity was determined in two phases using rats. In sub-acute toxicity studies, haematology, histopathology and evaluation of liver function indices were conducted. In the first phase of the toxicity study, the mice showed no observable toxic effects as high as at 1000 mg/kg. However, it caused sluggishness, respiratory distress at higher doses in the second phase. On haematological indices, white blood cell, lymphocyte, erythrocytes, haemoglobin, haematocrit rose significantly across the doses administered. The liver function test revealed significant increase in serum alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), albumin, total protein and conjugated bilirubin while 800 mg/kg extract reduced the aspartate transaminase. On histopathology, liver revealed normal hepatocytes but dilated central vein while in kidney, renal corpuscle, glomeruli and tubule were normal. On heart, revealed was active vascular congestion with normal bundle of myocardial fibres and coronary artery while in aorta, it revealed normal architecture. With the nature of the toxicity profile revealed especially at doses less than 500 mg/kg of *C. febrifuga* extract, the plant could be considered to be relatively safe. This study is significant as it provides information that may suggest some safe doses out of the doses used for clinical trial and application.

Keywords: *Crossopteryx febrifuga*, Toxicity, Histopathology, Haematology, Liver function indices

INTRODUCTION

Plants are essential sources of human needs such as for food, clothing, housing, and natural medicines for the well-being of man (Gurib-Fakim, 2006). Among the conventional drugs are those obtained from plant resources, such as quinine (*Cinchona officinalis*), aspirin (*Salix alba*), *digoxin* (*Digitalis purpurea*), and morphine (*Papaver somniferum*) (Gurib-Fakim, 2006). Several incidences of organs toxicity resulting from prolong ingestion of herbal medicine have already been reported (Ahmad, 2006, Bandaranayake, 2006). However, the demand and use of herbal medicine is rising possibly owing to its availability, affordability and the acclaimed safety (Hosseinzadeh *et al*., 2015, Mahomoodally, 2013). The rising patronage is not without its attendant consequences as some people tend to abuse it (Olayode *et al*, 2020). This therefore justify the need to establish and document the safety profiles. Moreover, the continuous call for translating herbal medicine into conventional medicinal (Agostinho, 2018, Krah, 2018) make it necessary to determine and document the safety profiles of every plant used for medication.

It is generally known that every drug is bound to be toxic at certain dose. Therefore, knowing the toxic status of any therapeutic agent is as important as its pharmacological effect. Essentially, *Crossopteryx febrifuga* Afzel (G.Don.) Benth. Rubiaceae has been widely used as traditional remedies in the Northern part of Nigeria without scientific basis of its safety. It is against this background that the studies on toxicity of *C. febrifuga* leaf was carried out to ascertain the toxic profile of the plant.

Toxicology is a science which deals with toxic or poisonous materials, their effects as well as management. Verifying the toxic nature of potential therapeutic agents is very critical in the development of novel drugs. The studies of toxicity started in (1493–1541) with a Physician by the name, Paracelsus. After his extensive research on toxicity of chemicals in plant and animals, he came up with the statement: "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy" (Hunter, 2008).

Several evidences of toxicity resulting from ingestion of drugs of plant origin have been documented. Previous studies on *C. febrifuga* extract reported that it has anti-malaria, anti-pyretic, analgesic and anti-inflammatory properties (Elufioye and Agbedahunsi, 2004; Salawu *et al*., 2008). The bark methanol extract of *C. febrifuga* was examined and found to be toxic at doses of 1000 mg/kg and above (Salawu *et al*., 2009). For the plant use in the local treatment of various ailments, it was essential to examine, confirm and make available its toxicity profiles.

Even though some pharmacological properties of *C. febrifuga* have been evaluated and reported, the toxicity profile especially on the leaf was before now not determined. In this report, the results of the evaluation of the toxic potential of the leaf extract of *C. febrifuga* using 7 day acute and 28 day subchronic toxicity approaches are presented. Furthermore, evidences of potential adverse effects associated with the plant leaf extract on haematological and liver function indices, as well as pathohistology on liver, kidney, heart and aorta of rats are presented.

MATERIALS AND METHODS

Toxicity studies of Methanol Extract of *C. febrifuga* Leaf

Experimental animals**:** Healthy Wistar rats of 180 g to 200 g were obtained and kept under standard environmental conditions (free aeration, free access to food and water *ad libitum*) to acclimatize for 7days in the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City, Nigeria.

Acute toxicity

The method described by Lorke (1983) was adopted. Thirteen rats were used in the investigation.

In the first phase, 3 different doses (10, 100, 1000 mg/kg) were administered to 3 groups each containing 3 rats. The rats were kept under the same conditions and observed for the first four hours for signs of toxicity such as licking of paw, stretching of the whole body, distress in respiration as well as death. The rats were thereafter observed daily for 7 days for the aforementioned signs.

In the second phase, more specific doses of 1600, 2900 and 5000 mg/kg were administered to 3 groups, each containing one rat as no death was recorded in the first phase and observed for 4 hours and then 7 days for signs of toxicity and mortality.

The median lethal dose (LD_{50}) value was obtained as a geometric mean of the highest non-lethal dose and the lowest lethal dose.

Sub-acute toxicity

This was conducted using the method described in WHO (1992). In this method, twenty rats (180 - 200 g) were randomly selected and then divided into four groups. The first group served as control while the remaining three groups were treated with 200, 400 and 800 mg/kg of plant extract orally for 28 days. The first day of dosing was taken as D_0 whereas the day of sacrifice was D_{28}. The weekly body weight of each rat was measured.

Blood was taken and the haematological analysis was conducted. Different organs namely the liver, kidney, heart and aorta were carefully dissected out and weighed in grams. Mortality, clinical signs and relative organ weight were determined. Gross pathology, microscopy and liver function test for ALP (alkaline phosphatase), ALT (alanine transaminase), AST (aspartate transaminase), TB (total bilirubin), CB (combine bilirubin), ALB (albumin), GLO (globulin) were conducted.

