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Investigations on the Effects of Various Oral Doses of *Croton zambesicus* Seeds' in Wistar Rats

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ABSTRACT

Different parts of *Croton zambesicus* have been identified traditionally to possess various medicinal uses in Africa, such as for treatment of hypertension, dysentery, fever, convulsions, malaria and diabetes mellitus. The aim of this study was to examine the effects of methanol and aqueous extracts of *C. zambesicus* seeds at a dose of 75 mg kg^{-1}/day and 300 mg kg^{-1}/day in Wistar rats. The body, biochemical parameters and histopathological changes were assessed for the treated animals as well as those in the control group. The result of this study showed that, the extracts of *C. zambesicus* present significant beneficial effects on erythropoiesis and lowering cholesterol level which confirms the use of this plant in ethnomedical practice. But, these extracts at the doses administered were found to cause varies body weight changes and significant increase in ALT, AST and ALP activities as well as pathological changes such as fatty changes and necrosis in liver and kidney and the lymphocytic infiltration in intestinal lamina are all indicate that the plant extracts has the potential to cause toxicity and intestinal damage at dose dependant manner. But the exact chemical compound(s) responsible for these effects in the plant extracts still remains speculative.

Key words: *Croton zambesicus*, ethnomedical, fatty change, necropsy, toxicity

INTRODUCTION

Herbal remedies used in traditional medicine provide an interesting and still largely unexplored source for the development of new drugs (Cos *et al*., 2006). *C. zambesicus* from family Euphorbiaceae locally known as *Umgelaela*, is a shrub distributed in the northern parts of South Africa, In Sudan widely distributed in Elnuba mountain in south kordofan state (Coates Palgrave, 2002). It contains alkaloids, terpenes, flavnoids, glycosides, saponins, volatile oil such as sesquiterpenes, monoterpenes and diterpenes and other chemicals (Block *et al*., 2006, 2002).This plant is extensively used in African traditional medicine (Watt and Breyer-Brandwijk, 1962). The leaf decoction is employed in Benin to treat hypertension, urinary infection and fever associated to malaria (Adjanohoun *et al*., 1989; Adjanohoun and Souza, 2002) and in Nigeria to treat diarrhea, dysentery and malaria (Ajibesin *et al*., 2008). The root is used in Nigeria as antidiabetic and antimalarial remedy (Okokon *et al*., 2005) and in Sudan for menstrual pain and constipation (El-Hamidi, 1970; Ngadjui *et al*., 2002). Antimalarial activity was observed with extracts from the stem bark (Boyom *et al*., 2009; Okokon and Nwafor, 2009) and from the seeds (Ali *et al*., 2002). The stem bark also displays antimicrobial activity (Abo *et al*., 1999).

Ngadjui *et al.* (2002) revealed that compounds isolated from *C. zambesicus* which are used in traditional medicine for the treatment of a number of diseases including malaria, were characterized and screened for anti-plasmodial activity using inhibition of growth of Plasmodium berghei in mice. The compounds include abiatane diterpenoids, quinines, triterpenoids and lavonoid. He concluded that the flavonoid exhibited strong anti-malarial activity against the multidrug resistant strain of *Plasmodium falciparum* (Ngadjui *et al.*, 2002).

Boyom *et al.* (2002) studied the composition of essential oils from the leaves, stem and roots of *C. 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 reported that the root extract whose LD50 is 273.86 mg kg^{-1} contains alkaloids, saponins, terpenes, tannins, phlobatannins, anthraquinones and cardiac glycosides, while flavonoids were reported to be absent. Despite these huge achievements on the successful isolation of some important phytochemicals, there is very little literature concerning the study of the effects of seeds of *C. zambesicus*, Therefore, this study was aimed to investigate the effects of methanolic and aqueous extracts of *C. zambesicus* seeds on haematological, serobiochemical and histopathological profile in Wistar rats and subsequently to evaluate whether its ethnopharmacological uses may have possible side effects. This will, in a way, guide the usage and dosage of this important plant.

