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Original article:

HEPATIC AND HEMATOLOGIC EFFECTS OF FRACTIONS OF *GLOBIMETULA BRAUNII* IN NORMAL ALBINO RATS

J. Okpuzor[1,*], H. A. Ogbunugafor[2], G. K. Kareem[1]

[1] Department of Cell Biology and Genetics, University of Lagos, Akoka – Lagos, Nigeria
[2] Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
* Corresponding author: Email: joyokpuzor@yahoo.com; Telephones: +234 802 306 5999, +234 803 361 1553

ABSTRACT

Globimetula braunii, a major medicinal plant used in traditional medicine in Nigeria, was evaluated for possible effects on hepatic and hematologic indices in Wister albino rats. The crude methanol extract of the plant was successively fractionated with hexane, chloroform, ethyl acetate, butanol and water. The MeOH extract of *G. braunii* and its fractions were administered to albino rats at a dose of 200 mg kg^{-1} body weight for 14 days. Assessments of liver function enzymes aspartate amino transferase (AST), alanine amino transaminase (ALT), as well as total and direct bilirubin levels were carried out.

We observed significant increases ($p \leq 0.05$) in packed cell volume (PCV) and hemoglobin (Hb) in the rats treated with chloroform extract ($CHCl_3$), ethyl acetate (EtOAc) and water (H_2O) fractions. The red blood cell (RBC) count increased ($p<0.05$) after administration of $CHCl_3$ and EtOAc fractions while white blood cell (WBC) count increased ($p<0.05$) in the crude and all its fractions except butanol. Significant decreases ($p<0.05$) in the activities of AST and ALT enzymes were observed in rats treated with chloroform fraction, while there was elevation in the activity of ALT in the butanol fraction group. There was no difference ($p>0.05$) in enzyme activities in the MeOH fraction. However, the administration of other fractions to the rats, led to increases ($p<0.05$) in the activities of both enzymes. Total and direct bilirubin showed increases ($p<0.05$) in EtOAc and hexane fractions while only direct bilirubin increased ($p<0.05$) in the crude fraction.

The present study demonstrates that *G. braunii* chloroform fraction has an influence on hematologic functions and liver enzyme levels in rats.

Keywords: *Globimetula braunii,* medicinal plant, fractions, hepatic, hematologic, toxic

INTRODUCTION

The use of medicinal plants for treatment and management of diseases has been gaining prominence world wide especially in the developing countries where 80 % of the population still depends on traditional healing methods (Iwu, 1993; De Silva, 1997). These plants are used primarily for the treatment of numerous diseases like hypertension (Adjanohoun et al., 1985), cardiovascular diseases (Ouedraogo et al., 2004), hepatic illness (Phillipson & Wright, 1991), arthritis (Dongmo et al., 2003) diabetes (Al-Ghaithi et al., 2004), malaria (Traore et al., 2000). This surge in the use of herbal medicines is probably due to the perceived failure of synthetic drugs in the treatment of some chronic diseases like hypertension, diabetes, arteriosclerosis etc; the side effects associated with most drugs and the incidence of drug resistance especially

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among the antibiotics family.

Globimetula braunii Engl van Tiegh (Loranthaceae) is a hemi-parasitic shrub that grows on dicotyledonous trees and attaches itself to the host by modified roots (Burkhill, 1985). The plant is widely distributed in tropical African countries such as Ghana, Cameroun and Nigeria and is a major ingredient used in herbal medicines. *G. braunii* commonly called "Afomo onishano" by the Yoruba tribe of south west Nigeria has gained importance as being useful in the treatment of headache, rheumatic pains and pulmonary troubles (Burkhill, 1985). Leaves, fruits and flowers of the plant are used to treat hypertension, while the roots are employed for other therapeutic uses such as ulcer and cancer treatment (Burkhill, 1985).

The liver plays a central role in metabolism of drug and xenobiotics, protein synthesis and in maintaining biologic equilibrium of organisms. Due to these important roles, liver enzymes are used as markers in assessment of drug or plant extract safety or toxicity (Tietz, 2000; Satyapal et al., 2008). The transaminases are involved in intermediary metabolism and are thus present in high concentration in the liver (Tietz, 2000). They are rapidly released into the serum in cases of acute destruction of tissues as in myocardiac infarction or hepatocellular necrosis (Tietz, 2000).

