International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 4 (1), pp. 001-006, January, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Acute and sub-acute toxicological evaluation of the methanolic stem bark extract of *Crossopteryx febrifuga* in rats

O. A. Salawu[1], B. A. Chindo[1], A. Y. Tijani[1]*, I. C. Obidike[1], T. A. Salawu[2] and A. James Akingbasote[1]

[1]Departments of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, P.M.B. 21, Garki, Abuja, Nigeria.

[2]Departments of Microbiology, Human Virology and Biotechnology National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B 21, Garki, Abuja, Nigeria.

Accepted 19 October, 2012

Crossopteryx febrifuga **(Afzel.) Benth. (Rubiaceae) widely used in Northern Nigeria for management of trypanosomiasis, malaria and pain, has been previously shown to possess analgesic, anti-pyretic and anti-plasmodial effects. In the present study, acute and sub-acute toxicity studies of the methanolic stem bark extract of *C. febrifuga* were carried out in rats. Using modified lorkes (1983) method. In the sub acute toxicity study, 4 groups of 5 rats per group were used. Group 1 rats (control) received normal 10 ml normal saline/kg body weight while rats in groups 2, 3 and 4 were given daily doses of 250,500 and 1000 mg extract/kg body weight for 28 days. The effect of the extracts on feed intake, water intake and body weight changes, haematological and biochemical parameters as well as histological studies of vital organs (heart, lungs, kidneys, liver, brain, spleen and gonads) were assessed. Treatment related mortality was observed in the rats at a dose of 4000 mg/kg orally and 600 mg/kg intraperitoneally in the acute toxicity study. The oral and the intraperitoneal median lethal dose (LD_{50}) values of the extract were calculated to be 2828.48 and 471.17 mg/kg body weight respectively. The extract at 500 and 1000 mg extract\kg body weight doses produced significant ($P < 0.05$) decrease in fluid and feed intake, as well as body weight. The haematological parameters were normal except a significant ($P < 0.05$) increase in platelet counts observed at all dose levels used. At 1000 mg extract/ kg body weight dose, the extract exerted a significant ($P < 0.05$) decrease that lies within the normal range values of serum aspartate, alanine transaminase and creatinine levels for this animal species. Histological findings indicated possible toxicity in the Liver, Lungs, Kidney, Testes and Uterus At 1000 mg/kg. These results suggest that oral administration of *C. Febrifuga* may not produce severe toxic effects at doses lower than 500mg extract/kg body weight.**

Key words: *Crossopteryx febrifuga*, sub-acute toxicity, haematology, biochemical and histopathology.

INTRODUCTION

The use of herbal medicines in the treatment of various disease conditions has expanded rapidly, globally. This is attributable to the affordability, accessibility and efficacy of herbal remedies (Eldin and Dunford, 1999). The increasing use of herbs therefore makes it pertinent that pre-clinical toxicological studies be carried out on these natural products. Growing as a straggling shrub or small tree, *Crossopteryx febrifuga* (Afzel.) Benth belongs to the Rubiaceae family; a large family of 630 genera and 10,200 species found worldwide, especially in tropical and warm regions (Dalziel, 1957). An infusion prepared from the leaves is used in the treatment of malaria and as a lotion for itching in Northern Nigeria while the fruits are used in Mali for infections of the respiratory apparatus, as an antitussive and a febrifuge (Bridson and Verdcourt, 1988). In French Guinea and Sierra Leone, a strong infusion of the bark is used for fevers, and as an

*Corresponding author. E-mail: ade_dat@yahoo.com. Tel: +234-8058123982. Fax: 234-9-5231043.

astringent for dysentery and diarrhoea (Tomas- barberan and Hostettman, 1988). Chemical compounds isolated from different parts of the tree include four quercetin and five non-quercetin containing flavonoids from the leaves (Gariboldi et al., 1990), two bisdesmonic saponins and one triterpene saponin from the stem barks (Norman et al., 2005), and traces of an alkaloid, crossopterine in the bark (Tona et al., 2000). *C. febrifuga* stem bark has been reported to be effective in the treatment of trypanosomiasis, malaria and *staphylococcus aureus* infection (Hostettmann et al., 2000; Yusuf et al., 2004). Previous studies in our laboratory using crude methanolic extract of *C. febrifuga* revealed that it has anti-malaria, anti-pyretic, analgesic and anti-inflammatory properties (Elufioye and Agbedahunsi, 2004; Salawu et al., 2008).

