SUBCHRONIC TOXICITY STUDIES OF THE ETHANOLIC ROOT EXTRACT OF CROTON ZAMBESICUS

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ABSTRACT

Subchronic toxicity study of the crude root extract of *Croton zambesicus* (27-81mg/kg), which is used traditionally as malarial remedy, was carried out in rodents to evaluate the safety profile. Effect of the extract on body weights, haematological indices as well as liver and kidney functions and histology of various organs were investigated. Subchronic treatment of rats for 21 days caused comparable increase in body weights of rats in extract treated and control groups. The extract caused a dose-dependent increases in RBC, PCV, Hb, WBC, bleeding time and clotting time. The increases were only significant ($P<0.05$) at the highest dose of the extract (81mg/kg) for RBC and WBC when compared to control. There was no significant ($P>0.05$) differences in the means of other haematological parameters in the extract treated groups compared to control. The extract caused significant ($P<0.05$-0.01) increases in the level of serum total protein, ALT, ALP, total bilirubin and total cholesterol. The was no significant ($P>0.05$) changes in the levels of albumin and AST. The extract did not produce any significant ($P>0.05$) changes in the mean concentrations of urea, creatinine, Na+, K+, and Cl- ions of rats in the extract treated groups compared to that of control. Histopathologic analysis of the vital organs revealed no significant lesions in the brain, liver, kidney, heart, spleen, ovary, and testis. The results suggest the extract to be safe when taken orally though with an insignificant effect on the liver.

Keywords: *Croton zambesicus*, subchronic, toxicology, haematology, liver, kidney.

INTRODUCTION

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Burch) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo – Congolese species widely spread in tropical Africa. Ethnobotanically, the leaf decoction is used in Benin as anti hypertensive and anti- microbial (urinary infections) (Adjanohoun *et al,* 1989) and in parts of Nigeria as antidiabetic and malarial remedy (Okokon *et al*., 2005a, 2006). The roots are used as antimalarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria (Okokon and Nwafor, 2009a). The root is also used in Sudan for menstrual pain (El-Hamidi, 1970) and as aperients (Ngadjui *et al*., 1999). Boyom *et al*. (2002) studied the composition of essential oils from the leaves, stem and roots of *Croton zambesicus* and found the three types of oils to be similar in composition, with those from the leaves and stem rich in monoterpenes, while that of the root bark contains sesquiterpenes. The root and stem bark oils were found to be rich in oxygen-containing compounds, with spathulenol and linalool as major components. Okokon and Nwafor (2009a) reported that the root extract whose LD50 is 273.86 mg/kg contains alkaloids, saponins, terpenes, tannins, phlobatannins, anthraquinones and cardiac glycosides, while flavonoids were reported to be absent. Block *et al. (*2002) isolated entrachyloban-3β-ol, an ent-trachylobane diterpene from dichloromethane extract of the leaves and reported that the diterpene has a cytotoxic activity on HeLa cells. Also two new trachylobane – and one isopimarane type diterpenoids; ent-18-hydroxy-trachyloban-3-one, ent-trachyloban-3- one, isopimara-7,15-dien-3β-ol, together with transphytol, β-sitosterol, α-amyrin and stigmaterol have been isolated from the leaves (Block *et al*., 2004). Crotonadiol, a labdane diterpenoid, clerodane, crotocorylifuran and two trachylobanes; 7β-acetoxytrachyloban – 18 – oic acid, trachyloban - 7β, 18 – diol, lupeol, β-sitosterol and its 3-β-glucopyranosyl derivative were isolated from the stem bark (Ngadjui *et al*, 1999). Ngadjui *et al*., (2002) further isolated three clerodane diterpenoids, crotozambefurans A, B and C from the stem bark. Studies have reported on the antimicrobial properties of the leaf and stem (Abo *et al*, 1999). The ethanolic leaf extract has been reported to possess antiplasmodial (Okokon *et al*., 2005a), antidiabetic (Okokon *et al*., 2006), anti-inflammatory, analgesic and antipyretic activities (Okokon *et al.,* 2005b), while the root extract has been reported to possess antimalarial (Okokon and Nwafor, 2009a) and anticonvulsant and antiulcer activities (Okokon and Nwafor, 2009b).

Information on biological activity of the root are scarce. We therefore investigated the antiiflammatory, analgesic and antipyretic activities of the root extract of the plant to ascertain the folkloric claim of its medicinal properties in

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the treatment of malarial symptoms such as fever and pains.

MATERIALS AND METHODS

Plant materials

The plant part (roots) was identified by Dr. Margaret Bassey, Taxonomist, Department of Botany, University of Uyo, Uyo. The roots were collected from compounds in Uyo metropolis, Akwa Ibom State of Nigeria and were authenticated. A voucher specimen (DPNM. 31c) of the plant was deposited in the herbarium of Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria

Table 1: Effect of subchronic administration of *C. Zambesicus* root extract on body weights of rats

Treatment R&G /Extract	Dose (mg/kg)	Initial body weight (Kg)	Final body weight (Kg)	Weight gain (Kg)
Control	0.2ml	180.2 ± 3.78	196.0 ± 2.82	14.83 ± 2.69
C. zambesicus	27	220.0 ± 4.56	238.6 ± 5.82	18.66 ± 2.18
	54	245.0 ± 2.87	269.0 ± 4.52	24.0 ± 2.63
	81	287.5 ± 8.80	302.0 ± 9.19	12.83 ± 3.38

Data are expressed as mean ± SEM. Not significant when compared to control P>0.05 .n = 6.

Table 2: Effect of ethanolic root extract of *Croton zambesicus* on haematological indices of rats after subchronic administration

Parameters treatment dose (cm/kg)	RBC (x 10^{12}/l)	PCV (%)	Hb (g/dl)	WBC (x 10^9/l)	Neut. (%)	Lymph. (%)	Eosin. (%)	Platelets (X 10^3/mm^3)	Bleeding Time (S)	Clotting Time (S)
Control	3.82 ± 0.32	34.66 ± 1.7	11.55 ± 0.41	4.50 ± 0.63	29.50 ± 4.26	69.16 ± 4.23	2.00 ± 0.70	200.37 ± 11.10	2.20 ± 0.40	2.81 ± 0.37
C. zambesicus 27	4.08 ± 0.34	33.4 ± 2.4	11.79 ± 0.64	6.94 ± 1.07	41.40 ± 6.11	54.40 ± 6.35	4.20 ± 1.46	199.32 ± 11.65	2.24 ± 0.78	2.32 ± 0.66
54	4.13 ± 0.26	35.16 ± 1.07	11.82 ± 0.80	8.96 ± 0.84	38.16 ± 5.36	68.50 ± 5.78	3.66 ± 1.49	190.41 ± 12.03	2.50 ± 0.47	2.37 ± 0.52
81	4.88 ± 0.2[a]	36.25 ± 1.01	12.19 ± 0.35	9.80 ± 1.74[a]	33.20 ± 4.03	66.20 ± 3.86	0.60 ± 0.80	183.71 ± 10.71	2.54 ± 0.12	2.46 ± 0.89

Data are represented as mean ± SEM. n = 6 . Significant at [a]P < 0.05 when compared to control.