Statistical Analysis

Data obtained were expressed as mean ± SEM and analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test with the aid of Graphpad prism6 software. P-value less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Effect of *Crossopteryx febrifuga* on haematological indices in sub-chronic toxicity

Toxicological effects of *C. febrifuga* on blood indices (Table). WBC showed a little rise by 11.11% at 200 mg/kg but significantly rose by 36.06% at 400 mg/kg while at 800 mg/kg it decreased by 18.78%. Lymphocyte got increase at every dose but only significant at 200 and 400 mg/kg. Both monocyte and granulocyte got reduced at every dose administered. The rise in RBC is 4.13% at 200 mg/kg, significantly rose by 10.77% at 400 mg/kg but showed a mild rise of about 3.13% at 800 mg/kg. Haemoglobin (Hgb) rose but the percentage increase was insignificant across the doses administered. Haematocrit (Hct), a measure of the volume % of RBC increased but the percentage rise was not significant. As observed in Hgb, mean corpuscular haemoglobin (MCH) also rise. (MCV) is a measure of the average size of RBC. Mean corpuscular haemoglobin concentration (MCHC) which means how much Hgb inside one RBC. It was noted that no significant change occurred. Red cell distribution width (RDW) meaning the measure of RBC size or volume. On platelets, significant reduction was noted by 11.35%, 16.22% and 23.71% at 200 mg/kg, 400 mg/kg and 800 mg/kg respectively. Procalcitonin (Pct) showed rise but not significant. Mean platelet volume (MPV) showed a relative decrease only at 800 mg/kg extract. In a similar scenario, Platelet distribution wide (PDW) did not show significant change across the doses used.

Effect of *Crossopteryx febrifuga* on liver function indices in sub-chronic toxicity

The liver function test revealed a dose dependent significant increase in serum ALP by about 22%, 43% and 51% at 400, 800, 800 mg/kg of the extract respectively (Figure 1). The extract at 200, 400 and 800 mg/kg caused increase in ALT by about 42%, 29% and 13% respectively (Figure 2). AST was raised significantly by 118%, 99% at 200 and 400 mg/kg *C. febrifuga* extract (Figure 4.63). On the contrary, the 800 mg/kg extract reduced the AST (Figure 3).

On TB, the three doses lowered it significantly by very large margin (Figure 4). The extract at 200 mg/kg increased the CB insignificantly while significant increase by about 23% was noted at 400 mg/kg (Figure 5). However, the extract at 800 mg/kg reduced CB by about 17% (Figure 5).

With regard to TP, the extract at 200, 400 and 800 mg/kg showed nearly 26%, 19% and 14% increase respectively (Figure 6). Also noted was increase in ALB by 32%, 18% and 26% caused by the extract doses of 200, 400 and 800 mg/kg respectively (Figure 7). On GLO, the extract showed insignificant effect across the period of the treatment (Figure 8).

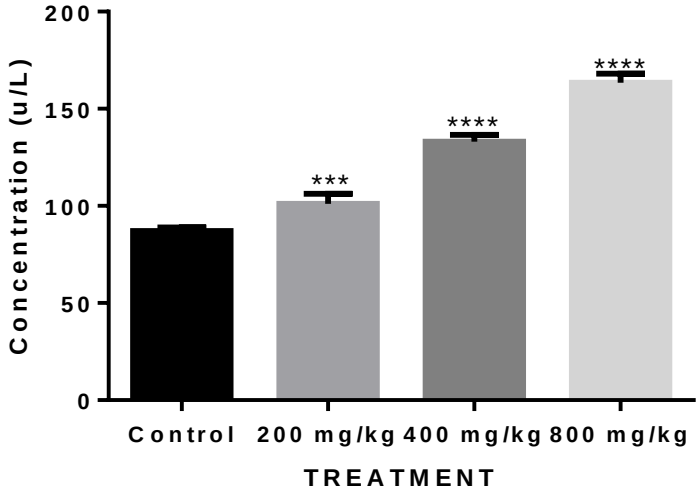


Figure 1: Effect of *Crossopteryx febrifuga* on serum alkaline phosphatase (ALP)

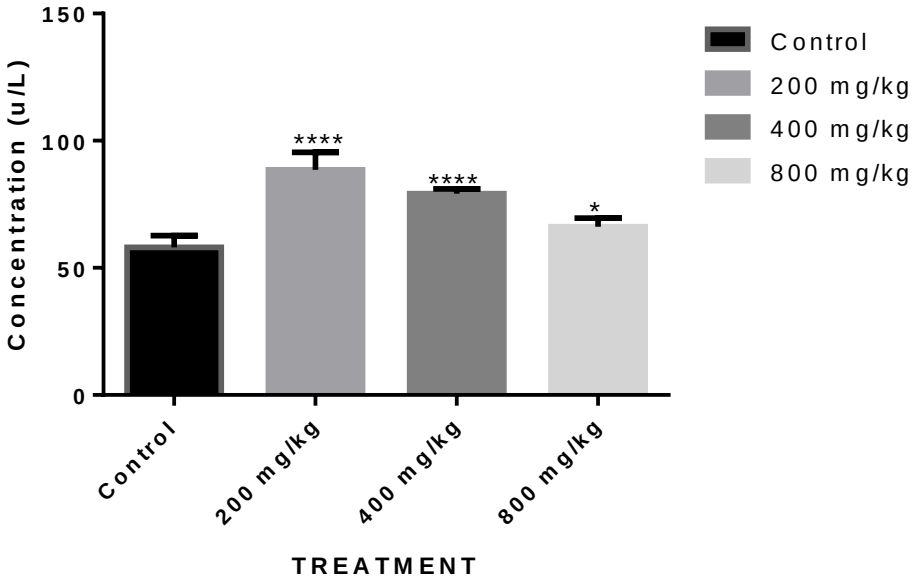


Figure 2: Effect of *Crossopteryx febrifuga* on serum alanine transaminase (ALT)

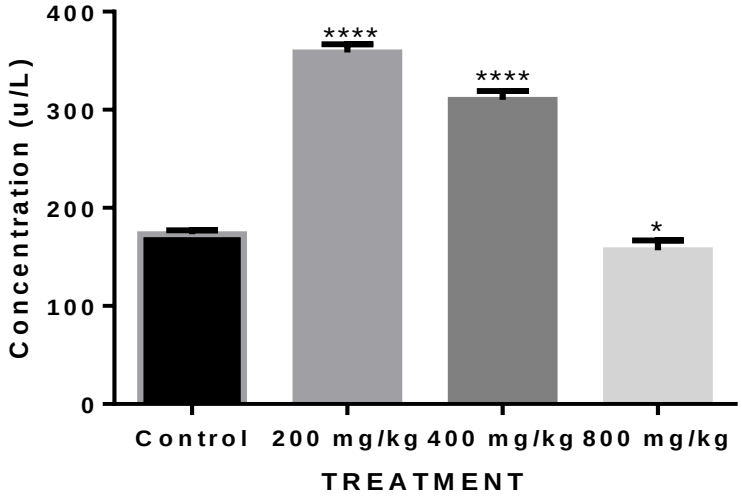


Figure 3: Effect of *Crossopteryx febrifuga* on serum aspatarte transaminase (AST)