Considering the widespread use of this plant in the management of various ailments, this study was carried out to evaluate and provide information on the sub-acute toxicity profile of its methanolic stem bark extract.

MATERIALS AND METHODS

Plant Material

Fresh stem bark of *C. febrifuga* was collected from Suleja, Niger State, Nigeria. It was identified and authenticated by Mallam Ibrahim Muazzan of the department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja where herbarium specimen (voucher number 4041) was deposited.

Preparation of the plant material

The stem bark was cleaned to remove adhering dirt, air-dried for two weeks and crushed into coarse powder using a pestle and mortar. Extraction was carried out by cold maceration of 500 g of the coarse powder with 2.5L of 70% $^{v}/_{v}$ methanol for 72 h, with constant shaking using the GFL shaker (no. 3017GBh, Germany). The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate was concentrated to dryness *in vacuo* at 40°C using rotary evaporator to give a yield of 20% $^{w}/_{w}$ of the extract. Aliquot portions of the extract were weighed and dissolved in distilled water for use in the study.

Animals

Swiss albino rats (100 - 120 g) of either sex maintained at the animal facility centre of NIPRD, Abuja were used. The animals were housed under standard conditions of temperature, (25 ± 2°C) and light, (approximately 12/12 h light-dark cycle), fed on standard diet and given water *ad libitum.* All experiments performed on the laboratory animals in this study followed the NIPRD approved Standard Operation Procedures (SOPs).

EXPERIMENTAL

Acute toxicity studies: The median lethal dose (LD_{50}) of the extract was determined in rats orally and intraperitoneally according to modified Lorke's method (1983). The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats each given 10, 100 and 1000 mg extract/kg body weight orally. The rats were kept under the same conditions and observed for signs of toxicity which include but not limited to paw-licking, stretching, respiratory distress and mortality for the first critical 4h and thereafter daily for 7 days. In the second phase of the study, 2000, 4000 and 6000 mg extract/kg body weight orally respectively were administered to another fresh set of three groups of three rats each. These rats were also observed for signs of toxicity and mortality for the first critical 4h and thereafter daily for 7 days. The oral median lethal dose was calculated as the geometric mean of doses that caused 0 and 100% mortality respectively. The first phase of the procedure was repeated using intraperitoneal route of extract administration. In the second phase of the study however, doses of 225,370 and 600 mg extract/kg body weight were given to another fresh set of three groups of three rats each. These rats were also observed for signs of toxicity and mortality for the first critical 4 h and thereafter daily for 7 days. The intraperitoneal median lethal dose was also calculated as the geometric mean of doses that caused 0 and 100% mortality respectively.

Sub-Acute Toxicity Study: Sub acute toxicity study was carried out in accordance to WHO (1992) and OECD 407(1995) guidelines. Twenty rats of either sex deprived of food for 24 h, were divided into four groups of five rats each. Group 1, which served as the control received normal saline, while rats in groups 2, 3 and 4 were given 250, 500 and 1000 mg extract/kg body weight respectively daily for 28-days. All the rats had free access to food and water throughout the duration of the experiment and were observed daily for general symptoms of toxicity and mortality.

Feed and water intake: The weight of feed and volume of water consumed by rats in each group were measured daily as the difference between the quantity of feed and water supplied and the amount remaining after 24 h respectively.

Body weight change: Rats in all the groups were weighed twice every week during the period of treatment and on the last day of study. Doses of the extract administered were adjusted accordingly.

