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Evaluation of the Safety Profile of the Leaves of *Gutenbergia nigritana* Benth

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Aim: This study was designed to evaluate the safety of aqueous extract of *Gutenbergia nigritana* leaves in Wistar rats.
Place and Duration of Study: Department of Biochemistry, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. Between July and September, 2014.
Methodology: The sub chronic toxicity experiment was conducted on the extract. Graded doses (100, 200, and 400 mg/kg) of the extract was administered orally to the rats on a daily basis for a period of 28 days. The animals were observed daily for signs of toxicity and mortality. Thereafter, alterations in the haematological parameters, serum lipid profile and some marker enzymes were then evaluated.
Results: The administration of the extract did not result in mortality of the rats at the tested doses and duration. There were no significant alterations in the levels of packed cell volume (PCV), heamoglobin (Hb), white blood cells (WBC) and platelets. However, there was a reduction in the concentration of red blood cells (RBC) at 200 mg/kg. The administration of aqueous extract of *G. nigritana* leaves resulted in significance increase the levels of total cholesterol and low density lipoprotein cholesterol. The triglycerides level reduced whereas that of high density lipoprotein cholesterol remained unaltered. The activities of aminotransferases were increased while that of alkaline phosphatase reduced. There was no significant alteration in the activity of acid phosphatase throughout the experimental period.

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Conclusion: Sub chronic administration of *G nigritana* leaf extract may predispose animals to cardiovascular risk.

Keywords: G. nigritana; safety; sub chronic; doses.

1. INTRODUCTION

Medicinal herbs have played vital roles in the treatment of various diseases in humans and animals [1]. A vast proportion of rural communities in the developing countries depend on crude extracts from herbs as their main source of drugs to meet their basic health care needs. This is probably due to the perceived therapeutic efficacy and lower side-effect profile of natural products from plant origin [2]. Growing evidence has shown that plants used in traditional medicine contain wide range of metabolites that can be harnessed to treat chronic as well as infectious diseases [3]. The therapeutic value of herbs lies in the bioactive compounds that elicit a definite physiological action on the human body [4]. However, their acceptability by some cultures of the world has been limited by lack of specific dose regimen and thorough scientific information on toxicity profile to validate their safety [5]. In recent times, concerns were raised about the adverse effects of herbal preparations used in the management of diseases. This is because some of these preparations had been reported to be hepatotoxic [6,7]. Consequently, putting into consideration the indiscriminate use of these plants, it is expedient to provide information on the safety of herbal preparations used in the management of diseases. One of these herbs is *Gutenbergia nigritana.*

Gutenbergia nigritana Benth (Family *Asteraceae*) locally known as "bush bitter leaf" in Western Nigeria, is an annual herb that grows in the tropical and subtropical regions of Central America, Eastern Brazil and west Africa [8].The leaf sap is widely used in traditional folk medicine in Nigeria for the treatment of hypertension, convulsion and asthma [9]. It is also an essential ingredient of a decoction used for the treatment of anemia and skin infections. Aluko and Alli Smith [9] reported the antioxidant activity and free radical scavenging potential of its hydroalcoholic extract.

Despite the ethno therapeutic usage of this plant in Nigeria by some tribes, no scientific information has been documented on the safety of consuming the extract of *G. nigritana* leaves. This present investigation was therefore undertaken to assess the potential toxic risks incurred following the ingestion of aqueous extract of *G. nigritana* leaves in Wistar rats.

2. MATERIALS AND METHODS

2.1 Assay Kits and Reagents

The assay kits for total cholesterol, triacylglycerol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alkaline phosphatase (ALP), acid phosphatase (ACP), alanine and aspartate aminotransferases (ALT and AST, respectively) were obtained from Randox Laboratories United Kingdom. All other reagents used were of analytical grade.

2.2 Collection of Plant Material

G. nigritana, was collected during its blossoming stage in the month of May from a local farmland in Ikere Ekiti South-Western Nigeria. The whole plant was identified by Mr Omotayo Felix Oluwafemi (herbarium curator) at the Department of Plant Science, University of Ado Ekiti, Nigeria. A voucher specimen (Aluko Med 12/02) was deposited at the Herbarium of the University. The leaves were separated and dried under shade for ten days.