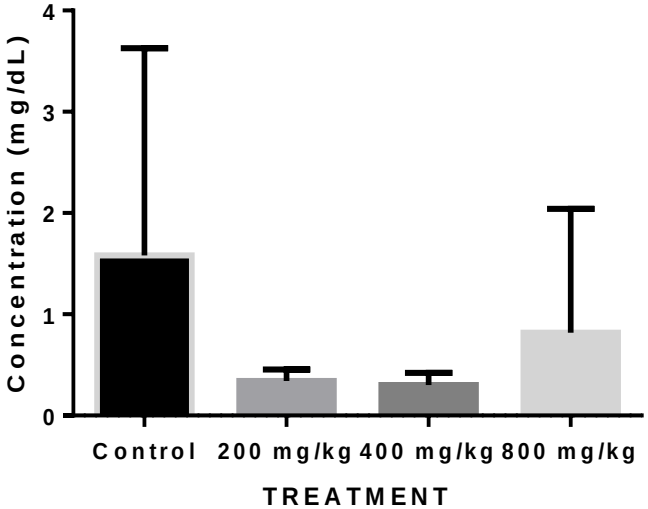


Figure 4: Effect of *Crossopteryx febrifuga* on serum total bilirubin (TB)

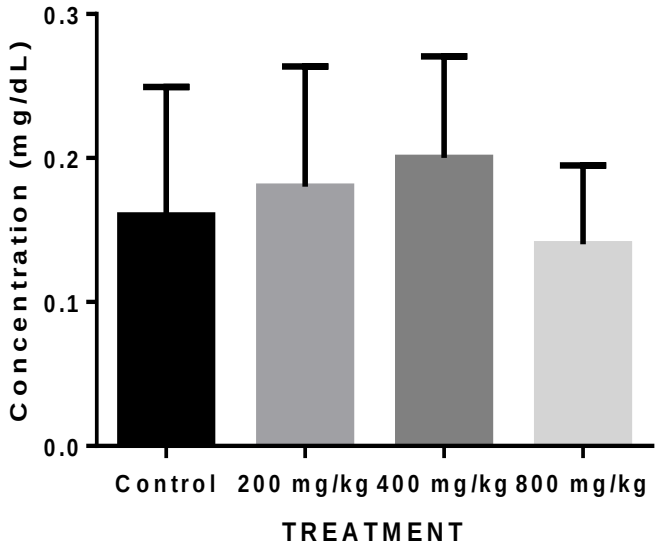


Figure 5: Effect of *Crossopteryx febrifuga* on serum conjugated bilirubin (CB)

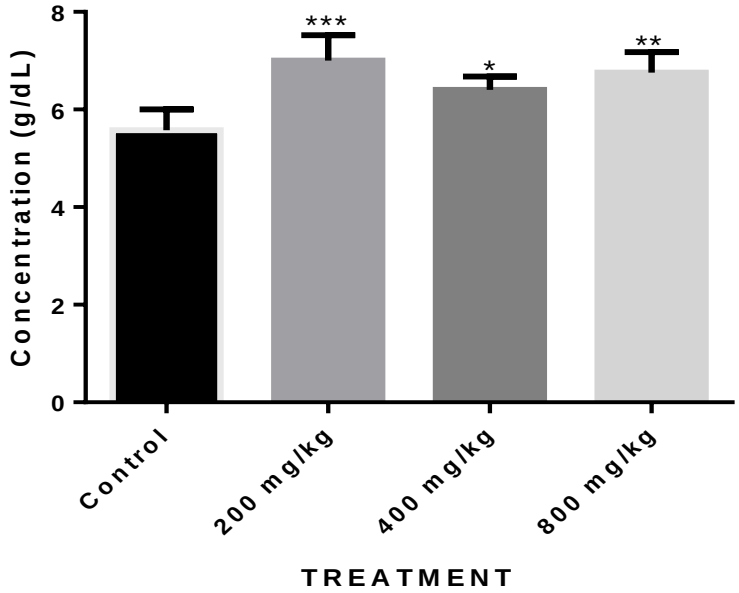


Figure 6: Effect of *Crossopteryx febrifuga* on serum total protein (TP)

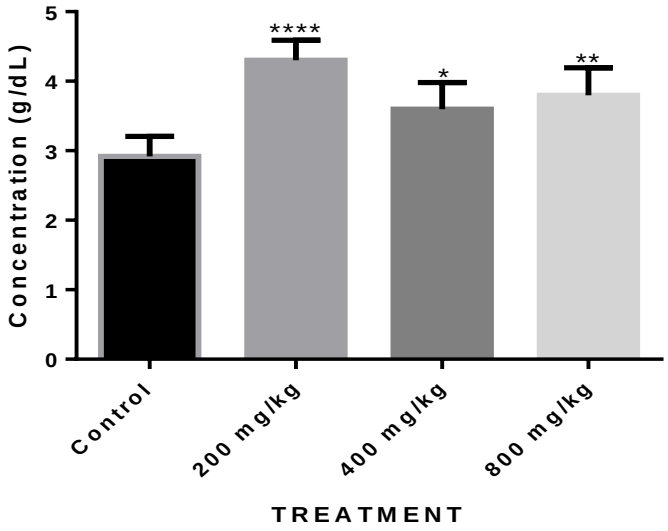


Figure 7: Effect of *Crossopteryx febrifuga* on serum albumin (ALB)