Haematology and clinical chemistry: On the 29th day of the experiment, all the rats were sacrificed by cervical dislocation and blood samples were collected by cardiac puncture after opening up the rats surgically. One portion was collected into K^{+} EDTA bottles for estimation of Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), platelets, white blood cell count (WBC) and differentials, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), using an automated haematological machine(Cell-Dyn^{TM} Abbot, US). Another portion was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera were separated, stored at -4°C used for evaluation of biochemical parameters which include, alanine transaminase (ALT), aspartate transaminase (AST) levels and alkaline phosphatase (ALP) levels, total cholestrol ,total and conjugated bilirubin , serum urea nitrogen and creatinine using commercial kits obtained from Randox Laboratories, UK.

Relative organ weight ratio: Different organs namely the brain, heart, spleen, liver, lungs, kidneys, brain, intestine and testis or uterus were removed, weighed and observed macroscopically. The relative organ body weight ratio (ROW) of each rat was calculated as follows:

$$ROW = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day}}$$

Table 1. Effect of *C. febrifuga* on fluid intake (ml)/ 24 h.

Dose (mg/kg)	Week 1	Week 2	Week 3	Week 4
control	141.43 ± 5.2	119.29 ± 3.7	129.64 ± 66	121.00 ± 6.9
250	132.86 ± 5.7	125.71 ± 5.1	111.43 ± 2.8*	113.00 ± 3.4
500	117.21 ± 4.6*	105.00 ± 6.2*	97.14 ± 3.9*	93.33 ± 3.3*
1000	129.57 ± 9.6*	111.43 ± 3.6*	112.86 ± 5/2*	88.33 ± 6.3*

*significantly different from the control at $p < 0.05$, n = 5.

Table 2. Effect of *C. febrifuga* on feed consumption (g)/24 h.

Dose(mg/kg)	Week 1	Week 2	Week 3	Week 4
contr	75.21 ± 6.7	92.57 ± 10.7	121.96 ± 10.1	120.01 ± 4.8
250	72.74 ± 4.6	88.06 ± 5.4	116.26 ± 7.7	120.23 ± 1.7
500	61.23 ± 2.3*	82.91 ± 6.3*	99.43 ± 8.6*	83.86 ± 4.7*
1000	64.93 ± 1.3*	75.34 ± 5.8*	98.67 ± 6.5*	69.69 ± 5.7*

*significantly different from the control at $p < 0.05$, n = 5.

Histopathological studies: The organs removed from the rats were fixed in 10% formal saline for at least 48 h. They were then processed routinely, and the tissues were embedded in paraffin wax. Histological sections were cut at 5 - 6 µm and stained with routine haematoxylin and eosin (HE). These were then examined by a consultant histopathologist. The lesions observed were assessed for the following: Infiltration of lymphocyte into portal and central veins, congestion of the spleen, mucosal atrophy, the presence of inflammatory cells in the wall, oesinophils, lymphocytes and plasma cells., emphysematous changes in the lungs and decreased spermatogenesis These were graded according to mild (+), moderate (++) or severe (+++). Photomicrographs of representative lesions were taken at various magnifications.

Statistical analysis: All quantitative data were expressed as the mean ± standard error of mean (SEM). Statistical analysis was carried out using one way analysis of variance (ANOVA). Significant difference between means was assessed by student's t-test at 95% level of significance.

RESULTS

Acute toxicity studies

In the first phase of the oral acute toxicity study, the rat treated orally did not show any remarkable behavioural changes. In the second phase of the study, the extract produced paw licking, reduced activity, sedation, convulsion and death in groups given 4000 mg /kg body weight orally.

The oral LD_{50} value of the extract was calculated to be (2000 × 4000) mg/kg = 2828.43 mg extract/kg. However, in both phases of intraperitoneal route of administration, paw licking, reduced activity, sedation, convulsion and death were observed at 1000 mg/kg body and at 600mg extract/kg body weight in the second phase of the study within the first 4 h. The intraperitoneal LD_{50} value was calculated to be (370 × 600) mg/kg = 471.17 mg extract/kg.

Sub-Acute Toxicological Assessment

Clinical signs and mortality: All the rats used for the study appeared normal before, during and post treatment. Mortality was not recorded at all dose levels used for the study.