Table 3: Effect of ethanolic root extract of *Croton zambesicus* on some kidney function parameters of rat (subchronic study)

Extract	Dose (mg/kg)	Urea (mmol/l)	Creatinine (mmol/l)	Na+ (mmol/l)	K+ (mmol/l)	CL- (mmol/l)
Control (dist water)	-	3.18 ± 0.09	41.83 ± 2.91	128.6 ± 4.59	5.20 ± 0.49	49.16 ± 1.85
	27	3.38 ± 0.33	41.83 ± 2.91	133.0 ± 7.37	5.20 ± 0.28	47.83 ± 4.07
C. zambesicus	54	3.26 ± 0.26	41.16 ± 0.79	133.3 ± 4.62	5.25 ± 0.47	49.00 ± 5.45
root extract	81	3.10 ± 0.27	41.00 ± 0.79	132.4 ± 4.73	5.24 ± 3.02	48.33 ± 2.68

Not significant when compared with control P>0.05. Data are expressed as mean ± SEM. n = 6.

Table 4: Effect of ethanolic root extract of *croton zambesicus* on some liver function parameters of rats (subchronic study)

Treatment/ extract	mg/kg	Total Protein (g/l)	Albumin (g/l)	AST (mmol/l)	ALT (mmol/l)	ALP (mmol/l)	Total Bilirubin (μmol/l)	Conjugated Bilirubin (μmol/l)	Total cholesterol (mmol/l)
Control(dist.water)	-	50.0 ± 1.02	38.8 ± 0.43	13.6 ± 7.05	10.6 ± 1.37	27.80 ± 0.65	3.16 ± 0.31	2.56 ± 0.17	2.56 ± 0.17
	27	60.80 ± 3.14[a]	39.20 ± 0.52	13.2 ± 4.51	10.6 ± 1.21	28.8 ± 0.59[a]	5.00 ± 0.42[a]	2.74 ± 0.17	2.82 ± 0.12[a]
C. zambesicus	54	64.16 ± 1.07b	39.16 ± 0.39	13.0 ± 1.20	15.38 ± 0.75	27.33 ± 0.55	5.23 ± 0.27[a]	3.28 ± 0.27[b]	2.98 ± 0.07[b]
root extract	81	60.60 ± 1.73a	38.60 ± 0.35	13.60 ± 1.00	12.6 ± 1.25[a]	27.60 ± 0.67	4.88 ± 0.26 [a]	3.18 ± 0.27 [b]	3.00 ± 0.15[b]

Data are expressed as mean ± SEM. Significant at [a]P < 0.05, [b]P < 0.01 when compared to control n = 6.

Extraction

The roots were shade dried for 2 weeks. The dried roots were further chopped into small pieces and reduced to powder. The powdered root was macerated in 97% ethanol for 72 hours to give the crude ethanolic extract. The liquid filtrates were concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator. The yield of the extract was calculated. The dry extract was stored in a refrigerator at 4°C until use for the proposed experiment.

Animals

The animals (mice and rats) both male and female that were used for these experiments were obtained from University of Uyo animal house. The animals were housed in standard cages and were maintained on a standard pelleted Feed (Guinea Feed) and water *ad libitum*. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo.

Subchronic toxicity studies of the extract

Animal treatment

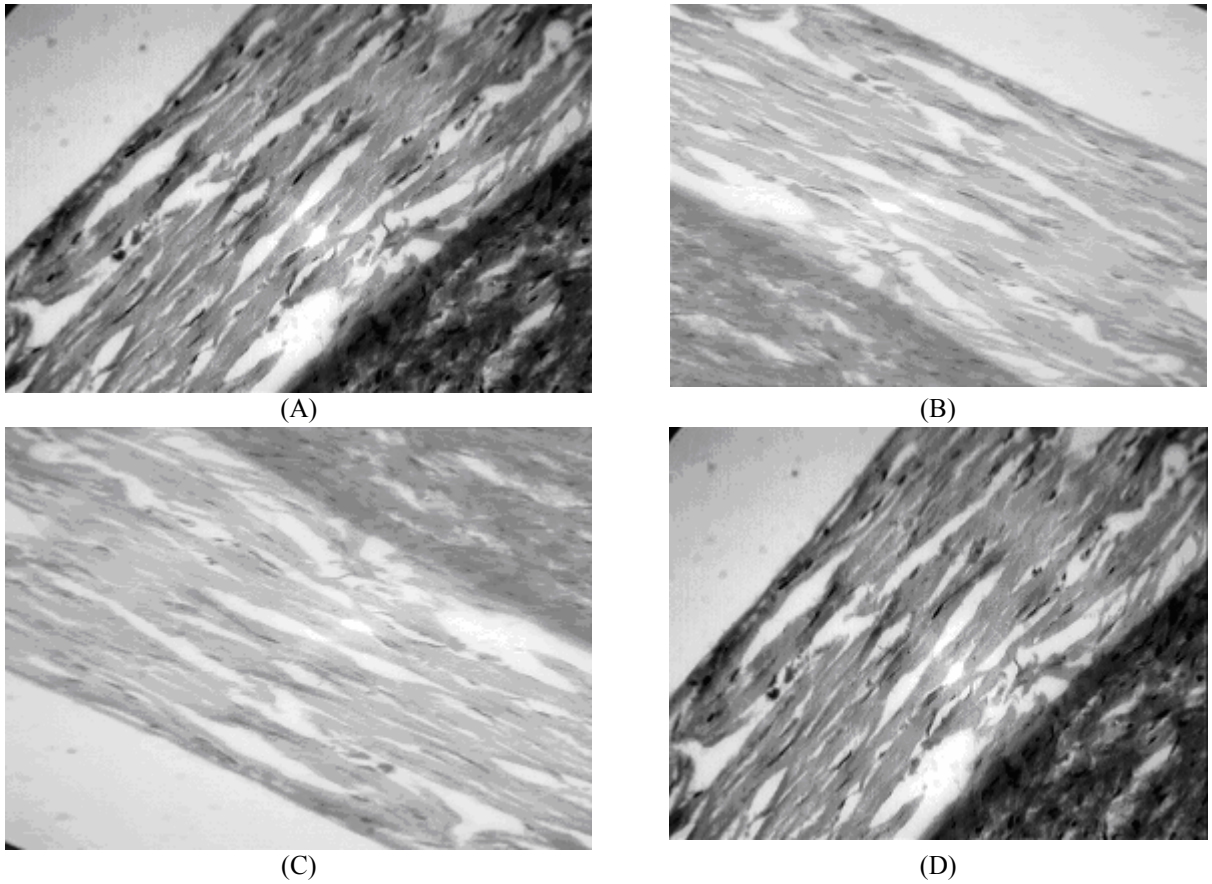
A total of 24 rats of both sexes were weighed and divided into four groups of 6 animals each and treated as follows: Groups A, B and C were administered orally with 27, 54 and 81 mg/kg of *C. zambesicus* root extract respectively in divided doses for 21 days. Group D was administered with distilled water 0.2 ml/kg for the same period of time .At the end of the treatment period, the animals were weighed again and sacrificed under light chloroform vapour. Blood were collected by cardiac puncture and used immediately for haematological testing. Serum was separated from the remaining blood and stored at -20^{o}C until used for biochemical determinations.