MATERIALS AND METHODS

Experimental animals: Thirty male Wistar rats with average body weight ranged from 85 to 90 g were used in this study. The rats were apparently clinically healthy and housed within the premises of El-Neelain University Animal House under standard husbandry conditions (30°C±2°C, 60-70% relative humidity and 12 h: 12 h day-night cycle) and fed on the rat diet (flour 75.3%, meat 15%, edible oil 7.5%, sodium chloride 1.5% and vitamins and minerals 0.7) and water provided *ad Libitum*. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee.

Plant materials: *C. zambesicus* seeds were purchased from Khartoum herbal market (April 2011). The plant seeds were cleaned and coarsely ground through mechanical grinder and the methanol and aqueous extracts were prepared and used in this study.

Preparation of plant extract: Hundred gram of the coarsely powdered seeds were weighed precisely and subjected to extraction with 250 mL methanol for 2 h through soxhlet apparatus, then, the extract was separated from solvent using rotary evaporator and the air dried powder were used for the treatment (methanol extract). The residues of plant seeds were further dried, weighed and extracted with 500 mL of distilled water overnight at room temperature, filtered and further dried by freeze drier (aqueous extract) (Mudeser, 2004).

Experimental design: The rats were divided randomly to five groups, each of 6 rats:

- **Group 1:** Served as normal control and were given only distilled water (DW) daily for 14 days
- **Groups 2 and 3:** received aqueous extract dissolved in DW at a dose of 75 and 300 mg kg^{-1}/day respectively for 14 days given orally through cathedral tube
- **Group 4 and 5:** Received methanol extract dissolved in DW at a dose of 75 and 300 mg kg^{-1}/day (BW) for 14 days given orally through cathedral tube

Body weights of rats were measured on day 0, 7th and day 14th of the treatment. Blood samples for biochemical parameters and tissue samples for histopathology were taken at day 7th and 14th after scarifying three animals from each group under mild chloroform anaesthia in the 1st week (day 7th) and the remaining three animals in the 2nd week (day 14th).

Blood collection for hematological and serobiochemical parameters: The blood samples for measurement of Complete Blood Count (CBC) were collected in EDTA blood containers and immediately analyzed through automated Haematology analyzer (Sysmex KX-21, Japan, 1999). while blood samples for the other parameters were collected in heparinized sample containers and centrifuged immediately at 3500 rpm for 5 min, the plasma was separated in a new plain sample container labeled according to the study group, rat number, time and date of collection and stored in refrigerator at -4°C until the parameters were analyzed within one week of collection thought Roche diagnostic Hitachi 902 analyser (Germany, 1996).

Organs harvest and tissue processing for light microscopy: Necropsy was conducted to study the effects of the plant extracts on the histology of some vital organs, namely, liver, kidneys, heart, spleen and small intestines. After scarifying the rats, specimens of about 1 cubic cm of each organ were immediately fixed in 10% neutral buffered formalin to prevent the post mortem changes. The fixed tissues were dehydrated via transferred them into different concentration of alcohol solutions for 17.5 h in an automated tissue processor, after which the tissues were passed through a solution of xylene. Following clearance in xylene, the sections were then infiltrated and embedded in molten paraffin wax, then sections of 5 μm thickness were cut using a rotary microtome, floated onto clean slides coated with 2% formaldehyde for proper cementing of the sections to the slides and were then stained with Haematoxylin and eosin stains (Shittu *et al.*, 2006, 2007). The stained sections were examined under light microscope.

Statistical analysis: Statistical Package for Social Science (SPSS) was used for the analysis of the data, the values of body weight and biochemical estimations were expressed as Mean±standard error of mean (SEM) and analyzed through *hoc* Dunnet's simple *t*-test. Differences between groups were considered significant at $p<0.05$ levels (Snedecor and Cochran, 1989).

RESULTS

Growth changes: The effects of treatments of plant extracts on body weight of treated rats were presented in Table 1. There was significant decrease ($p<0.05$) on the body weight of treated groups (G2, G3 and G5) when compared to normal control (G 1). No death among the rats recorded along the treatment periods.