Water intake: Within each group, a decrease in water intake with time was recorded. A significant ($P < 0.05$) reduction in water intake was observed at 250 mg extract/kg body weight in the third week and throughout the 28-days study at 500 and 1000 mg extract/kg body weight (Table1).

Feed consumption: Feed consumption was significantly ($p < 0.05$) reduced in groups that received 500 and 1000 mg/kg of the extract throughout the duration of the study when compared to the control in the corresponding week (Table 2).

Body Weight: Significant ($P < 0.05$) decrease in weight was observed in the group treated with 500 mg extract/kg body weight in the 1st week and throughout the 4 weeks of study in the group treated with 1000 mg extract/kg body weight (Table 3).

Haematological indices: A significant ($p < 0.05$) increase in the platelet count which was not dose dependent was observed in all the extract treated groups. All other haematological parameters were not significantly different from the control (Table 4).

Renal indices: The decreases in the serum urea nitrogen were not significantly different from the control while significant ($P < 0.05$) and dose dependent decrease

Table 3. Effect of *C. febrifuga* on average body weight (g).

Dose (mg/kg)	Week 0	Week 1	Week 2	Week 3	Week 4
control	106.34 ± 9.2	112.43 ± 9.0	121.26 ± 9.3	132.60 ± 8.9	136.30 ± 9.6
250	113.5 ± 7.2	118.60 ± 8.0	126.19 ± 9.6	136.96 ± 10.5	146.30 ± 11.7
500	102.70 ± 5.0	107.10 ± 5.0*	118.34 ± 5.9	132.54 ± 6.4	131.26 ± 7.8
1000	100.98 ± 5.0*	102.81 ± 7.2*	96.74 ± 7.8*	119.04 ± 8.5*	116.59 ± 8.7*

*significantly different from the control at $p < 0.05$, n = 5.

Table 4. Effect of *C. febrifuga* on Haematological indices.

	Control	250 mg/Kg	500 mg/Kg	1000 mg/Kg
PCV (%)	42.5 ± 1.32	43.00 ± 0.75	37.71 ± 2.31	39.33 ± 2.93
HB (g/dL)	9.73 ± 0.55	11.49 ± 0.55	10.80 ± 0.45	9.79 ± 0.55
WBC(X 10^9/L)	7.90 ± 1.47	4.67 ± 0.58	3.54 ± 0.79	5.1 ± 1.51
MCHC (g/dL)	24.13 ± 1.92	26.57 ± 1.27	29.86 ± 2.30	25.43 ± 1.02
PLT (X10^9/L)	198.25 ± 28.21	307.57 ± 29.10*	215.14 ± 36.45*	259.57 ± 40.74*
NEUT (%)	39.5 ± 3.17	37.00 ± 5.06	37.29 ± 3.60	36.71 ± 4.11
LMPH (%)	59.0 ± 3.25	62.43 ± 5.24	61.86 ± 3.51	61.43 ± 4.14
EOS (%)	1.13 ± 0.40	0.14 ± 0.13	0.71 ± 0.39	0.85 ± 0.38
MONO (%)	0.38 ± 0.26	0.57 ± 0.40	0.14 ± 0.13	1.00 ± 0.46

*significantly different from the control at $p < 0.05$, n = 5.

Table 5. Effects of the methanolic extract of *C. febrifuga* on renal indices.

	Electrolytes	
	Serum urea nitrogen (mmol/l)	Creatinine (mmol/l)
Control	4.47 ± 0.20	72.48 ± 2.23
250	4.19 ± 0.22	70.95 ± 3.04
500	3.98 ± 0.08	66.63 ± 3.09*
1000	4.39 ± 0.27	62.10 ± 3.45*

*significantly different from the control at $p < 0.05$, n = 5.

in serum Creatinine level was obtained (Table 5).

Hepatic indices: There was Significant ($P < 0.05$) decease in the levels of AST and ALT at the dose of 1000 mg extract/kg. Significant ($P < 0.05$) decrease in direct bilirubin level was observed at 500 mg extract/kg, while Alkaline phosphatase, total bilirubin, albumin and cholesterol levels were not significantly different from the control at all dose levels used for the study (Table 6).