2.3 Preparation of Aqueous Extract of *G. nigritana* Leaves

The dried leaves of *G. nigritana* were pulverized into fine powder using an electric blender. 100 g of *G. nigritana* leaf powder was extracted in 2 liters of cold sterile distilled water for 24 h. The extract was filtered using a Buchner funnel with Whatman's No 1 filter paper. The filtrate was concentrated to dryness in the water bath at 40ºC. The dried extract was reconstituted in cold distilled water to give doses of 100, 200 and 400 mg/kg body weight.

2.4 Experimental Design and Treatment of Animals

Eight weeks old Wistar rats (*Rattus norvegicus*) were were randomly divided into 4 groups with 6

animals per group. The rats were kept in standard cages and laboratory conditions. They were fed with normal rat pellets and water *ad libitum*. Group A (control) was administered with 1 ml of distilled water while groups B, C and D were given 100, 200 and 400 mg/kg body weight of aqueous extract of *G. nigritana* leaves respectively. The administration was done repeatedly on daily basis for four weeks using metal oropharyngeal cannula. The animals were monitored daily for any abnormal clinical signs and death during the study period. At the end of the study, all animals fasted overnight (water *ad libitum*) and, on the 29th day, the animals were weighed. The animals were sacrificed under chloroform anesthesia 24 hrs after their 28 doses of distilled water and extract. Blood was collected by cardiac puncture with or without EDTA for haematological and biochemical analysis respectively.

2.5 Determination of Biochemical Parameters

The method described by Tietz et al. [10] was adopted for the determination of total cholesterol, LDL-C, HDL-C and triacylglycerol. The activities of ALT, AST, ALP and ACP were determined in the serum according to the method described in the assay kits. The atherogenic index was computed from the expression of Panagiotakos et al. [11]. The heamatological parameters analyzed include: red blood cells (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cell (WBC) and platelets.

2.6 Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD) and subjected to one – way analysis of variance (ANOVA) followed by Duncan multiple range test. Values were considered statistically significant at $P < 0.05$.).

3. RESULTS

The aqueous extract of *G. nigritana* leaves in either of the doses (100, 200 and 400 mg/kg body weight) did not significantly alter the haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC) and platelet throughout the experimental period (Table 1). In contrast, the extract significantly decreased the red blood cell count (RBC) at a dose of 200 mg/kg body weight. All the doses significantly increased the serum concentrations of total cholesterol and low-density lipoprotein cholesterol (Table 2). The serum concentration of high-density lipoprotein cholesterol remained unaltered throughout the period of administration. However, the triglycerides levels decreased significantly after the administration of all the tested doses.

The administration of the aqueous leaf extract significantly increased the activities of ALT and AST throughout the period of administration at all doses investigated (Fig. 1). Although, the extract did not alter the activities of ACP, that of ALP decreased throughout the experimental period (Fig. 2).

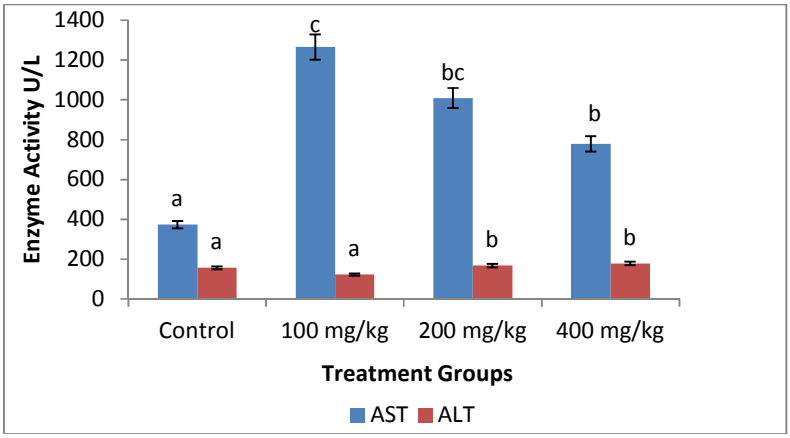

Fig. 1. Effect of *G. nigritana* leaf extract on the activities of aspartate transaminase (AST) and alanine transaminase (ALT) in the serum rats