Evaluation of effect on haematological parameters

The determination of various blood parameters such as RBC counts, Hb, PCV, WBC counts (total and differential), platelets counts, bleeding and clotting time were done according to standard methods (Baker *et al*., 1985).

Determination of the effect of extract on Biochemical parameters of rats

The various serum samples collected after subchronic treatment of the animals were analysed according to standard methods for effect of the extract on various biochemical parameters of rats such as Total protein,



(A) (B)

(C) (D)

Plate 1: Normal morphology of histological section of rat heart (A) control (B) low dose, 27 mg/kg (C) median dose, 54 mg/kg (D) high dose, 81mg/kg. X 400

albumin, aspartate aminotransferases (AST), alanine aminotransferase (ALT), alkaline phosphotase (ALP), total bilirubin, conjugated bilirubin, total cholesterol, urea, creatinine as well as some ions like sodium, potassium and chloride. This analysis were done at Department of Chemical Pathology,University of Uyo Teaching Hospital, (UUTH), Uyo using various diagnostic kits like Randox Laboratory kits, Dialab diagnostic kits, HUMAN diagnostic kits and TECO analytical kits.

Determination of the effect of extract on Histology of some organs of rats

A total of 24 adult rats (male and female) were divided into four groups of six animals each and treated as follows; Group 1 served as control and group 2-4 received 27-81 mg/kg of *Croton zambesicus* root extract for 21 days in divided doses. At the end of the treatment period, the rats were sacrificed under light chloroform vapour. Various organs such as brain, heart, liver, spleen, kidney, ovary and testis of the animals were surgically removed and fixed in 10% formaldehyde. The organs were processed, sectioned and stained with Heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Uyo Teaching Hospital, Uyo.

Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students' t-test and ANOVA (One- or Two-way) followed by a post test (Tukey-kramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance i.e. $P \leq 0.01$and 0.05.