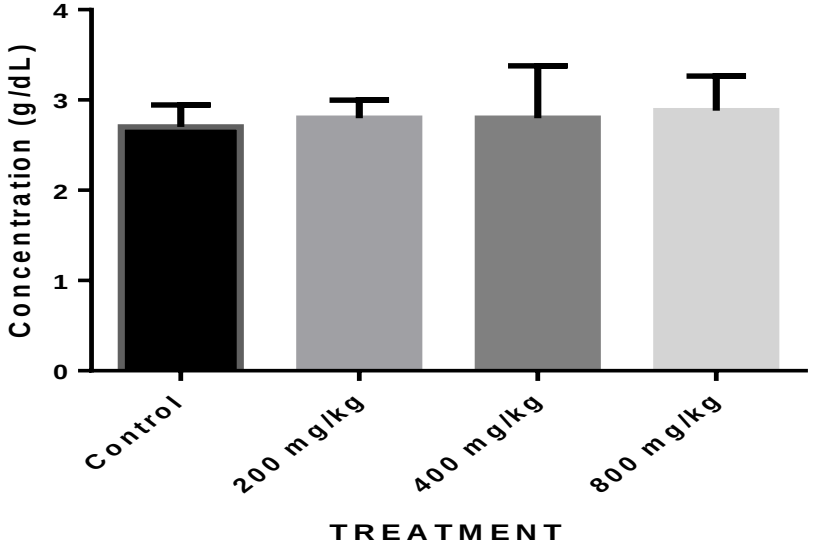


Figure 8: Effect of *Crossopteryx febrifuga* on serum globulin (GLO)

Effect of *Crossopteryx febrifuga* on haematological indices in sub-chronic toxicity

WBC showed a little rise by at 200 mg/kg and significantly rose by at 400 mg/kg while at 800 mg/kg it decreased by (Table 1). Lymphocyte got increase at every dose but only significant at 200 and 400 mg/kg (Table 1). Both monocyte and granulocyte got reduced at every dose administered (Table 1). There was rise in RBC at 200 mg/kg, significantly further rose at 400 mg/kg but showed a mild rise of about at 800 mg/kg (Table 1). Haemoglobin (Hgb) rose but the level of increase was insignificant across the doses administered (Table 1). Haematocrit (Hct), a measure of the volume % of RBC increased but the percentage rise was not significant (Table 1). As observed in Hgb, mean corpuscular haemoglobin (MCH) also rise. (MCV) is a measure of the average size of RBC. Mean corpuscular haemoglobin concentration (MCHC) which means how much Hgb inside one RBC. It was noted that no significant change occurred (Table 1). Red cell distribution width (RDW) meaning the measure of RBC size or volume. On RBC, no significant change was observed across the doses applied (Table 1). On platelets, significant reduction was noted at 200 mg/kg, 400 mg/kg and 800 mg/kg respectively (Table 1). Procalcitonin (Pct) showed rise but not significant (Table 1). Mean platelet volume (MPV) showed a relative decrease only at 800 mg/kg extract (Table). In a similar scenario, platelet distribution wide (Pdw) did not show significant change across the doses used (Table 1).

Table 1: Effect of *C. febrifuga* on haematological indices of Wistar rats

S/No	Parameters	Control	200 mg/kg	400 mg/kg	800 mg/kg
1	WBC	11.23±0.7067	12.59±0.9514	15.28 ± 1.071**	9.12 ± 0.2267
2	Lymphocyte	6.12±0.9216	8.12±1.070	11.02±2.341	6.25±0.6501
3	Monocyte	1.32±0.2956	1.0±0.1000	1.08±0.1393	0.9±0.2168
4	Granulocyte	4.38±1.140	2.96±0.5750	3.68±1.146	1.98±0.6240
5	RBC	7.98±0.1491	8.31±0.2208	8.84±0.1244*	8.23±0.2232
6	Hgb	15.74±0.3829	16.42±0.2801	16.04±0.3172	15.12±0.3652
7	HCT	42.08±0.8885	45.44±1.202	47.12±0.6003	36.04±5.566
8	MCV	51.66±0.6337	53.4±1.004	52.86±0.8262	52.76±0.8897
9	MCH	18.28±0.2498	18.8±0.1703	18.62±0.2035	18.54±0.2249
10	MCHC	35.12±0.3569	34.66±0.6329	34.88±0.1800	34.66±0.2731
11	RDW	17.70±0.3886	17.76±0.1030	17.4±0.1643	17.96±0.3172
12	PLT	1683.2±168.6	1492.8±111.4	1410.8±47.88	1284±43.94
13	PCT	4.345±0.1281	0.914±0.08931	0.799±0.02446	0.878±0.03785
14	MPV	6.8±0.1140	6.86±0.4285	6.94±0.4226	6.06±0.06782
15	PDW	3.54±1.487	4.4±1.555	7.08±0.2035	7.44±0.2315

Effect of *Crossopteryx febrifuga* on histology of liver, kidney, heart and aorta in sub-chronic toxicity

Liver: On control, the methanol extract revealed normal central vein (long arrow) and normal hepatocytes (short arrow) and sinusoid (medium arrow) (Plate 1 A). The 200 mg/kg revealed central vein (long arrow) with normal hepatocytes (short arrow) and Kupffer cells in sinusoid (median arrow) (Plate 1 B). The 400 mg/kg revealed histological structure with dilated central vein appearing large (long arrow) with normal hepatocytes (short arrow) (Plate 1 C) while the 800 mg/kg treated group revealed dilated central vein (long arrow) with normal hepatocytes (short arrow) (Plate 1 D).

Kidney: the control revealed normal renal corpuscle (long arrow) and normal tubule (short arrow) (Plate 2 A). The extract at 200 mg/kg revealed normal glomeruli (long arrow)

and normal tubule (short arrow) (Plate 2 B). At 400 mg/kg the methanol extract revealed normal glomeruli (long arrow) and normal tubule (short arrow) (Plate 2 C) while at 800 mg/kg, it revealed normal glomeruli (long arrow) and normal tubule (short arrow) (Plate 2 D).

Heart: Control revealed normal coronary artery (long arrow) with normal myocardial fibres (short arrow) (Plate 3 A).

