MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Medicine and Medical Sciences (ISSN: 2354-323X) Vol. 3(9) pp. xxx-xxx, September, 2015
Available online http://www.meritresearchjournals.org/mms/index.htm

Original Research Article

Effect of Methanol leaf extract of *Croton zambesicus* on Haemoglobin, Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities and liver histology of albino mice

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Abstract

The present study was aimed at evaluating the effect of methanol leaf extract of *Croton zambesicus* on hemoglobin (Hb), serum aspartate aminotransferase (AST), alanine aminotransferase ALT) and liver histology of albino mice. Mice of both sexes (n=24), weighing between 20-25g were distributed into four groups, I-IV. Group I orally received 5 ml/kg normal saline and served as the control. Groups II-IV received the extract orally at respective doses of 500 mg/kg, 700 mg/kg and 900 mg/kg. Treatments lasted for five days, and on the sixth day blood samples were taken from the sacrificed animals in order to evaluate the levels of the parameters. Carefully excised liver samples were fixed in 4% formaldehyde for detailed examination. The parameters were assessed for the treated animals as well as those in the control group. The Hemoglobin value was observed to increase from 90.83 g/L (for the 500 mg/kg extract group) to 95.17 g/L (900 mg/kg extract group). The AST values decreased from 28.50 iu/L (500 mg/kg extract group) to 22.00 i.u/L (900 mg/kg extract group).The ALT values were also observed to decrease from 25.00 i.u/L(at 500 mg/kg extract dose) to 22.0 i.u/L(at 900 mg/kg extract dose). The differences in the values were not significant (p>0.05) as compared with the controls. Histological examinations showed that all liver samples showed well preserved hepatocytes. It was thus concluded that the extract did not elicit any deleterious effect on the hepatic tissues/hematology of the animals.

Keywords: AST, ALT, Croton zambesicus, hemoglobin, histology, liver, mice, serum

INTRODUCTION

Medicinal plants are those plants whose one or more organs contain substances that can be used for therapeutic purpose, or which are precursors for the synthesis of useful drugs (W.H.O.1977).

Plants serve as rich sources of medicines because they produce a host of bioactive molecules, most of which have probably evolved as chemical defense against predation or infection (Cox and Ballick, 1994).

Medicinal plants have been identified and used throughout human history because they have the ability to synthesize a wide variety of chemical compounds that are used to perform important physiological functions. These chemical compounds also defend the plants against attack from predators such as insects, fungi and herbivorous mammals. Herbal remedies used in traditional medicine provide an interesting and still largely unexplored source for the development of new drugs (Cox *et al.,* 2006). Traditional medicine (also known as indigenous or folk

Figure 1. Picture of *Croton zambesicus*

medicine) comprises knowledge system that developed over generations with various societies before the era of modern medicine.

Croton zambesicus is a shrub or small tree that is about 16m high in fringing forest and savannah, the Gambia to south Nigeria and widely distributed elsewhere in tropical Africa. The tree has a scaly bark and silvery leaves rusty scaly below and has an attractive appearance (Figure 1). The leaves which are silvery have greenish colour on top and some of the leaves are orange in colour. It has a sweet smell and it is often planted in towns and villages. The wood is pale yellow, fine grained, hard and gives a good polish. The stems are used in parts of West Africa for hunt posts and in Yoruba houses for beams in default of other timbers. The bark slash emits an aromatic smell also. Croton zambesicus is extensively used in African traditional medicines (Watt and Breyer-Brandwijk, 1962) and other parts of the world. It contains alkaloids, terpenes, flavonoids, glycosides, saponins, volatile oils such as sesquiterpenes, monoterpenes and diterpenes and other chemicals (Block *et al*., 2006). It has been shown to be a free radical scavenger and to protect against lipid peroxidation. This ability has been reported to increase peripheral testosterone level in swiss albino mice (Okokon *et al*., 2005).The components are appropriate for detoxification and antioxidants (Okokon *et al*., 2005). Boyom *et al*., (2002) studied the composition of essential oils from the leaves, stems and roots of *croton zambesicus* and found three types of oils to be similar in composition, with those from the leaves and stem rich in monoterpenes while that of 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. Despite these huge achievements on the successful isolation of some important phytochemicals, there is very little literature on safety/toxicity profile of *Croton zambesicus* leaves. Thus, in this research work, an attempt has been made to evaluate whether its ethno pharmacological uses have negative effects on hepatic/haematological systems. The aim of the study is thus to investigate the effects of methanol extracts of *Croton zambesicus* leaves on aspartate aminotransferase, alanine aminotransferase, haemoglobin levels and liver histology in albino mice.