Table 1: Body weight and body weight gain in rats orally given *C. zmbesicus* extract for 2 weeks

	Body weight (g)		
Treatment groups	0 week	One week	Two weeks
Control (Normal diet)	85.0±2.2	94.0±2.9	105.7±5.0
75 mg kg^{-1} day^{-1} aqueous extract	90.8±3.5	92.8±2.8*	91.8±5.6*
300 mg kg^{-1} day^{-1} aqueous extract	90.8±3.5	83.3±2.8*	81.3±5.0*
75 mg kg^{-1} day^{-1} methanolic extract	90.8±3.5	85.8±3.7*	80.8±7.2*
300 mg kg^{-1} day^{-1} methanolic extract	90.8±3.5	88.4±1.2*	86.7±1.7*

Values are expressed as mean±S.E, *Significant = ($p<0.05$)

Hematological and serobiochemical changes: Hematological and serobiochemical changes of rats treated with 75 and 300 mg kg^{-1} of each extracts were showed in Table 2 and 3. There was significant increase ($p<0.05$) in the values of Hb, RBCs, PCV and MCV of the treated groups (G2-G5) when compared to normal control group (G1) on day 7th of the treatment and at the end of experiment, with the dose of 75 mg kg^{-1} of both extracts produced a marked increases than the dose of 300 mg kg^{-1}. WBCs of treated groups were significantly ($p<0.05$) increased during the first week of treatment as compared to normal control, while it found to be significantly reduced at the end of the experiment (Table 2).

Also, there was significant ($p<0.05$) increase in the AST, ALT and ALP levels of the treated groups (G2-G5) as compared to normal control group (G1) at the end of experiment, whereas, there was significant ($p<0.05$) decrease of total cholesterol level (Table 3). No significant changes were observed in the other biochemical parameters measured.

Pathological changes: Histology of liver, kidney and small intestine of group 3 and group 5 showed some pathological changes when compared to normal control. In group 3, there were Cytoplasmic fatty vaculation and necrosis of centrilobular hepatocytes (Fig. 1), whereas, there were disquamination of small intestine epithelium cells with lymphocytic infiltration (Fig. 2). In group 5, there was necrosis, fatty change, packing, segmentation and dilatation of kidney (Fig. 3). No lesions were observed in spleen or heart of the test rats.

Table 2: Hematological analysis of rats given *C. zambesicus* aqueous and methanolic extracts for 2 weeks