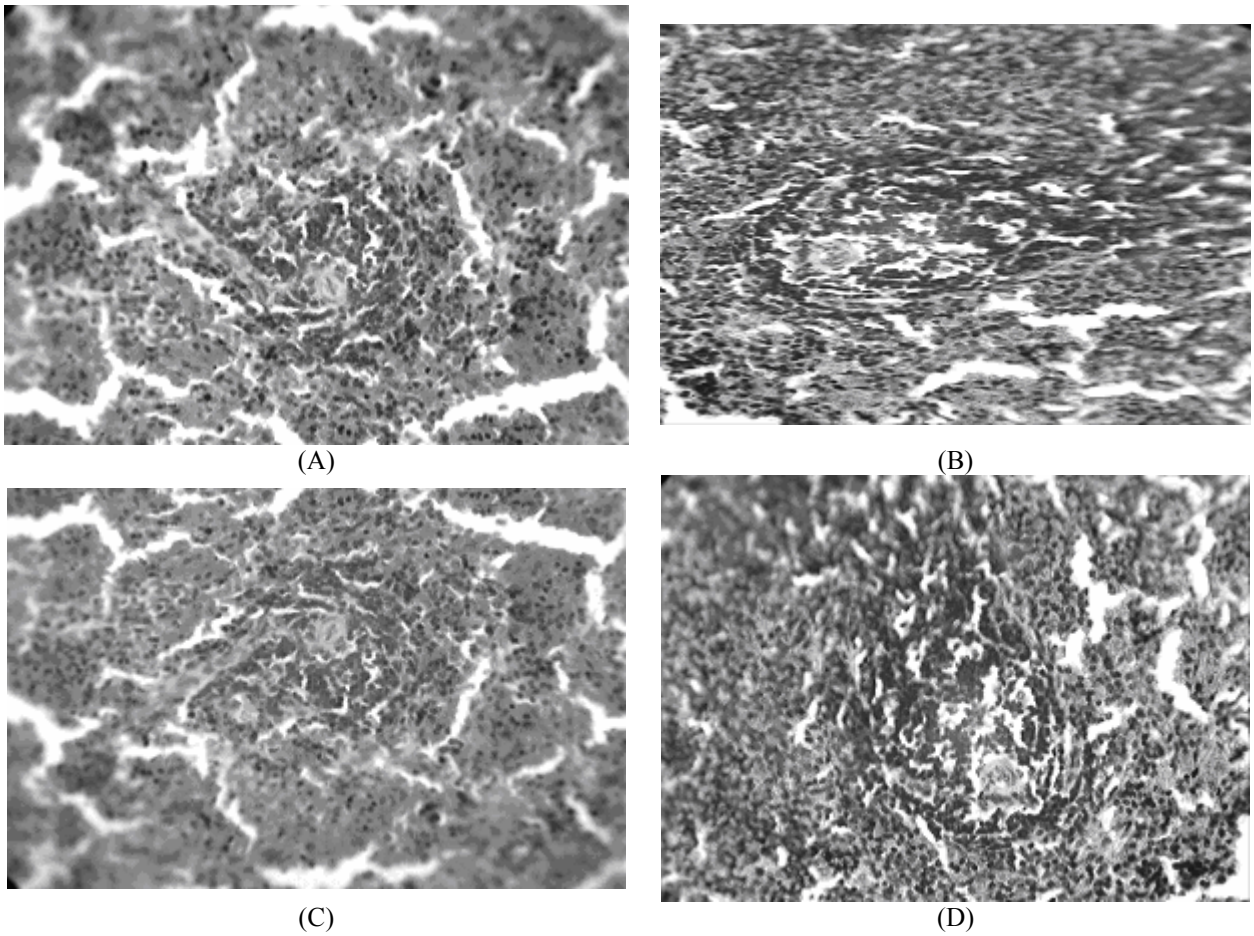
RESULTS

Body weight

The effect of the extract on body weight of rats treated subchronically with root extract of *C. zambesicus* is shown in table 1. There was a comparable increase in body weight of the extract treated groups and that of control with the group administered with the median dose of the extract having the highest weight gain.

Effect on biochemical parameters of rats

Table 1 shows the effect of subchronic administration of



(A) (B) (C) (D)

Plate 2: Normal morphology of histological section of rat spleen (A) control (B) low dose, 27 mg/kg (C) median dose, 54 mg/kg (D) high dose, 81mg/kg. X 400

the ethanolic root extract of *C. zambesicus* on biochemical parameters of rats.

Total protein and albumin

Subchronic administration of the root extract of *C. zambesicus* (27, 54 and 81 mg/kg) caused a significant (P<0.05- 0.001) increase in the level of serum total protein levels of rats when compared to control untreated rats. The increase which was non dose dependent was highest with the median dose (54mg/kg) of the extract. However, there was no significant difference (P>0.05) between the mean levels of albumin of rats treated with the extract and that of control (table 1).

Effect on AST, ALT and ALP

Subchronic administration of ethanolic extract of *C. zambesicus* did not exert any significant (P>0.05) effect on the level of AST of the extract treated rats. There was no statistical significant (P>0.05) difference between the means of treated groups and that of the control (table 1).

There was a significant (P<0.01-0.001) increase in the level of ALT of rats administered with ethanolic root extract of *C. zambesicus* for 21 days compared to control. The increase which was non dose dependent was highest in the group administered with the median dose of the extract (54mg/kg). The extract administered caused significant (P<0.05) increase in the level of ALP of rats treated with the lowest dose (27mg/kg) of the extract. Higher doses of the extract did not produce any statistical difference in means of the treated groups and that of control.

Total and conjugated bilirubin

Administration of ethanolic extract of *C. zambesicus* for 21 days to rats caused sinificant(P<0.05-0.001) increase in the level of total and conjugated bilirubin when compared tto control.The increase which was non dose dependent was highest with the median dose (54mg/kg) of the extract.

Total cholesterol

There was a progressive increase in the level of the total cholesterol of the extract treated rats. The increase was dose dependent and significant (P<0.05-0.001) when compared to control.

Effect on haematological parameters

The results of the effect of subchronic administration of ethanolic root extract of *C. zambesicus* on haematological

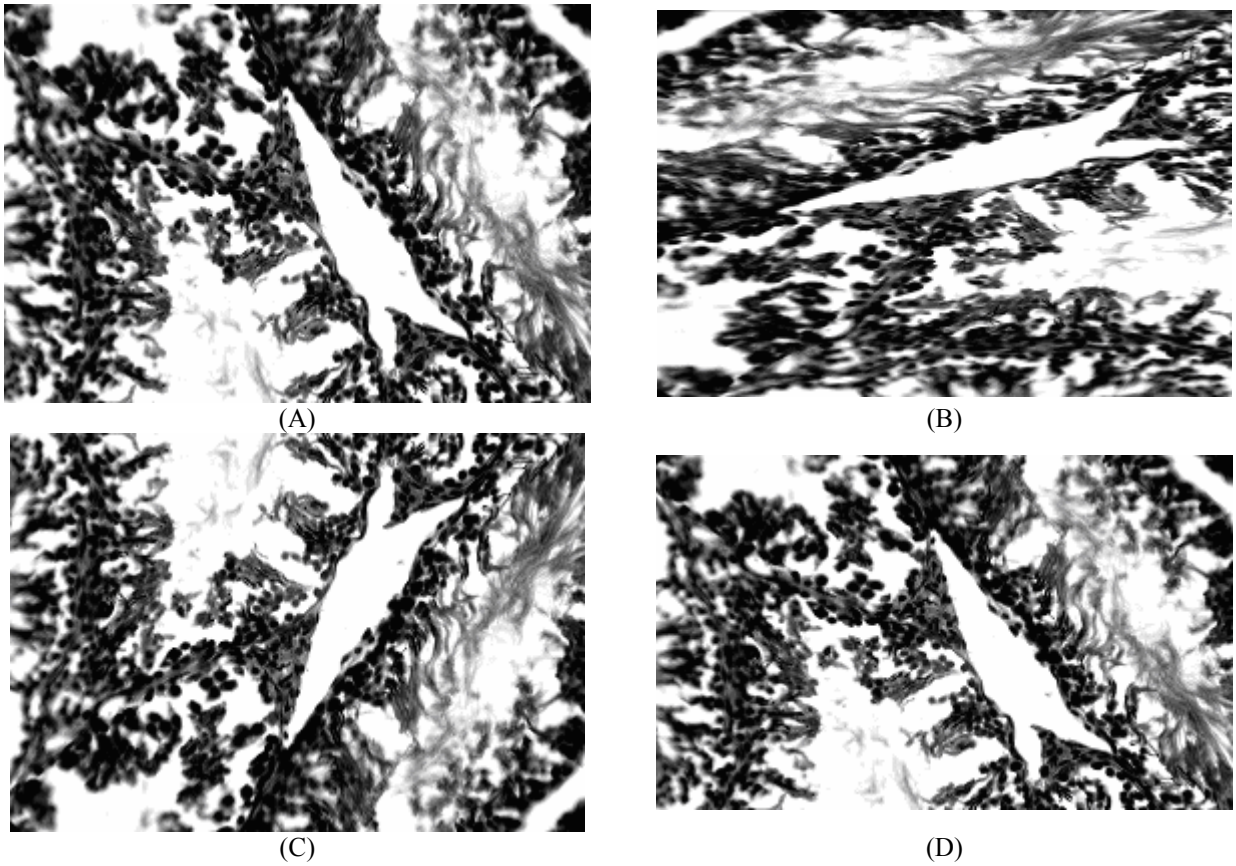


Plate 3: Normal morphology of histological section of rat testis (A) control (B) low dose,27 mg/kg (C) median dose, 54 mg/kg (D) high dose, 81mg/kg. X 400