MATERIALS AND METHODS

Collection and identification of plant

Croton zambesicus leaves were collected from a tree on the campus- Federal Polytechnic Nekede, Imo State. The leaves were identified by a taxonomist in the department of Biology, Federal University of Technology Owerri. The fresh leaves of *Croton zambesicus* were washed, and dried under shed for two weeks. These were ground to a coarse powder using a mechanical blender. The methanol extract was prepared and used for this study.

Preparation of plant extract

500g of the coarsely powdered leaves was weighed precisely and was soaked with 1500ml of 95% methanol in a beaker and covered with aluminum foil. The mixture was stirred intermittently and allowed to stand for 72hours. Filtration was carried out using filter paper and the filtrate was subsequently concentrated in a rotary evaporator at the temperature of 45-50^{o}c. The extract obtained was packaged in an air tight container and stored in a refrigerator at 4^{o}C until it was used for the study.

Table 1. Effect of methanol extract of *Croton zambesicus* on serum AST activity of albino mice.

Group	Treatment	Dosage	AST activity (i.u/l)
I	Normal saline (control)	5ml/kg	28.83±1.06[a]
II	Extract	500mg/kg	28.50±1.12[a]
III	Extract	700mg/kg	27.00±0.98[a]
IV	Extract	900mg/kg	26.50±1.23[a]

Values are mean ± SEM (n=6)
Differences in AST activities are not significant (P>0.05).

Table 2. Effect of methanol extract of *Croton zambesicus* on serum ALT activity of albino mice.

Group	Treatment	Dosage	ALT activity (i.u/l)
I	Normal saline (control)	5ml/kg	24.50±0.98[a]
II	Extract	500mg/kg	25.00±2.01[a]
III	Extract	700mg/kg	25.17±1.96[a]
IV	Extract	900mg/kg	22.00±0.78[a]

Values are mean ± SEM (n=6)
Differences in ALT activities are not Significant (p>0.05).

Experimental animals

A total of 24 albino mice of both sexes with average body weight ranging from 20- 25g were obtained from a local breeder in Owerri for use in the study. The animals were apparently clinically healthy and were housed in plastic cages with sawdust as beddings and they were maintained under standard husbandry conditions (30±2 ^{o}C, 60-70% relative humidity, 12 hours light and 12 hours darkness cycle) for two weeks.

During the period of acclimatization, the animals were fed with animal feed and clean water was provided *Ad libitum.*

Experimental design

The study was designed according to the method of Onwusonye *et al.,* (2014).

Animal Treatment

The albino mice were divided randomly into four groups, each of six mice:
Group i: served as control and were given only normal saline (5mlkg^{-1}/day)
Group ii: received methanol extract at a dose of 500mg kg^{-1}day
Groupiii: received methanol extract at a dose of 700mg/ kg^{-1}/day.
Group iv: received methanol extract at a dose of 900mg/ kg^{-1}/day

These treatments were given once daily for five consecutive days. All experimental procedures followed the recommendations provided in the "Guide for the care and use of laboratory animals" (National Academy Press 1996). On the 6th day, the animals were sacrificed and the blood collected by cardiac puncture. The blood sample was taken in two aliquots, one in anticoagulant sample bottles for the measurement of haemoglobin concentration and the second portion in plain sample bottles for preparation of sera for evaluation of AST and ALT. Haemoglobin concentrations were determined by the cyanomethaemoglobin method (Jain,1986), while AST and ALT activities were determined by the method of Reitman and Frankel (1957).

Histological Procedure

Histological examination was done by fixing the organs (liver) in 4% formaldehyde. They were subsequently processed and embedded in Paraffin wax. Tissue blocks were sectioned 5µm thick and stained with haematoxylin and eosin for detailed observation

Statistical analysis

Data collected were subjected to analysis of variance implemented in SPSS statistics 17.0. The means were separated using Duncan Multiple Range Test at the 0.05 level of significance.

RESULTS

The result of the effect of methanol leaf extract of Croton zambesicus on serum AST, ALT and Haemoglobin concentration of albino mice are presented in tables 1-3 while the photomicrograph results of the histological examinations are presented in (Figure 2-5).

Table 3. Effect of methanol extract of *Croton zambesicus* on Haemoglobin levels of Albino mice.