		Aqueous		Methanol	
Parameters	G1. Control	G2. *C. zambesicus* (75 mg/kg/day)	G3. *C. zambesicus* (300 mg/kg/day)	G4. *C. zambesicus* (75 mg/kg/day)	G5. *C. zambesicus* (300 mg/kg/day)
One week					
Hb (g dL^{-1})	14.1±0.9	17.9±0.75*	15.6±1.7*	17.7±1.9*	16.7±1.8*
RBCs ($X10^6 mm^3$)	9.2±0.5	11.3±0.7*	11.0±2.0*	12.4±0.5*	11.9±0.7*
PCV (%)	61.2±3.9	75.6±3.7*	69.6±3.8*	71.9±3.2*	67.2±1.9*
MCV (m3)	61.6±1.2	68.5±0.5*	67.3±0.3 *	65.0±1.0 *	69.6±0.3 *
MCH (pg)	14.2±0.2	16.2±0.4*	15.5±0.3^{NS}	14.3±2.0^{NS}	14.9±0.2^{NS}
MCHC (%)	23.0±0.1	24.7±0.2^{NS}	24.5±0.6^{NS}	22.9±3.4^{NS}	23.2±0.4^{NS}
WBCs ($X10^3 mm^3$)	8.4±0.8	14.9±1.1*	13.8±0.8*	10.8±0.9*	6.3±0.6*
Lymphocytes (%)	67.4±3.4	68.7±3.3^{NS}	63.7±3.0^{NS}	62.1±1.9^{NS}	70.6±2.4^{NS}
Granulocytes (%)	32.5±3.4	31.2±3.3^{NS}	36.2±3.2^{NS}	37.8±1.9^{NS}	29.4±2.4^{NS}
Two weeks					
Hb (g dL^{-1})	13.5±1.2	15.4±0.8*	16.3±0.4*	16.2±0.5*	17.1±1.2*
RBCs ($X10^6 mm^3$)	9.2±0.8	8.4±0.3*	8.3±0.7*	11.5±0.8*	11.8±1.7*
PCV (%)	59.0±5.6	61.7±3.3^{NS}	66.3±4.2*	75.3±5.8*	65.3±1.6*
MCV (m^3)	60.0±2.0	65.5±1.5*	63.0±1.7*	65.0±0.5*	64.0±1.0*
MCH (pg)	14.2±0.3	17.0±0.3*	15.8±0.6*	14.0±0.8^{NS}	14.7±1.5^{NS}
MCHC (%)	22.9±0.1	25.9±0.0^{NS}	25.2±0.3^{NS}	21.6±1.4^{NS}	23.5±2.7^{NS}
WBCs ($X10^3 mm^3$)	9.0±0.5	5.4±0.7*	6.4±1.7*	8.3±1.5^{NS}	9.1±0.9^{NS}
Lymphocytes (%)	64.9±4.1	55.3±4.5*	52.6±7.0*	59.2±2.3*	59.3±8.4*
Granulocytes (%)	35.0±4.1	44.6±4.5*	37.3±7.0*	40.8± 2.3*	40.7±8.4*

Values are expressed as Mean±SE; NS: Not significant; *Significant at $p<0.05$

Table 3: Serobiochemical analysis of rats given *C. zambesicus* aqueous and methanolic extracts for two weeks

		Aqueous		Methanol	
Parameters	G1. Control	G2. *C. zambesicus* (75 mg/kg/day)	G3. *C. zambesicus* (300 mg/kg/day)	G4. *C. zambesicus* (75 mg/kg/day)	G5. *C. zambesicus* (300 mg/kg/day)
One week					
AST (iu)	31.2± 3.9	30.2±2.6^{NS}	30.1±2.6^{NS}	36.6±4.2*	38. 3±2.4*
ALT (iu)	48.0±5.0	47.0±2.9^{NS}	54.3±1.4^{NS}	44.3±0.8^{NS}	65.3±0.3*
ALP (iu)	364.0±4.4	341.0±6.1*	206.3±8.1*	196.3±5.5*	161.0±5.2*
Total protein (g dL^{-1})	8.2±0.1	8.5±0.2^{NS}	9.0±0.5^{NS}	7.6±0.2^{NS}	7.5±0.2^{NS}
Albumin (g dL^{-1})	4.3±0.1	4.6±0.2^{NS}	4.9±0.6^{NS}	4.3±1.5^{NS}	4.2±0.1^{NS}
Globulin (g dL^{-1})	3.9±0.2	3.9±0.2^{NS}	4.2±0.4^{NS}	3.3±0.1^{NS}	3.2±0.1^{NS}
Bilirubin (mg dL^{-1})	0.13±0.0	0.1±0.0^{NS}	0.2±0.0^{NS}	0.1±0.0^{NS}	0.13±0.1^{NS}
Cholesterol (mg dL^{-1})	98.3±0.3	91.0±4.1*	88.3±4.6*	86.0±10.1*	77.6±5.4*
Two weeks					
AST (iu)	35.0±2.5	34.2±4.2^{NS}	35.4±3.7^{NS}	49.1±2.7*	41.9±2.8*
ALT(iu)	44.6±5.8	54.6±5.7*	62.6±5.2*	89.3±7.4*	72.6±5.3*
ALP (iu)	443.3± 9.8	351.0±1.1*	291±8.8*	258±8.4*	173.6±27.3*
Total protein (g dL^{-1})	8.3±0.9	7.9±0.7^{NS}	8.1±0.3^{NS}	8.3±0.4^{NS}	8.7±0.5^{NS}
Albumin (g dL^{-1})	4.5±0.8	4.1±0.2^{NS}	4.6±0.1^{NS}	4.6±0.1^{NS}	4.8±0.3^{NS}
Globulin (g dL^{-1})	3.8±0.5	3.8±0.5^{NS}	3.4±0.3^{NS}	3.6±0.3^{NS}	4.1±0.4^{NS}
Bilirubin (mg dL^{-1})	0.2±0.0	0.2±0.1^{NS}	0.2±0.0^{NS}	0.2±0.1^{NS}	0.2±0.0^{NS}
Cholesterol (mg dL^{-1})	141.6±26.1	96.3±22.4*	99±19.2*	107±15.1*	85.3±14.4*