parameters rats is shown in table 2. The extract caused a progressive increase in the red blood cell count (RBC), Packed cell volume (PCV) percentage, Haemoglobin (Hb) concentration,White blood cell (WBC) counts, bleeding time and clotting time. However, the dose- dependent increases were only significant ($P<0.005$) at highest dose of the extract (81mg/kg) in RBC, PCV and WBC. There was no statistical difference in the mean percentages of neutrophils, lymphocytes and eosinophils of the extract treated groups and that of the control.

Effect on kidney function test

Subchronic administration of the root extract of *C. zambesicus* (27-81mg/kg) to rats for 21 days did not produce any significant ($P>0.05$) difference between the mean serum concentrations of urea, creatinine, Na^+, K^+ and Cl^- ions of the extract treated groups and that of control.

Effect on histology of organs

Table 4 shows the effect of subchronic administration of ethanolic root extract of *C. zambesicus* to rats for 21 days on histology of some organs. The extract did not produce any deffect in the histology of the brain, liver, kidney, heart, spleen, ovary and testis. The morphology of these organs were normal as that of the control (Plates 1-7).

DISCUSSION

The body weights of the animals were found to be affected by extract treatment as they gained considearble weight just as the control group. This indicate that the extract does not interfres with growth processes and may have promoted growth by stimulating the synthesis of body proteins. The extract was observed to caused significant increases at the highest dose (81mg/kg) in RBC, PCV and WBC. This indicates that the extract stimulates erythropoiesis and leucocytosis. This could be attributed to alkaloids present in the extract. Alkaloids have been implicated to cause similar effect by inhibiting phosphodiesterase leading to the accumulation of cAMP which in turn stimulates protein synthesis (Eteng *et al*., 2003). Administration of the extract did not affect platelets count , bleeding and clotting time reflecting zero toxicity on the blood mechanism.

Significant levels of serum total protein and cholesterol were observed in the study. Serum protein and cholesterol are largely regulated via synthesis in the liver and reflect synthetic ability of the liver (Kouitchev *et al.,* 2007). An increase in serum concentration of total protein and cholesterol resulting from treatment with the extract may suggest stimulation of their synthesis or inference in

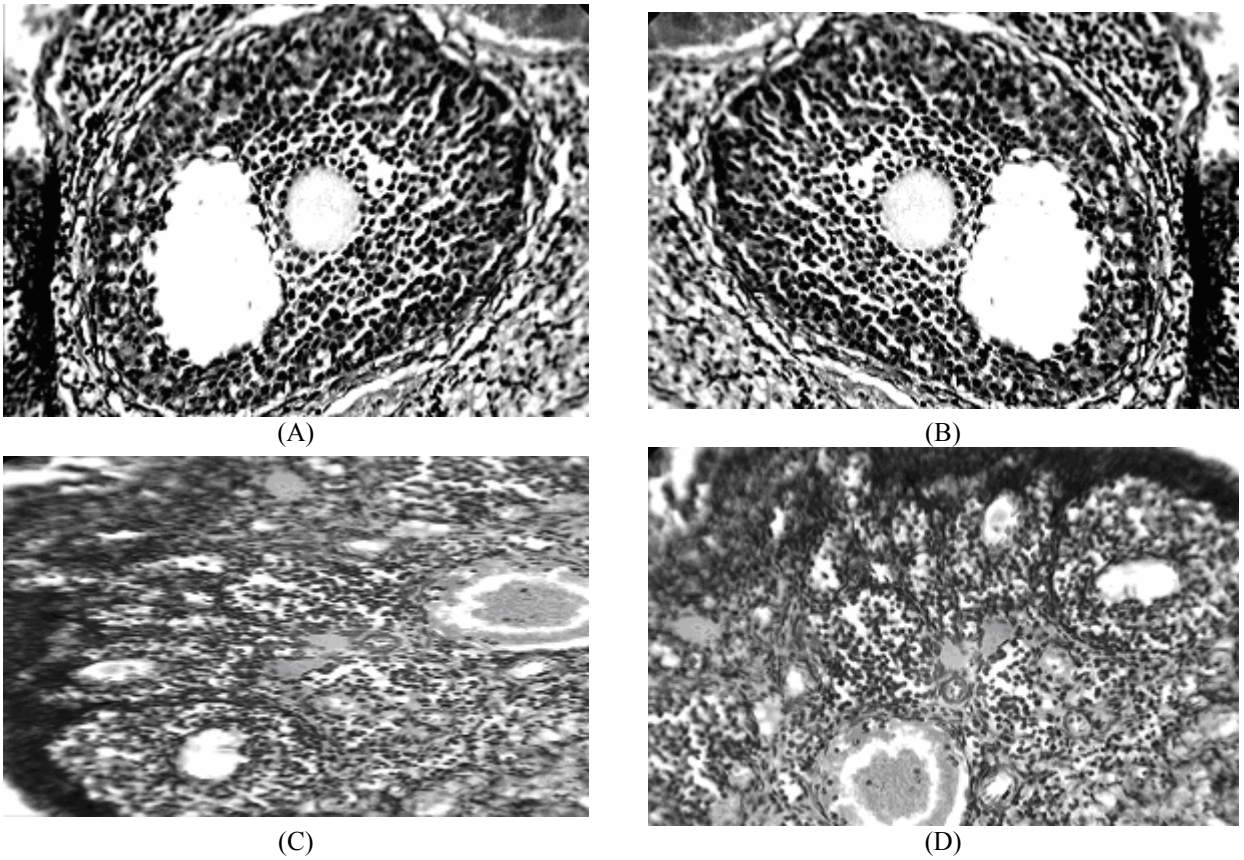
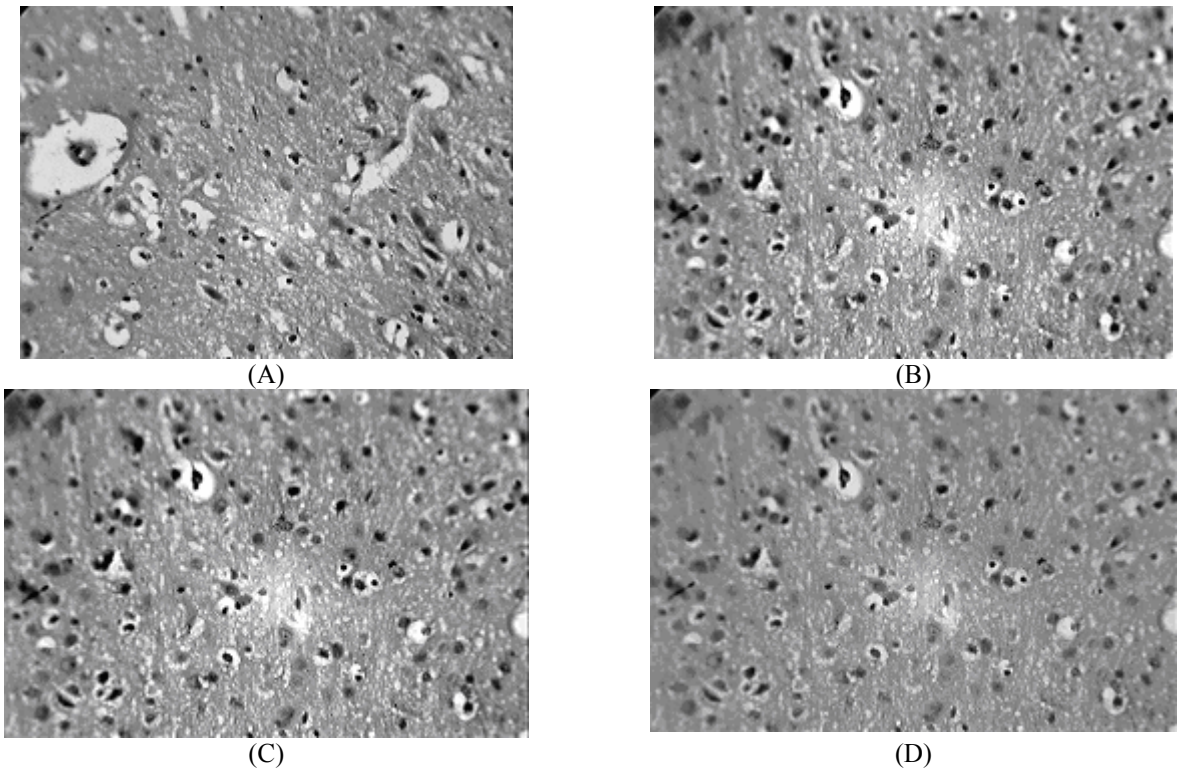