Results are expressed as means ± SD (n=6). Test values carrying superscripts (b - c) are significantly different (P <0.05) from the control (a) for each parameter

Table 1. Effect of administration of aqueous extract of *G. nigritana* leaves on heamatological parameters of rats

Dose	PCV (%)	Hb (g/dl)	WBC ($x 10^9$/L)	RBC ($x 10^6$/L)	Platelet ($x 10^3$/µL)
Control	42.4±2.88^a	15.12±2.31^a	3.63±0.51^a	8.28±1.00^a	176.33±19.86^a
100 mg/kg	42.00±3.08^a	16.48±1.36^{ab}	2.37±0.32^a	8.15±1.73^a	195.33±7.23^a
200 mg/kg	41.4±5.32^a	18.42±2.41^a	3.07±0.42^a	1.13±2.08^b	194.67±13.32^a
400 mg/kg	38.4±3.13^a	16.50±1.19^{ab}	3.47±0.42^a	7.95±1.15^a	198.00±18.33^a

Results are expressed as means ± SD (n=6)
Test values carrying superscripts (b) are significantly different (P <0.05) from the control (a) for each parameter

Table 2. Serum lipid levels in rats following the administration of aqueous extract of *G. nigritana* leaves

Dose	Total cholesterol mmol/L	Triglyceride mmol/L	LDL-C mmol/L	HDL-C mmol/L	Atherogenic index
Control	1.95±0.06^a	0.60±0.14^a	0.36±0.06^a	1.33±0.12^a	0.27
100 mg/kg	3.20±0.12^d	0.58±0.10^{ab}	0.87±0.18^c	1.40±0.10^a	0.62
200 mg/kg	2.35±0.06^b	0.48±0.10^{ab}	0.53±0.01^{ab}	1.60±0.15^a	0.33
400 mg/kg	2.63±0.26^c	0.40±0.12^b	0.73±0.12^{bc}	1.60±0.21^a	0.46

Results are expressed as means ± SD (n=6)
Test values carrying superscripts (b - d) are significantly different (P <0.05) from the control (a) for each parameter

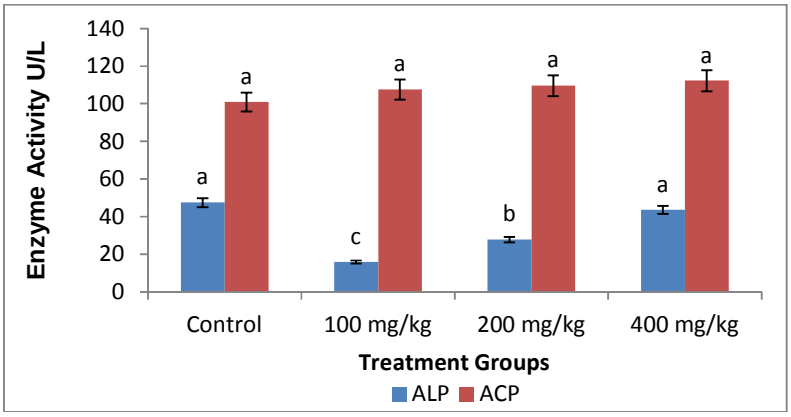

Fig. 2. Effect of *G. nigritana* leaf extract on the activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) in the serum of rats

Results are expressed as means ± SD (n=6). Test values carrying superscripts (b - c) are significantly different (P <0.05) from the control (a) for each parameter