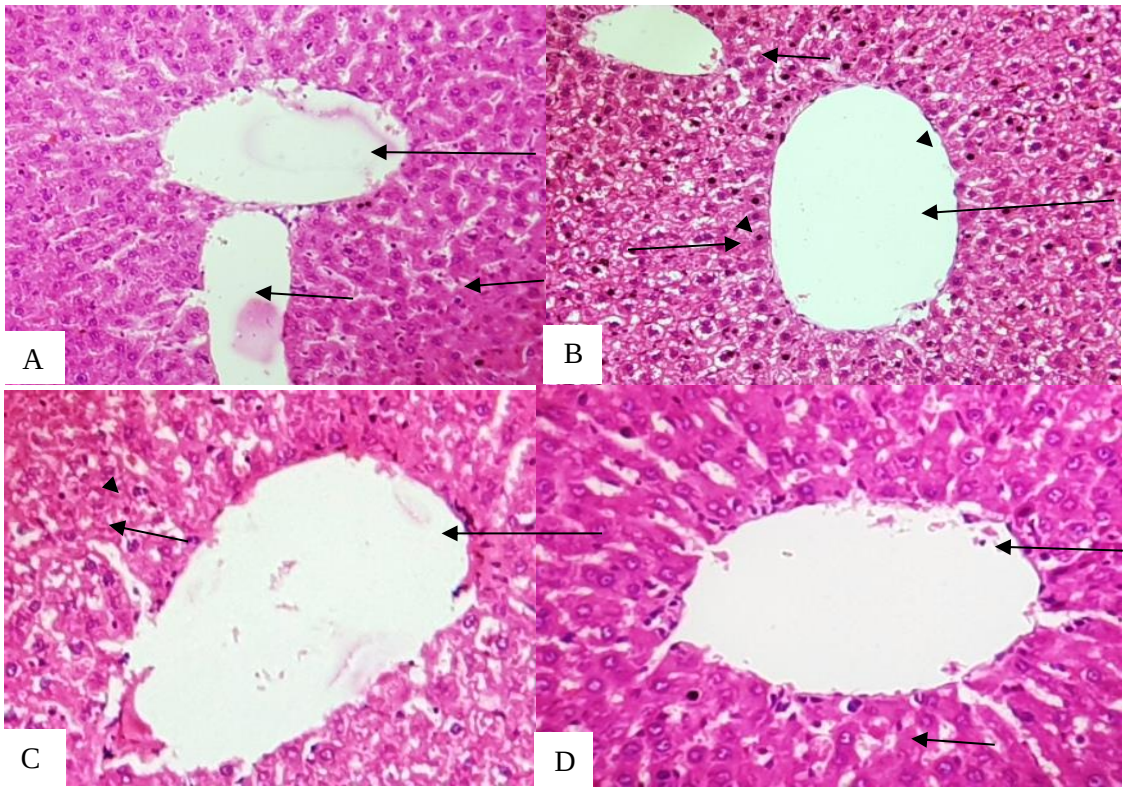


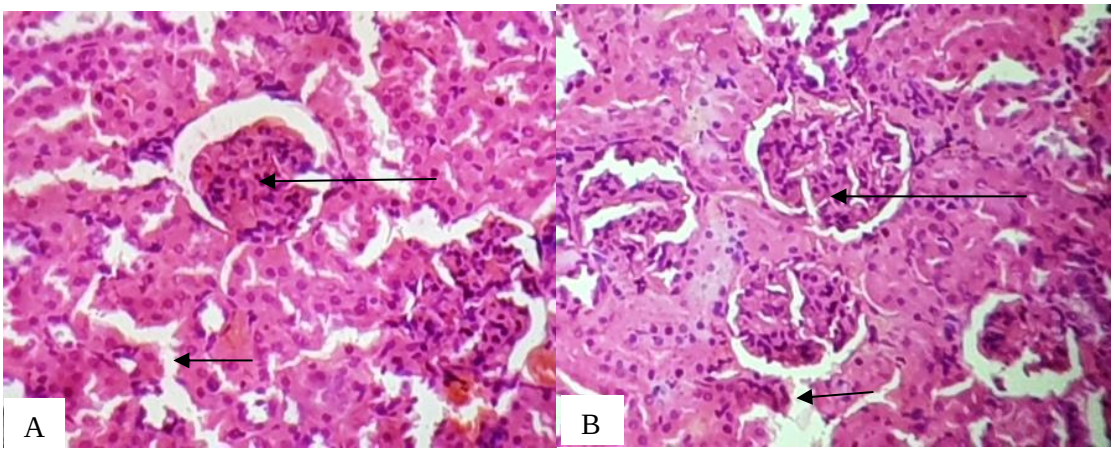
Plate 1: Photomicrographs of the effect of *Crossopteryx febrifuga* methanol extract on the liver of rats (H&E X100):

(A) Control (revealed normal central vein - long arrow, normal hepatocytes - short arrow and sinusoid - medium arrow).

(B) 200 mg/kg methanol extract (revealed central vein - long arrow with normal hepatocytes - short arrow and Kupffer cells in sinusoid - median arrow)

(C) 400 mg/kg methanol extract (dilated central vein appears large - long arrow with normal hepatocytes - short arrow).

(D) 800 mg/kg methanol extract (revealed dilated central vein - long arrow with normal hepatocytes - short arrow)



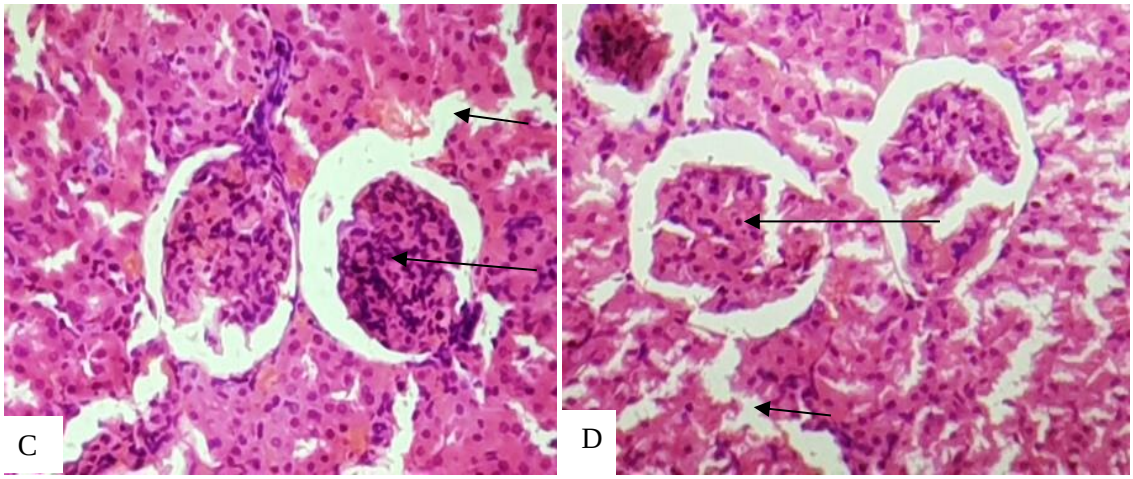


Plate 2: Photomicrographs of the effect of *Crossopteryx febrifuga* methanol extract on the kidney of rats (H&E X100):

(A) Control (normal renal corpuscle - long arrow and normal tubule - short arrow).
(B) 200 mg/kg methanol extract (normal glomeruli - long arrow and normal tubule - short arrow).
(C) 400 mg/kg methanol extract (normal glomeruli - long arrow and normal tubule - short arrow).
(D) 800 mg/kg methanol extract (normal glomeruli - long arrow and normal tubule - short arrow).