Group	Treatment	Dosage	HB(g/dl)
I	Normal saline (control)	5ml/kg	93.33±3.45[a]
II	Extract	50mg/kg	90.83±2.72[a]
III	Extract	700mg/kg	91.67±3.05[a]
IV	Extract	900mg/kg	95.17±2.86[a]

Values are mean ± SEM (n=6)
Difference in Hb values are not significant (P>0.05)

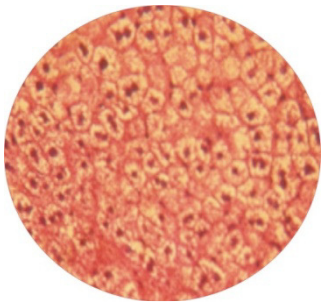

Figure 2. Photomicrograph of liver treated with Normal saline (5ml/kg body weight) showing well preserved hepatocytes H&E x 400

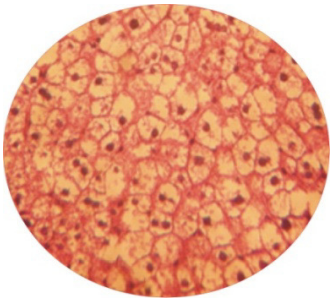

Figure 3. Photomicrograph of liver treated with *C. Zambesicus* extract (500mg/kg body weight) showing well preserved hepatocytes H&E x 400

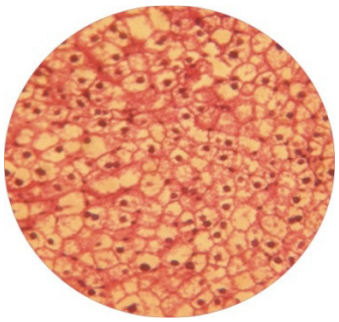

Figure 4. Photomicrograph of liver treated with *C. Zambesicus* extract (700mg/kg body weight) showing well preserved hepatocytes H&E x 400

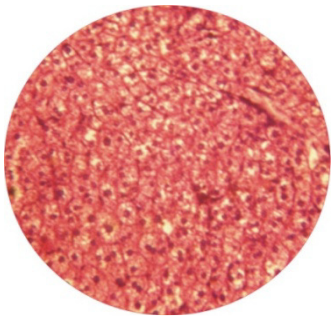

Figure 5. Photomicrograph of liver treated with *C. zambesicus* extract (900mg/kg body weight) showing well preserved hepatocytes (H & E x 400)

DISCUSSION AND CONCLUSION

In this study, different doses of the extract of *Croton zambesicus* leaves were administered orally to albino mice in order to determine its effect on haemoglobin, aspartate and alanine transaminases levels as well as liver histology. There was no significant difference in the values of AST, ALT and Hb. as compared with the control at doses of 500mg/kg, 700mg/kg and 900mg/kg of the extract (Tables 1, 2 and 3)

Haemoglobin is the protein molecule in red blood cells that transports oxygen from the lungs into tissues of the body. Any pathological condition that affects the red blood cell alters its functions and thus may be detrimental to the body (Agbor *et al.,* 2005). There were no obvious haemolytic changes rather the extract increased the haemoglobin level at the highest dose of 900mg/kg (Table 3). The increase in the haemoglobin level suggests that the extract does not possess toxic substances that can cause anaemia. This may also suggest that the leaf extract posseses haematopoeitic properties. Alanine and aspartate transferases (ALT and AST) are well known transaminases used as biomarkers to predict possible toxicity in the blood of sick animals (Akdogan et al., 2003) .It is known that an increase in concentration of AST and ALT in the serum directly reflects liver disease. However, ALT is more hepatospecific than AST because it is more sensitive to hepatic damage. Elevation in ALT levels is rarely observed except in chronic liver diseases (Gad, 2001). Therefore, the decrease in AST and ALT levels implies that oral administration of the extract did not cause any impairment to the liver function and thus none of the liver markers(AST and ALT) was altered suggesting that *Croton zambesicus* leaf extract did not induce hepatocellular damage. Histological examination of liver samples excised from treated and control mice showed that in all samples, the hepatic cells were well preserved with normal cyto-architecture (Figure 2-5), also in agreement with the results of the haematological and biochemical tests.

From the results of this study, it could be opined that leaves of *croton zambesicus* do not contain deleterious substances capable of altering haematological or serum biochemical values in mammals. The leaves may be used as an agent for the stimulation of the production of red blood cells and also as antihepatotoxic agent. The present investigation suggests that intake of *croton zambesicus* has no deleterious or lethal effects on the liver of albino mice. Further studies are thus recommended on its effects on other mammalian systems. The active principle behind the hepatoprotective as well as haematological effects should be isolated and characterized.

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