Relative organ weights: The relative organ weights were not significantly different from the control except the significant increase observed in the heart in the 250 mg extract/kg group (Table 7).

Histopathology: Splenic congestion was seen at all dose levels used in the study. Lymphocytic infiltration of the portal and central vein of the liver was noted at 500 and 1000 mg extract/kg body weight. At 500 and 1000 mg extract/kg, vascular congestion and focal granulomata were noted in the lungs and with emphysematous changes at 1000 mg extract/kg. The kidneys showed focal glomerular atrophy and the testes showed decreased spermatogenesis at 1000 mg extract/kg (Table 8).

DISCUSSION

The need to evaluate the toxicity profile of *Crossopteryx febrifuga* stem bark extract was prompted by its widespread use in the management of various disorders in Northern Nigeria. The observed behavioural signs of sedation, convulsion and mortality in the rats that received 4000 mg extract /kg suggests the involvement of central nervous system in its toxic effect. The solvent system used for the extraction which is capable of extracting both polar and non-polar constituents, the extracted bioactive hydrophilic components capable of penetrating the blood brain barrier may have been responsible for the observed central nervous mediated toxic effects. The oral and intraperitoneal median lethal doses of 2828.43 and 471.17 mg/kg body weight respectively obtained indicates that the extract is slightly and moderately toxic by these routes (Cobett et al. 1984). These values are 28 and 5 times greater than the minimum effective dose of 100 mg/kg in rats respectively reported by Salawu et al. (2008). Earlier reports have shown that if the median lethal dose of a test substance

Table 6. Effects of the methanolic extract of *C. febrifuga* on Liver function indices.

Drug/dose (mg/kg)	Alkaline (U/L) phosphate	AST (U/L)	ALT (m/L)	Total biluribin (mmol/L)	Direct biluribin (mmol/L)	Albumin (g/L)	Cholesterol (mmol/L)
Control	806.75 ± 67.43	126.61± 4.63	57.62 ± 4.72	4.39 ± 0.25	1.43 ± 0.36	26.86 ± 1.86	1.89 ± 0.10
250	906.14 ± 138.40	120.94 ± 5.93	44.18 ± 2.82*	4.92 ± 0.51	1.45 ± 0.26	33.21 ± 2.95	2.80 ± 0.28
500	768.00 ± 76.65	135.52 ± 8.20	51.72 ± 3.39	3.82 ± 0.47	0.93 ± 0.29	32.89 ± 1.58	1.95 ± 0.11
1000	759.71 ± 67.34	111.66 ± 3.76*	46.82 ± 1.82*	5.13 ± 0.44	1.53 ± 0.59	30.0 ± 1.86	1.93 ± 0.19

*significantly different from the control at $p < 0.05$, n = 5.
ALT: Alanine-aminotransaminase., AST: Aspartate-aminotransaminase., ALP: Alkaline phosphatase.

Table 7. Effect of *C. febrifuga* on relative organ weights (g).

	Control	250 mg extract/kg	500 mg extract/kg	1000 mg extract/kg
HEART ($X10^{-3}$)	0.41 ± 0.02	0.64 ± 0.04	0.51 ± 0.10	0.43 ± 0.02
LIVER($X10^{-3}$)	3.77 ± 0.02	3.78 ± 0.03	3.53 ± 0.04	3.93 ± 0.04
TESTES($X10^{-3}$)	3.18 ± 0.04	3.53 ± 0.02	2.96 ± 0.04	2.60 ± 0.09
UTERUS($X10^{-3}$)	0.48 ± 0.01	0.62 ± 0.05	0.56 ± 0.01	0.39 ± 0.02
SPLEEN($X10^{-3}$)	0.51 ± 0.03	0.68 ± 0.04	0.54 ± 0.12	0.59 ± 0.08
LUNGS($X10^{-3}$)	0.83 ± 0.04	0.91 ± 0.08	0.73 ± 0.12	0.84 ± 0.05
KIDNEYS($X10^{-3}$)	0.76 ± 0.06	0.92 ± 0.06	0.70 ± 0.09	0.71 ± 0.08
BRAIN($X10^{-3}$)	0.92 ± 0.05	1.14 ± 0.08	1.19 ± 0.12	1.13 ± 0.12

*significantly different from the control at $p < 0.05$, n = 5.