Therefore, the extensive use of this plant underscores the need to evaluate its toxic and hematologic effects. Our search on the previous use of the plant did give an insight on previous toxic and hematologic properties of the leaf extract and fractions of *G. braunii*. Thus, the aim of this study is to evaluate the effect of the methanol extract of the leaf and fractions of *G. braunii* on liver and blood parameters in rats.

MATERIALS AND METHODS

Plant material

The leaves of *Globimetula braunii* were collected in March 2008 from Olokemeji forest reserve in Ogun State, Nigeria. They were authenticated at Forestry Research Institute (FRIN) Ibadan, Nigeria and a specimen was prepared and deposited at the Institute's herbarium (FHI107741).

Animal's source and sorting

Thirty five adult Wistar albino rats were purchased from the animal colony of the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria. The rats were allowed to acclimatize for two weeks in the animal house of University of Lagos. Approval for the use of the animals was obtained from the Ethical Committee of University of Lagos for experimental purposes only.

The rats weighing between 180-200 g were sorted into 7 groups of 5 rats each. They were housed in standard rat cages under standard laboratory conditions and allowed feed and water *ad libitum*.

Chemicals

Assay kits for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) were from Randox Laboratories Ltd, Ardmore, UK and the kit for bilirubin determination was from Human Gesellschaft für Biochemica und Diagnostica, Wiesbaden, Germany. They were purchased from Nzemat Pharmaceuticals Company, Fola-Agoro, Lagos, Nigeria. All other reagents and solvents used were of analytical grade.

Extraction of plant

The dried leaves of *Globimetula braunii* were pulverized into a fluffy mass. 303 g of the powdered leaves was extracted with 250 ml of 80 % MeOH using Soxhlet extractor for 8 hours. The extract solution was evaporated to dryness under reduced pressure (below 40 °C) to yield 35.88 g crude extract.

Fractionation of the extract

The method described by Yesilada and Kupeli (2002) was employed. The methanolic extract of *G. braunii* was reconstituted with 200ml of a mixture of MeOH:H_20 (9:1) and shaken with hexane (3 x 100 ml). Combined hexane fraction was evaporated under reduced pressure to yield "hexane

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fraction" (4.95 g). MeOH was evaporated from the remaining extract and diluted with distilled water to 200 ml and further fractionated by successive solvent extraction with chloroform ($CHCl_3$) (4 x100 ml), ethyl acetate (EtOAc) (2 x 100 ml) and *n*-butanol saturated with H_20 (3 x 100 ml). Each extract was evaporated to dryness under reduced pressure to yield $CHCl_3$ fraction (4.29 g), EtOAc fraction (2.87 g), butanol fraction (1.59 g) and remaining H_20 fraction (3.86 g).

Preparation of extract and fractions solutions

The extract and fraction solutions were prepared by dissolving 0.4 g of the extract and fractions in 10 ml of 2 % Tween 80, to give an effective concentration of 40 mg mL^{-1}. The formula: Dosage mg kg^{-1} /1000 x Wt of animal (g)/concentration (mg mL^{-1}) was used to calculate the volume of the extract/fractions solution to be administered to each animal. The solutions were prepared fresh daily before administration.

Animal treatment

The animals were distributed into seven groups consisting of five rats per group. One milliliter of the crude and different fractions were administered orally at a dose of 200 mg kg^{-1} body weight to the test groups for 14 days. The control group received 1 ml of 2 % Tween 80 only. The animals were fasted overnight, sacrificed and blood was collected. The blood for hematologic studies was stored in EDTA bottles. The blood for biochemical studies was left to coagulate and centrifuged at 3000 rpm for 10 min; and serum was separated.

Determination of hematologic parameters

Hematologic parameters were determined by standard methods. Packed cell volume (PCV) was determined by microhematocrit method as described by Dacie and Lewis (1991, 1995) using microhematocrit centrifuge (Uniscope Sm 112, Springfield Medicals, U.K.). Hemoglobin concentration was determined by the Cyanomethemoglobin method (Jain, 1986). The red cells (RBC) and white blood cells (WBC) were estimated using improved Neubauer counting chamber (Dacie & Lewis, 1991, 1995).

Determination of biochemical parameters

Assay of Alanine amino transaminase (ALT) and Aspartate amino transaminase (AST) was carried out by the procedure outlined in the diagnostic kit as described by Reitman and Frankel (1957). Total and direct bilirubin was determined by procedure outlined in the diagnostic kit as described by Jendrassik and Grof (1938).