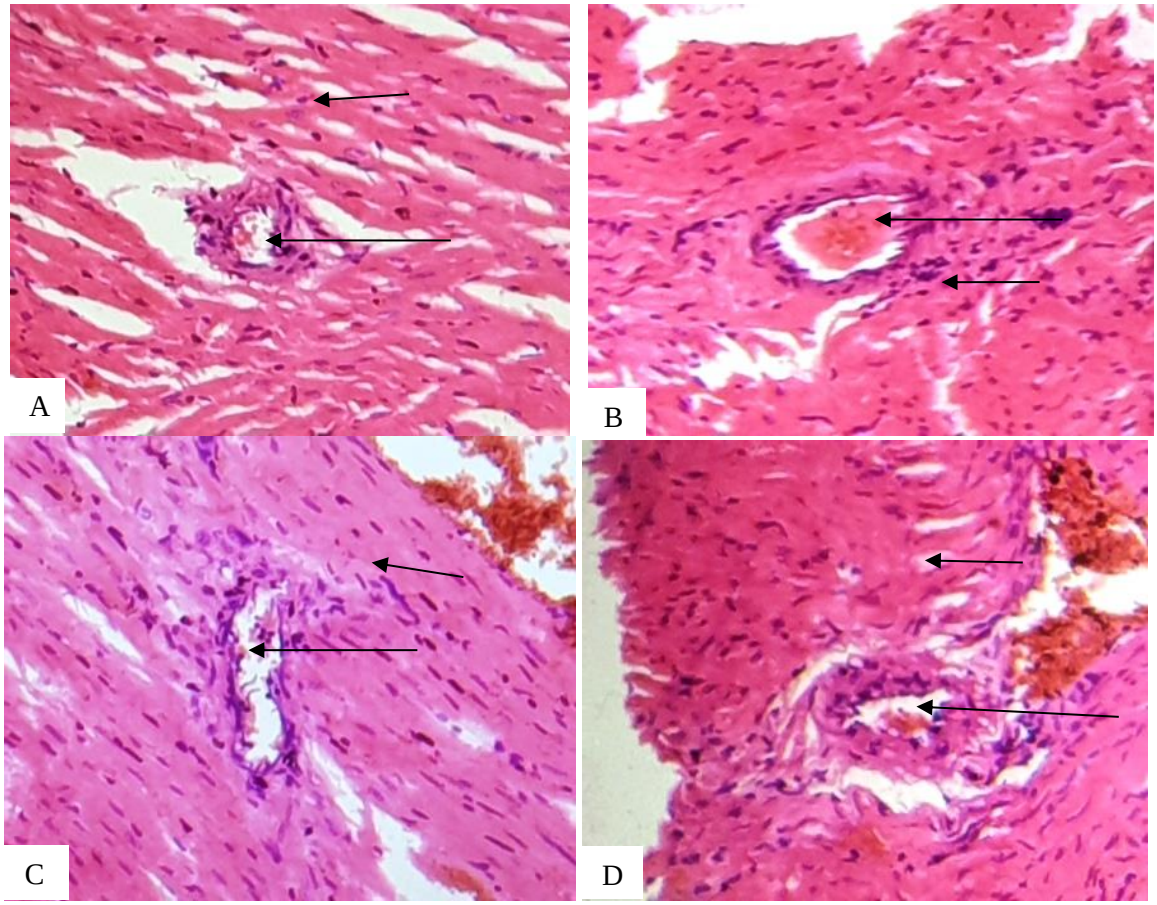


Plate 3: Photomicrographs of the effect of *Crossopteryx febrifuga* methanol extract on the heart of rats (H&E X100):

(A) Control (normal coronary artery - long arrow) with normal myocardial fibres - short arrow).
(B) 200 mg/kg methanol extract (active vascular congestion - long arrow and normal bundles of myocardial fibres - short arrow)
(C) 400 mg/kg methanol extract (reveal active vascular congestion (long arrow) with normal bundle of myocardial fibres (short arrow).
(D) 800 mg/kg methanol extract (coronary artery - long arrow with bundles of myocardial fibres - short arrow).

Moreover, 200 mg/kg revealed H&E (X100) active vascular congestion (long arrow) and normal bundles of myocardial fibres (short arrow) (Plate 3 B). On the other hand, 400 mg/kg: revealed active vascular congestion (long arrow) with normal bundle of myocardial fibres (short arrow) (Plate 3 C) while at

800 mg/kg revealed normal coronary artery (long arrow) with bundles of myocardial fibres (short arrow) (Plate 3 D).

Aorta: Control revealed normal tunica intima (short arrow), normal tunica media (medium arrow) and normal adventitia (long arrow) (Plate 4 A). At 200 mg/kg, it revealed normal tunica intima (short arrow) with bundles of muscle layer; tunica media (medium arrow) and adventitia comprising of connective tissue (long arrow) (Plate 4 B). At 400 mg/kg, revealed normal tunica media (long arrow) with visible adventitia comprising of connective tissue (short arrow) (Plate 4 C) while at 800 mg/kg it revealed normal tunica media with bundles of muscle layer (short arrow) and adventitia comprising of connective tissue (long arrow) (Plate 4 D).