Values are expressed as Mean±SE; NS: Not significant;*Significant at $p<0.05$

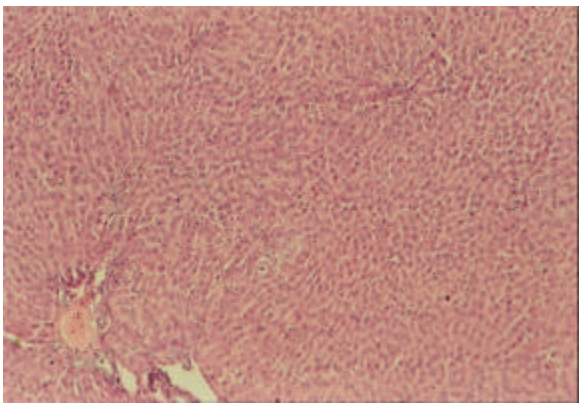

Fig. 1: Liver of rats received *C. zambesicus* aqueous extract 300mg kg^{-1} for 2 weeks showing fatty cytoplasmic vaculation, H×E X100

DISCUSSION

C. zambesicus has been used ethanomedicinally as a therapeutic agent for a variety of diseases. The young branches of *Croton* are pleasantly aromatic. The charred and powdered bark is used to treat bleeding gums. Although the plant is believed to be toxic, it is an important stock food in Namibia (Okokon *et al.*, 2004). It is reported that most type of *Croton* posses medical activity; The volatile oil from the bark of *Croton cajucara* exert gastric ulcer healing activity, as well as protection of the gastric mucos (Hiruma-Lima *et al.*, 2000). This study was aimed to investigate the effects of

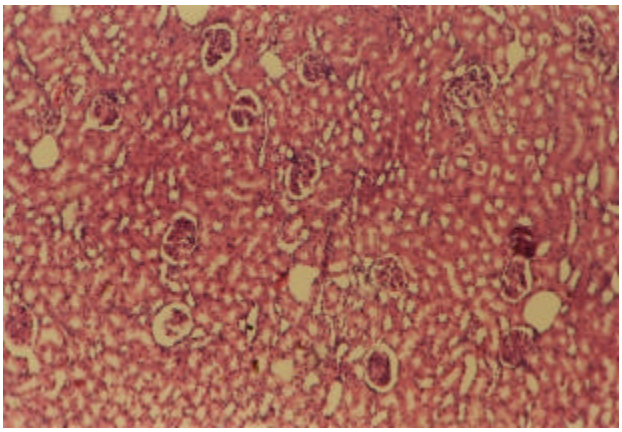

Fig. 2: Kidney of rats received *C. zambesicus* methanolic extract 300mg kg^{-1} for 2 weeks showing, necrosis, lymphocytic infiltration, segmentation, packing and dilatation. H×E X100