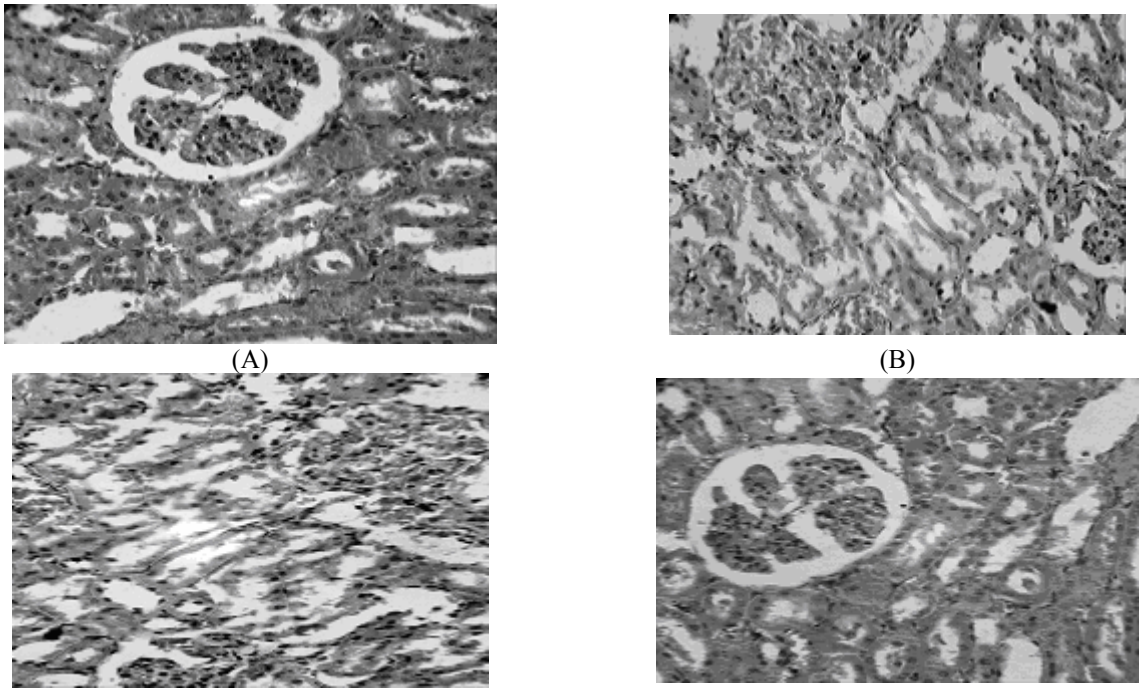
Plate 4: Normal morphology of histological section of rat ovary (A) control (B) low dose, 27 mg/kg (C) median dose, 54 mg/kg (D) high dose, 81mg/kg. X 400

feedback or in mobilization pathways associated with this organ. However, these increases can not be attributed to liver damage as no histological lesions were observed in the hepatic tissue during the administration of this extract. Moreso,the albumin levels of the rats were not affected by the extract as there were no statistical difference



(A) (B) (C) (D)

Plate 5: Normal morphology of histological section of rat brain (A) control (B) low dose, 27 mg/kg (C) median dose, 54 mg/kg (D) high dose, 81mg/kg. X 400



(A) (B) (C) (D)