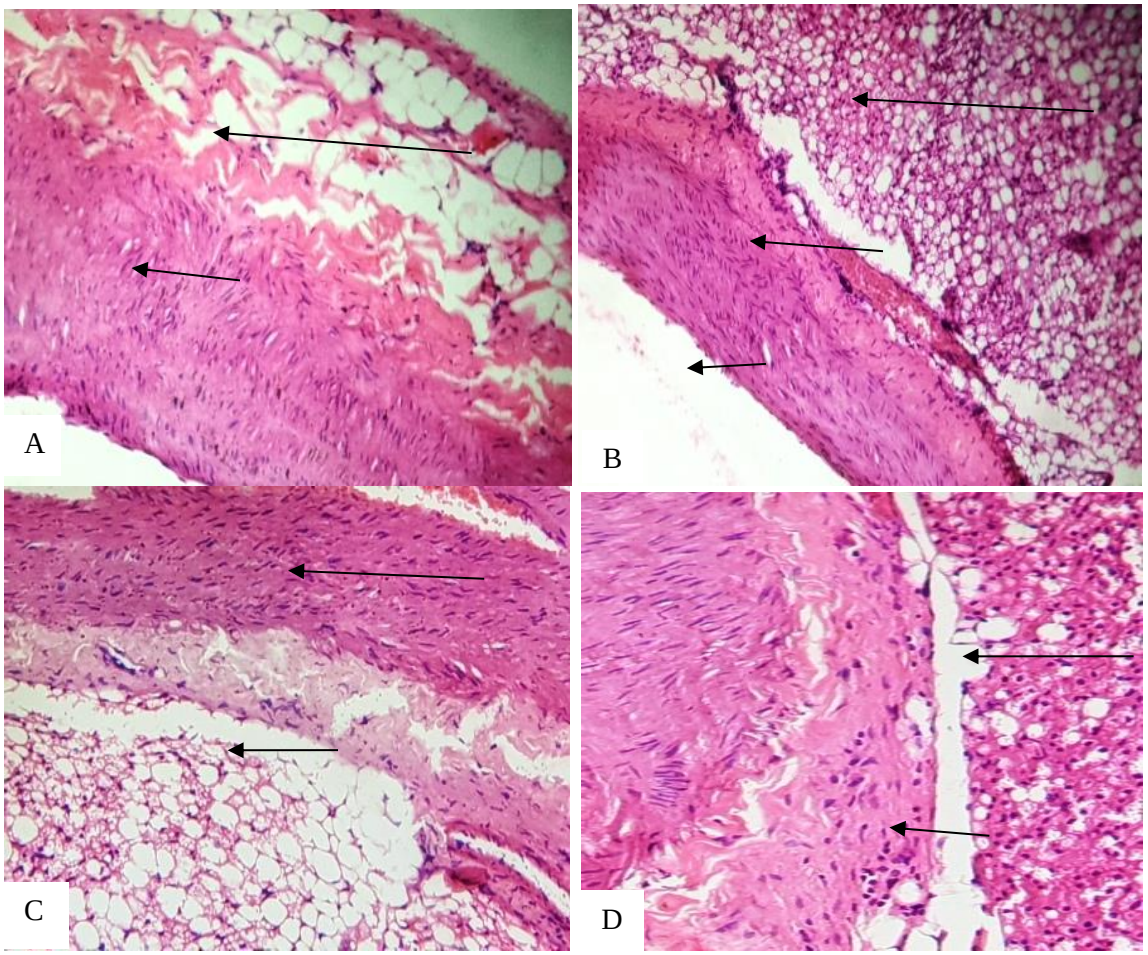


Plate 4: Photomicrographs of the effect of *Crossopteryx febrifuga* methanol extract on the aorta of rats (H&E X100):

(A) Control (normal tunica intima - short arrow, normal tunica media - medium arrow and normal adventitia - long arrow).
(B) 200 mg/kg methanol extract (tunica intima - short arrow) with bundles of muscle layer; tunica media - medium arrow and adventitia comprising of connective tissue - long arrow).
(C) 400 mg/kg methanol extract (tunica media with bundles of muscle layer - short arrow and adventitia comprising of connective tissue - long arrow).
(D) 800 mg/kg methanol extract (tunica media with bundles of muscle layer - short arrow and adventitia comprising of connective tissue - long arrow)

Since *C. febrifuga* is widely used traditionally in treating various ailments in the North central States of Nigeria, investigation of its toxicity cannot be overemphasized.

In the first phase of the toxicity study, the mice showed no observable side effects which probably suggests that the plant leaf extract is relatively safe for rats.

The leaf methanol extract of *C. febrifuga* showed some obvious toxic effects such as sluggishness, respiratory distress at 1600 mg/kg and mortality at 2900 and 5000 mg/kg in the second phase of acute toxicity study. This suggests that the plant leaf extract like other common drugs, conventional or non-conventional is not safe at overdose in rats. This as well indicated that the leaf extract of the plant may also not be safe at overdose for human consumption. It is a great advantage of any plant extract used as phytotherapeutic agent to have a very low toxicity, meaning high LD_{50}. In the current investigation, 1000 mg extract/kg/day was the greatest dose administered which is roughly equivalent to 70 g/day in a 70 kg person (Tan *et al*., 2007). At this dose of the extract, it is logical that any disorder arising during treatment may likely show toxic effect from the extract in man if taken in overdose.

According to Clarke and Clarke, (1979), any substance whose LD_{50} is higher than 1000 mg/kg is considered relatively safe.

The rise in ALP could be due to liver disease or bone disorder while rise in AlT could be due to necrosis, liver damage which necessitate release of more of it. The increased ALT is probably due to injury to liver cells, liver damage caused by the crude drug. More so, the rise in Albumin and globulin may be due to inflammation resulting from the effect of the crude drug.

Haematological indices from sub-acute toxicity study

With the significant rise noted in WBC at 400 mg/kg, it could be as a result of its immune response to injury caused by the

drug leading to immune boosting. Similarly, lymphocyte rise may be due to injury caused by the extract. Granulocyte reduction is probably due to injuries while monocyte rise is perhaps due to bone marrow suppression and injury (Adedapo *et al*., 2007, Adedapo *et al*., 2008, Mohajeri, 2007).

Increase in RBC could be due to hypoxia as a result of heart problem caused by drug toxic effect. This therefore, in turn supports the rise in Hgb which could be due to a corresponding rise in RBC. Also, rise in RBC, Hb and pcv was suggestive of polycythemia and erythropoiesis (Kuppasi *et al*., 2009, Okpuzor *et al*., 2009). Haematocrit (Hct) is a measure of the volume of the percentage of RBC. Rise could as well be due to rise in RBC. Mean corpuscular haemoglobin (MCH) is a measure of the average size of RBC. Its rise could also be due to rise in hgb. MCHC is mean corpuscular haemoglobin concentration. It is a measure of the quantity of hgb inside one RBC. *C. febrifuga* at all doses administered showed insignificant toxic effect on it.