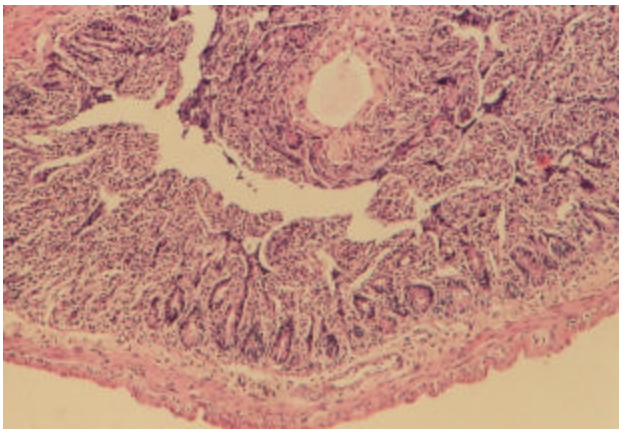

Fig. 3: Intestine of rats received *C. zambesicus* aqueous extract 300mg kg^{-1} for one week showing, lymphocytic infiltration and disquamination, H×E X100

methanolic and aqueous extracts of *C. zambesicus* seeds on haematological, serobiochemical and histopathological profile in Wistar rats and subsequently to evaluate whether its ethnopharmacological uses may have possible side effects which is common with the use of most chemotherapeutic agents.

The body weights of the animals were found to be affected by seed extracts treatment as they loss of considerable weight in dose dependant manner when compared to the control group. This indicates that, the extracts may interfere with growth processes and may have suppressed growth by stimulating the breakdown of body proteins. This finding is contradict with the result of Jude E Okokon *et al.* (2010) whom reported that, the subchronic treatment of rats with root extract of *C. zambesicus* for 21 days caused comparable increase in body weights of rats in extract treated and control groups.

Administration of seeds extracts of *C. zambesicus* to rats for 14 days produced a dose-dependent increases in PCV, Hb, RBCs, MCHC and MCH, the observed increase in this parameters may have resulted from supportive action of the extracts on erythropoiesis, This could be attributed to

alkaloids present in the extract reported by Okokon *et al.* (2010). Alkaloids have been implicated to cause similar effect by inhibiting phosphodiesterase leading to the accumulation of cAMP which in turn stimulates protein synthesis and consequently erythropoiesis (Eteng *et al.*, 2003).

Total white blood cells were observed to be reduced following the administration of the extracts for two weeks to rats, the observed decrease may have resulted from suppression of leucocytosis by the extracts or from the suppression of their production in the bone marrow. This finding were also observed by Okokon *et al.* (2004), whom reported a decrease in WBCs following administration of ethanolic leaf extract of the *C. zambesicus*.

Significant increases in levels of ALT, AST and ALP 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 hepato-specific enzyme that is principally found in the cytoplasm in rats (Benjamin, 1978; Ringler 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 reflected on the histology of the liver. These increases could be attributed to liver damage as the histological lesions were observed in the hepatic tissue of treated rats. This finding was also reported by Okokon *et al.* (2010) whom observed a significant rise in serum total and conjugated bilirubin in rats treated with *C. zambesicus* root extracts suggesting hepatotoxicity. However, the total protein and albumin levels of the rats were not affected by the extract as there were no statistical difference suggesting the hepatic damage was not serious.

The decreases of serum cholesterol level observed in this study may be due to plant effects to stimulate cholesterol excretion into the intestine, oxidation of cholesterol to bile salts, blocking the reabsorption of cholesterol from the gastrointestinal tract, preventing the reabsorption of bile salt and/or inhibition of the pathway of cholesterol synthesis (Kaur *et al.*, 2011).

From these *in vivo* studies, we can conclude that the prepared seeds extracts of *C. zambesicus* present significant beneficial effects on erythropoiesis and lowering cholesterol level which confirms the use of this plant in ethnomedical practice. But the plant extract in the administered doses also showed an increase in ALT, AST and ALP activities as well as some pathological changes in liver, kidney and small intestine which indicate that the plant extracts have the potential to cause toxicity at dose dependant manner. But the exact chemical compound(s) responsible for these effects in the plant extracts still remains speculative, therefore more detailed studies using different doses, assessment of safety and covering longer period of observations are needed before reaching a clear-cut conclusion.

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