Plate 6: Normal morphology of histological section of rat kidney (A) control (B) low dose, 27 mg/kg (C) median dose, 54 mg/kg (D) high dose, 81mg/kg. X 400.

between the level in extract treated group and that of the control. Albumin level levels are usually reduced in chronic liver diseases, congestive heart failure and nephritis (Sclavo, 1987). This further indicate that the extract is not hepatotoxic. Significant increases in levels of ALT, AST and ALP without any effect on AST levels were observed in this study. It is known that an increase in the enzymatic activity of ALT, AST and ALP in the serum directly reflects a major permeability or cell rupture (Benjamin, 1978). ALT is a hepatospecific enzyme that is principally found in the cytoplasm in rats (Benjamin 1978; Ringer and Dabich 1979) and is a specific marker for hepatic injury. The increase in the level of ALT therefore indicates hepatic injury (biochemical or pathological) which has not reflected on the histology of the liver. The increase in ALP was only with the lowest dose of the extract and could reflect damage to the tissue in which it is localised. However,the damage was not serious as the level of AST was not affected. Bilirubin is derived mainly from the haem moiety of haemoglobin molecules and synthesized in the liver. High level of bilirubin is commonly found in bilirubin abnormal metabolism,which results in a chemical condition called jaundice (Mayne 1999; Nduka 1999). Increase in the serum bilirubin may arise from exessive haemolysis, cytotoxicity to the liver or from obsruction into bile ducts resulting in cholestasis (Klaassen and Watkins 1999). In this study significant rise in serum total and conjugated bilirubin was observed in extarct treated rats suggesting hepatottoxicity. However, histological study did not observed any lesion in the liver tissue with the adminstration of the extract. This could be due to the liver repair mechanism that must have corrected the the injury on the liver cells,thus none was spottted. Besides increases in transaminases and ALP without a substantial necrosis on the liver been reported previously (Heiberg and Svegaard 1981; Burges *et al*., 1994). Since AST level was unaffected the injury in the liver may have been at the cellular level and was corrected through the repair mechanism of the liver.

The electrolytes, urea and creatinine are markers of kidney function (Jesse *et al.,* 1982), throughout the study, the serum levels of Na^+, K^+, Cl^-, urea and creatinine were not affected by the treatment of rats with the root extract of *C. zambesicus*. This is an indication that the extract is not nephrotoxic. Furthermore, histological studies of the kidney, brain, heart, spleen, testis and ovary revealed no pathological lesions. These are supported by unaffected levels of urea, creatinine and electrolytes (for the kidney) as well as the level of AST for the heart and other tissues. These findings are consistent with that reported on the leaf by Ofusori *et al. (*2008) in which no histological deffect was observed in the liver of rats treated with the leaf extract of *C. zambesicus*. Moreso, the finding in the histology of the testes correlate well with the reports of

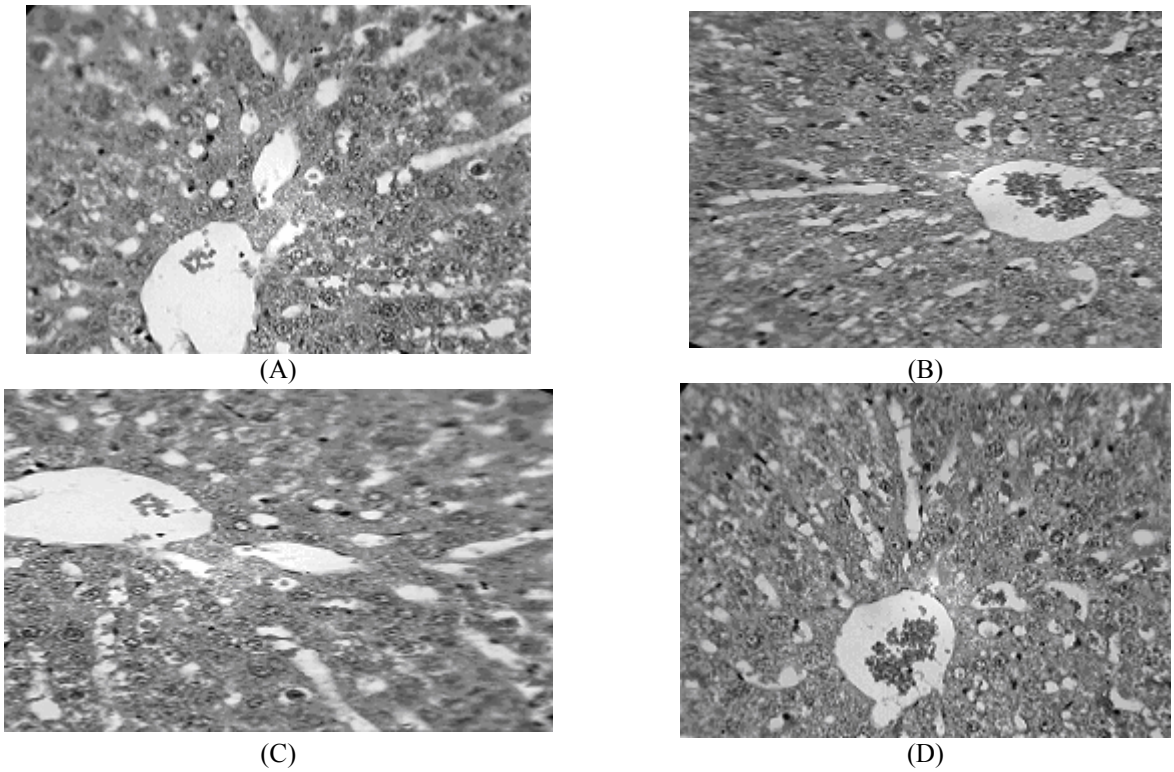


Plate 7: Normal morphology of histological section of rat liver (A) control (B) low dose, 27 mg/kg (C) median dose, 54 mg/kg (D) high dose, 81mg/kg. X 400

Ofusori *et al*. (2007) on the effect of the leaf extract on the testes.

CONCLUSION

The results of this investigation show that ethanolic root extract of *Croton zambesicus* (27-81mg/kg) is safe though with an insignificant effect on the liver.

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REFERENCES

Abo KA, Ogunleye VO and Ashidi JS (1999). Antimicrobial potential of *Spondias mombin, Croton zambesicus,* and *Zygotritonia crocea. Phytotherapy Research*, **13**(6): 494-497.