On red cell distribution width (RDW), that is measure of RBC size or volume, the *C. febrifuga* methanol extract as well showed insignificant toxic effect.

Platelets reduction is due to some drug ability to destroy platelets probably by confusing the immune system. Therefore, the plant showed the capacity of decreasing the platelets at the doses applied. However, Adedapo *et al*., 2008 and Adeniyi *et al*., 2010 suggested that reduced platelets reduced the viscosity of blood which consequently reduced blood pressure. In line with this, the property could be seen as a possible side effect of the crude drug. So, this should be taken care of if it is to be used as curing agent of an ailment.

Procalcitonin is a peptide precursor of the hormone calcitonin which is involved in calcium homeostasis. Rise is perhaps due to tissue injury and of course is a mild toxic effect since it is insignificant but worthy of note.

Mean platelet volume is only relatively low at 200 mg/kg extract, indicating that the drug expressed mild effect on it. On platelet distribution wide which normally reflects how uniform platelets are in size. It is high which means that platelet size varies greatly, a clue of the disorder caused by the extract toxicity.

The haematological indices are veritable tools for determining the level of lethal effect of foreign agents in the blood such as plant material introduced to the body through any means. In addition, it explains blood associated effect subjected to chemical constituent.

More so, such results from past investigation have been highly responsive, exact and consistent which remains the basis for coherent and ethical research, disease diagnosis, prophylaxis and cure (Yakubu *et al*., 2005). From the foregoing, the facts revealed is in tandem with that of Adedapo *et al.* (2008) that the normal values of these indices were possibly distorted by the intake of toxic quantity of plant material. The result has it that plant extract at graded doses showed no much significant changes on the leucocytes, erythrocytes and thrombocytes in the extract treated groups after 28 days of oral administration. Also in the present study, a significant increase in WBC, RBC and lymphocytes were observed.

The extract at 200 mg/kg revealed prominent centriole wall with visible mononuclear cells. The hepatocytes revealed nuclei with pyknotic appearance showing the irreversible condensation of chromatin in them thereby leading to necrosis. At 400 mg/kg, the centriole appears large with mild mononuclear cells surrounding it as evidence of invasion or attack occasioned by foreign agents. The hepatocytes also revealed slightly vacuolated nucleus with evident mild steatosis, a condition in which there is infiltration of liver cells with fats usually associated with metabolic disorder. At 800 mg/kg, revealed was centriole, prominent and mild fatty changes resulting from crude drug toxic effect. On the normal control, revealed was distinct centriole and hepatocytes with pyknotic nucleus, an irreversible condensation of chromatin in them thereby leading to necrosis or apoptosis and well fenestrated.

The methanol extract at 200 mg/kg revealed visible large renal corpuscle and interstitial space and tubules with prominent mononuclear cells in the parenchyma. At 400 mg/kg, revealed was visible renal corpuscle with lesser inflammatory cells surrounding the tubules with appearance not distinct. This slight inflammation observed when compared to the normal control is a clear attestation of the toxic effect resulting from the drug. At 800 mg/kg, revealed was physically distinct healthy renal corpuscles. However, the mild distortion in the tubules may be due to the toxic effect of the crude drug. This mild distortion is in tandem with the report of Salawu *et al.* (2008) who revealed that the distortion was unremarkable at 250 and 500 mg/kg but showed especially glomerular atrophy at 1000 mg/kg. However, the normal control revealed normal renal corpuscles containing slight granulation and prominent tubules with normal interstitial spaces.

The extract at 200 mg/kg revealed bundles of myocardial fibres with mononuclear cells, interstitial space and congested coronary artery which may be due to the extract ability to increase LDL along the arterial walls as seen in antihyperlipidaemia activity study of the plant extract. Similarly, the ability of the drug at 400 mg/kg to make coronary artery become atrophied and at 800 mg/kg made coronary artery become visibly congested with bundles of myocardial fibres with mild infiltrates, interstitial proved that the drug has direct impact on heart. From all indications, *C. febrifuga* at the three doses administered over the 28-day period affected the coronary artery negatively by causing congestion and muscular atrophy. On the normal control, revealed was coronary artery with visible myocardial fibres, interstitial space.

It is however a possibility that the extract at nontoxic dose perhaps might have metabolized to toxic substances which consequently interfered with gastric function and reduced the efficiency of catabolic action on food in line with the observations made by Salawu *et al*., 2008.

It could be inferred that *C. febrifuga* doses below 400 mg/kg showed no toxic effect in Wistar rats and this gives credence to its vast folkloric use in treating various ailments in Nigeria. To this end, the safety profile obtained from the toxicity study of the plant extract could prove its safety for conventional marketing and utilization.

At 200 mg/kg, revealed were tunica intima with bundles of muscle layer; tunica media with visible mononuclear and histiocytic infiltrates and adventitia comprising of connective tissue. At 400 mg/kg, revealed was thickened tunica media with visible mononuclear and histiocytic infiltrates and mild angiogenesis with visible adventitia comprising of connective tissue. On 800 mg/kg dose, revealed were bundles of muscle layer; tunica media with visible mononuclear, inflammatory and histiocytic infiltrates probably resulting from massive production of histiocytes due to immune response which precipitated into necrosis. Adventitia comprising of connective tissue with diffused mononuclear infiltrates due to inflammatory lesions where white blood cells especially the macrophages and lymphocyte collect at site of injury to aid in clearing debris. This is a sign of the immune response to indicate the rejection of the foreign agents. However, revealed of control group was normal tunica intima with bundles of muscle layer; tunica media and adventitia comprising of

connective tissue. This is in contrast to what were noted of histology in the rats treated with *C. febrifuga* extract. Histological investigation was said to be a golden standard for evaluating pathological alterations which arose from treatment in organs and tissues (OECD, 1995). The results of this study therefore suggest that the extract caused treatment related disorders.

From the forgoing, the study suggests that oral application of *C. febrifuga* at doses lower than 500 mg/kg may not produce serious toxic effects and so could be relatively safe for human consumption. With the wide use of *C. febrifuga* traditionally for the treatment of variety of ailments in Nigeria, the present toxicity profiles provide information on its level of safety which can be used in obtaining regulatory right for public consumption and trading.

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