Table 8. Effects of the methanolic extract of *C. febrifuga* on selected tissues.

Tissue	Control	250 mg/Kg	500 mg/Kg	1000 mg/Kg
Liver	Unremarkable	Unremarkable	Lymphocyte at portal and central veins	Lymphocyte at portal and central veins
Spleen	Unremarkable	Congestion	Congestion	Congestion
Intestine	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Kidney	Unremarkable	Unremarkable	Unremarkable	Focal glomerular atrophy
Brain	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Uterus/Testes	Unremarkable	Unremarkable	Unremarkable	Decreased spermatogenesis
Lung	Unremarkable	Unremarkable	Unremarkable	Expanded alveola space, Vascular congestion, Focal granuloma, and emphysematous changes
Heart	Unremarkable	Unremarkable	Unremarkable	Unremarkable

is three times more than the minimum effective dose, the substance is considered a good candidate for further studies.The extract is therefore safe for oral use and this could explain the safe use of the plant by the local people in traditional management of various ailments in northern part of Nigeria. A 28-days oral toxicity study was carried out in rats to determine the potential of *C. febrifuga* stem bark extract to produce toxicity in man. Dose levels of 250, 500 and 1000 mg extract/kg body weight were selected for the study. These doses were intended to support human oral intake of approximately 3000 mg extract per person per day which is equivalent to 42.87 mg extract/kg/day assuming a 70 kg human. The highest dose given to rats in this study (1000 mg extract/kg/day) would be equivalent to approximately 70 g/day in a 70 kg person (Tan et al., 2007). Any treatment related disorder observed at this dose level may show direct possible toxic effect of the extract in man when an overdose is taken. The body weight changes serve as a sensitive indication of the general health status of animal. The observed decline in food consumption and water intake at a dose of 1000 mg extract/kg may have contributed to the observed reduction in body weight. The crude extract may have also been metabolised to a more toxic end product that interferes with gastric function and decrease food conversion efficiency (Chokshi, 2007). Earlier reports have shown that if the median lethal dose of a test substance Increase in platelet count observed in the study is usually associated with post inflammatory changes, accompanying organ damage or anaemia. This

observation is supported by the marked histopathological changes observed in the lungs, liver, spleen and kidney at 1000 mg extract/kg. The slight decrease in serum urea nitrogen and dose dependent significant decrease in creatinine levels may have contributed to the loss in weight observed in the rats due to muscle wastage as decrease in serum creatinine level usually precedes renal impairment and muscle wasting. Renal function may have to be monitored on long-term administration of the extract. This is supported by histopathological lesions of focal glomerular atrophy observed at 1000 mg extract/kg body weight.Histological examination is the golden standard for evaluating treatment related pathological changes in tissues and organs (OECD, 1995). We, hereby propose that the extract did cause treatment related disorder in kidney structure.

Daily administration of the extract at different dose levels to rats resulted in decrease in serum level of liver function markers such as Aspartate amino transferase (AST), alanine amino transferase (ALT), direct bilirubin while other parameters such as alkaline phosphatase, total bilirubin, albumin and cholesterol levels were not different from the controls. Inflammatory changes seen histologically (characterised by infiltration of lymphocyte at portal and central veins of the liver) at 500 and 1000 mg extract/kg body weight respectively showed that the extract exerted deleterious effect on the liver. The liver is capable of regenerating damaged tissue hence liver function may not be impaired early following an insult from a toxicant. The observed lesions in the lungs and spleen of rats given 500mg and 1000 mg extract/kg daily for 28 days showed that the extract exerted multiple organ toxicities at these doses; it should therefore be used with caution in patients with known history of liver, lungs and spleen related disorders. Though there was no significant change in relative organ weight ratio between group given 1000 mg extract/kg and the control, presence of reduced spermatogenesis in the testes indicates that the extract exerted significant toxic effects on the physiological function of the male reproductive system. It is suggested that the reproductive toxicological studies of the extract should be carried out. These results suggest that oral application of *C. febrifugal* may not produce severe toxic effects at doses lower than 500mg extract/kg body weight. Given the wide ethno-medical applications of *Crossopteryx febrifuga,* the present toxicity results constitute safety information that can be used in obtaining regulatory approval for its commercialisation.