Adjanohoun EJ, Adjakide V and deSouza S (1989). Contribution to ethnobotanical and floristic studies in Republic of Benin.Vol.1. Agency for Cultural and Technical Cooperation, p.245.

Baccelli C, Block S, VanHolle B, Schancke A, Chapon D, Tinant B, Van Meervelt L, Morel N and Quertin-Leclkercq J (2005). Diterpenes isolated from *Croton zambesicus* inhibit KCl-induced contraction. *Planta Medica*, **71**: 1036-1039.

Baker FJ, Silverton RE and Kilshaw D (1985). Introduction to Medical Laboratory Technology. 6th ed. Butter Worth, London, pp.316-348.

Benjamin MN (1978). Outline of Veterinary Clinical Pathology, University Press, Iowa, USA, pp.229-232.

Bessiere JM (2002). Essential oil from *Croton zambesicus* Muell. Arg. growing in Cameroun. *Flavour and Fragrance Journal*, **17**: 215-217.

Block S, Baccelli C, Tinat B, Meervelt L, Rozenberg R, Jiwan J, Llabres G, DePauw-Gillet M and Quetin-Leclercq J (2004). Diterpenes from the leaves of *Croton zambesicus*. *Phytochemistry*, **65**: 1165-1171.

Block S, Stevigny C, Llabres G, deHoffman E, Adjakide V, DePauw-Gillet M and Quetin-Leclercq J (2002). Ent-Trachyloban-3β-ol, a new cytotoxic diterpene from *Croton zambesicus. Planta Medica*, **68**: 647-648.

Boyom FF, Keumdjio F, Dongmo PM, Ngadjui BT, Amvam-Zollo PH, Menut C and Bessiere JM (2002). Essential oil from *Croton zambesicus* Muell. Arg. growing in Cameroun. *Flavour and Fragrance Journal,* **17**: 215-217.

Burges R, Hancke J and Wikman G (1994). Toxicological assessment of *Aralia mandshurica* (Araliaceae) root extract after subchronic administration in rats. A biochemical and histological study. *Phytotherapy Research*, **8**: 1-9.

El-Hamidi A (1970). Drug Plants of the Sudan Republic in native medicine. *Plantae Medica.* **18**: 278-280.

Eteng MU, Etarrh RR and Owu DU (2003). Effect of theobromine exposures on haematological parameters of rats. *Nigerian Journal of Physiological Sciences*, **18**(1-2): 4-6.

Heiberg JK and Svejgaard E (1981). Toxic hepatitis during ketoconazol treatment. *Britain Medical Journal,* **283**: 825- 826.

Jesse B (1982). Animal anatomy and Physiology. Reston Publishing Company, Reston, USA, p.521.

Klaassen CD and Watkins JB (1999). Casarett and Doull's Toxicology. McGraw Hill, New York, p.231.

Lorke D (1983). A new approach to practical acute toxicity testing. *Achieves of Toxicology*, **54**: 275-286.

Mayne PD (1999). Clinical Biochemistry in Diagnostics and Treatment. 6th edn., Oxford University Press, New York, p.193.

Mekkawi AC (1985). The essential oil of *Croton zambesicus. Fitoterapia*, **56**(3): 181-183.

Menut C, Lamaty G, Bessere JM, Sulean AM, Fendro L, Maidou E and Denamgania J (1985). Aromatic plants of Tropical Central Africa. *Journal of Essential oil Research,* **7**(A): 419-422.

Nduka N (1997). Clinical Biochemistry for students of Chemical Pathology. 1st Edn, Longman, Lagos, Nigeria, p.79.

Nduka N (1999). Clinical Biochemistry for Students of Pathology. Animo Press, Lagos, Nigeria p.81.

Ngadjui BT, Abeghaz BM, Keumedjio F, Folefoe GN and Kapche GW (2002). Diterpenoids from the stem bark of *Croton zambesicus*. *Phytochemistry*, **60**: 345-349.

Ngadjui BT, Folefoc GG, Keumedjio F, Dango E, Sondegam BL and Conolly JD (1999). Crotondiol, a lablane diterpenoid from the stem bark of *Croton zambesicus*. *Phytochemistry*, **51**: 171-174.

Ofusori DA, Oluwayinka OP, Adelakun AE, Keji ST, Oluyemi KA, Adesanya OA, Ajeigbe KO and Ayoka AO (2007). Evaluation of the effect of ethanolic extract of *Croton zambesicus* on the testes of swiss albino mice. *African Journal of Biotechnology*, **6**(2): 2434-2438.

Ofusori DA, Adelakun AE, Ayoka AO, Ibeh JA, Adeeyo AO, Adesanya OA and Ajeigbe KO (2008). Histological investigation of the effects of *Croton zambesicus* on the liver of swiss albino mice. *The Internet Journal of Gastroenterology*, **7**(1): 15-20.

Okokon JE, Nwajuakwu N and Udokpoh A (2005b). Preliminary study of analgesic, anti inflammatory and anti pyretic activities of ethanolic leaf extract of *Croton zambesicus*. *Journal of Pharmacy and Bioresources*, **2**(2): 75-79.

Okokon JE, Bassey AL and Obot J (2006). Antidiabetic activity of ethanolic leaf extract of *Croton zambesicus* on alloxan diabetic rats. *African Journal of Traditional,Complementary and Alternative Medicine*, **3**(2): 21-26.

Okokon JE and Nwafor PA (2009a). Antiplasmodial activity of root extract and fractions of *Croton zambesicus. Journal of Ethnopharmacology*, **121**: 74-78.

Okokon JE and Nwafor PA (2009b). Antiulcer and anticonvulsant activities of *Croton zambesicus* root extract. *Pakistan Journal of Pharmaceutical Sciences,* **22**(4): 384-390.

Okokon JE, Ofodum KC, Ajibesin KK, Danladi B and Gamaniel KS (2005a). Pharmacological screening and antiplasmodial activity of *Croton zambesicus* against *Plasmodium berghei berghei* infection in mice. *Indian Journal of Pharmacology*, **37**(4): 243-246.

Ringer DH and Dabieh L (1979). Haematology and Clinical Biochemistry. *In*: The Laboratory Rats. Vol.1 edited by HJ Baker, JR Lindsey, SH Weisbroth, Academic Press, London, pp.105-116.

Sclavo SPA (1987). Sclavo Diagnostics, Science, Italy, SPA, pp.47-92.