4. DISCUSSION

The heamatological parameters are important in the assessment of physiological and pathological status of an animal. The ingestion of toxic compounds has been reported to alter the normal range of heamatological parameters in experimental animals [12]. The absence of significant alterations in the levels of PCV, Hb, WBC and platelets of rats following the administration of aqueous extract of *G. nigritana* leaves might be an indication that the animals adjusted to the assault of the extract [13]. However, the significant decrease in the levels of RBC at a dose of 200 mg/kg body weight) of the extract administration is an indication that there was destruction of matured RBC's or impairment in the rate of production of RBCs (erythropoiesis). There is tendency that the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues at this dosage will be affected [14]. This may also be as a result of adverse effect on the average size of RBC (microcytes) as well as weight of Hb per

RBC at this dose. This implies that this dose has the potential to induce anaemia. Anaemia is a reduction in the number of erythrocytes in the circulating blood. It results from excessive red blood cell (RBC) destruction, RBC loss, or decreased RBC production and is a manifestation of an underlying disease condition [15].

The serum lipid profile is a useful index to assess the susceptibility of an animal to coronary artery diseases [16]. Elevated levels of total cholesterol, low density lipoprotein cholesterol and triglycerides have been implicated in the pathogenesis of atherosclerosis [17]. The increase in total cholesterol observed with the extract may be due to increase in the concentration of acetyl CoA as a result of enhanced _-oxidation of fatty acid. Acetyl CoA is a key substrate in the biosynthesis of cholesterol [18]. Low-density lipoprotein cholesterol (LDL-C) is associated with plasma cholesterol transport. The significant increase in the serum LDL-cholesterol is understandable since an increase in total cholesterol should normally result in elevated LDL- cholesterol. This observation may be due to impairment in the transportation of plasma cholesterol. These elevated levels may be dangerous to the animals as it may enhance atherosclerosis and increases the risk of high blood pressure. Triglycerides are metabolic fuel deposits stored in the adipocytes of the adipose tissue. The elevated levels of serum triglyceride represent a risk factor for cardiovascular diseases [19]. The administration of aqueous extract of *G. nigritana* leaves reduced the level of triglycerides in the rats. This can be attributed to the inhibition of lipolysis [20]. The administration of the extract had no observable effect on HDL-cholesterol. HDL-c plays a vital role in protecting against cardiac disorders by acting in reverse cholesterol transport. It also protects the endothelial function, fibrotic, inflammatory and oxidative processes [21]. It has been reported that an increase in the concentration of HDL-cholesterol correlates inversely with coronary heart disease [22].

Some marker enzymes are found in appreciable quantities in the serum. These enzymes do not originate from the extracellular fluid [23]. Their concentration in the blood is dependent on the leakage from the tissues as a result of compromised membrane integrity. Serum aminotransferases are indicators of lesions in liver cells [21]. Therefore, the observed increase in the levels of ALT and AST at the tested doses in this study may be an indication of altered membrane permeability of the hepatocytes [24]. Acid phosphatase is a 'marker' enzyme for lysosomal membrane. The activity of this enzyme in tissues following the administration of a chemical compound can be used to assess the status of the membrane [25]. Alkaline phosphatase is a marker enzyme of the plasma membrane. It is also found in the biliary duct of the liver [26]. The activity of ACP was not altered throughout the experimental period. However, the observed decrease in the activity of ALP following the administration of the extract could be attributed to inactivation of the enzyme molecules [23].

5. CONCLUSION

In conclusion, the alterations of some biochemical indices following the administration of aqueous extract of *G. nigritana* leaves suggested parameter and dose-selective toxicity of the extract. The elevation in LDL and total cholesterol by the extract may predispose consumers to cardiovascular risk when repeatedly consumed on daily basis at the investigated doses for 28 days. Therefore, the aqueous extract of *G. nigritana* leaves may not be completely safe as oral remedy in rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As regards the issue of ethical approval, there is no constituted ethical committee for animal use that we know in Ekiti State University, Ado Ekiti Nigeria.

However, in the animal house of where this study was conducted, any experiment involving the use of animals is monitored directly by the Head of department of biochemistry and Dean of faculty of science to ensure the guidelines for principle of laboratory animal care were followed throughout the experimental period.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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