CONFLICT OF INTEREST STATEMENT

The authors do not have conflict of interest over this research work.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical support of the entire staff of the Animal Facility centre of the Department of Pharmacology and Toxicology and the management of National Institute of Pharmaceutical Research and Development (NIPRD), Idu, Abuja, Nigeria for providing enabling environment for this research.

REFERENCE

Bridson D, Verdcourt B (1988). Rubiaceae: in: flora of tropical east africa, aa balkema, rotterdam p. 2.

Chokshi D (2007). Sub-chronic oral toxicity of a standardized white kidney bean (*Phaseolus vulgaris)* extract in rats. Food and Chemical Toxicol. 45: 32-40.

Cobett JR, Wight K, Baille AC (1984). The Biochemical mode of action of Pesticides. 2nd Ed. Academic Press, London and New York.

Dalziel JM (1957). The useful plants of West Tropical Africa. 2nd edition. Crown agents, London p. 396

Elufioye TO*,* Agbedahunsi JM (2004). Antimalarial activities of Tithonia diversifolia (Asteraceae) and Crossopteryx febrifuga (Rubiaceae) on mice *in vivo*. J. ethnopharmacol. 93: 2-32-3, 167-171.

Gariboldi P, Verotta l, Abetta B (1990). Saponins from Crossopteryx febrifuga. Phytochem. 29(8): 2629-2635.

Hostettmann K, Marston A, Ndjoko K, Wolfender J (2000). The potential of African plants as a source of drugs, Current Organic Chemistry 4: 973-1010.

Norman NO, Frederick FM, Ark M (2005). Toxicity of pesticides. http://edis.ifas.ufl.edu/pdffiles/pi/pi00800.pdf.

OECD (1995). Repeated Dose 28- day Oral Toxicity Study in Rodents. OECD guideline for testing of chemicals 407: 1-8.

Salawu OA, Chindo BA, Tijani AY, Adzu B (2008). Analgesic,anti-inflammatory,anti-pyretic and antiplasmodial effects of the methanolic extract of *Crossopteryx febrifuga*. J. Med. Plant Res. 2(8): 213-218.

Tan VP, Boda M, Enow-Orock GE, Francois-Xanvier BP (2007). Acute and sub-acute toxicity profile of the aqueous stem bark extract of *Enantia chlorantha* Oliver (Annonaceae) in laboratory animals. Pharmacol. 1: 304-313.

Tomas-barberan FA, Hostettmann F (1988). A cytotoxic triterpenoid and flavonoids from crossopteryx febrifuga. Plant Med. 54(3): 266-267.

Tona L, Kambu K, Ngimbi N, Mesia K, Penge O, Lusakibanza M, Cimanga K, De BT, Apers S, Totte J, Pieters L, Vlietinck AJ (2000). Antiamoebic and spasmolytic activity of extract of some antidiarrhoeal traditional preparations used in Kinshasa Congo. Phytomed. 7: 31-38.

WHO (1992). Research guidelines for evaluating the safety and efficacy of herbal medicine. WHO regional office for western pacific Manila, Philippine p. 38.

Yusuf AB, Iliyasu B, Abubakar A, Onyekwelu EL, Bot DY (2004). Preliminary Evaluation for Anti-trypanosomial Activity of Aqueous Stem Bark Extract of *C. febrifuga* in *T. congolensis* infection in Rats. Presented at the 31st West African Society of Pharmacology Conference held in Kano.