RESULTS

Fractions of *Globumetula braunii* were administered to experimental rats to evaluate their toxic and hematologic effects. Results revealed significant increases ($p<0.05$) in PCV (%) values and RBC levels in $CHCl_3$, EtOAc and H_2O fractions (Figures 1 & 2) while there were no significant changes in the levels of these two parameters in methanol, hexane and butanol fractions.

The Hb concentration in chloroform, ethyl acetate and water fractions (Table 1) showed significant increase $p\leq0.05$ but no significant increases were evident in methanol, hexane and butanol fractions compared to control.

Table 1: Effect of fractions of *G. braunii* on Hb concentration (mg/dL) in rats

Groups	Hemoglobin concentration (mg/dL)
Control	8.50 ± 0.11
MeOH	10.18 ± 1.01
Hexane	11.01 ± 1.04
$CHCl_3$	12.72 ± 0.70*
ETOAc	10.59 ± 0.42*
Butanol	9.11 ± 0.39
H_2O	10.53 ± 0.63*

*significant at $p\leq0.05$

Furthermore, there were significant increases ($p<0.05$) in WBC (cells/mm^3) in all the fractions except in butanol fraction (Figure 3).

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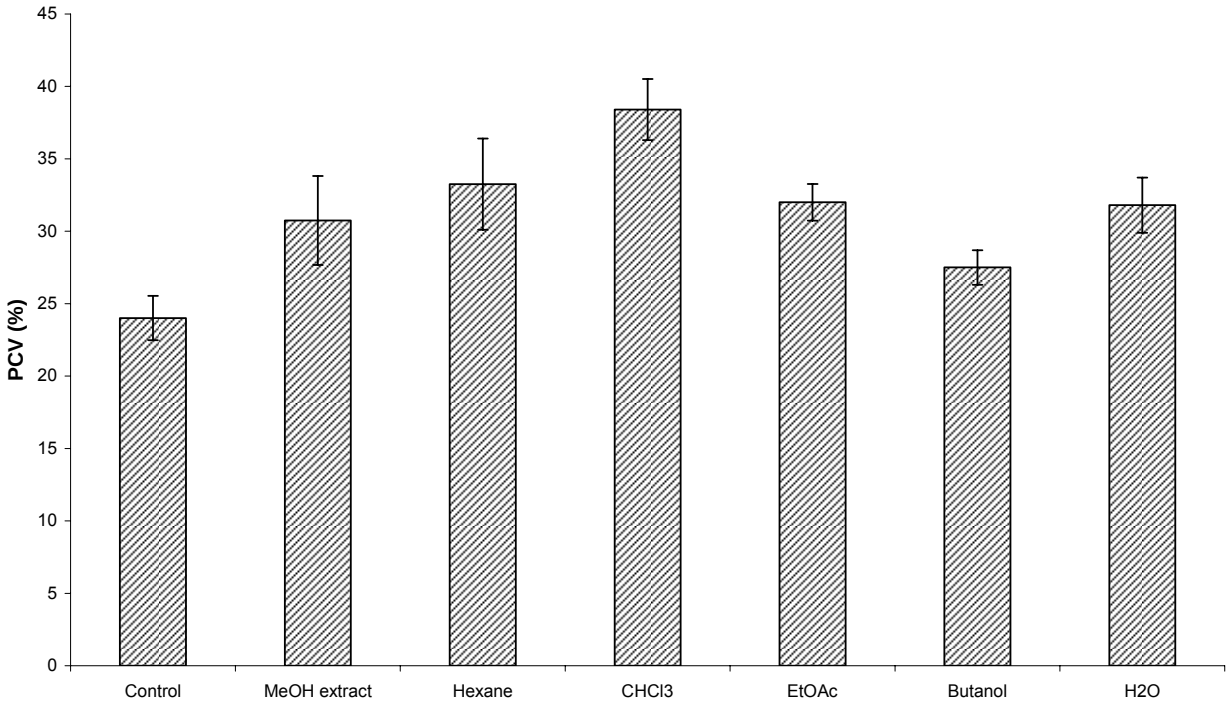

Figure 1: The effect of fractions of *G. braunii* on PCV level in albino rats

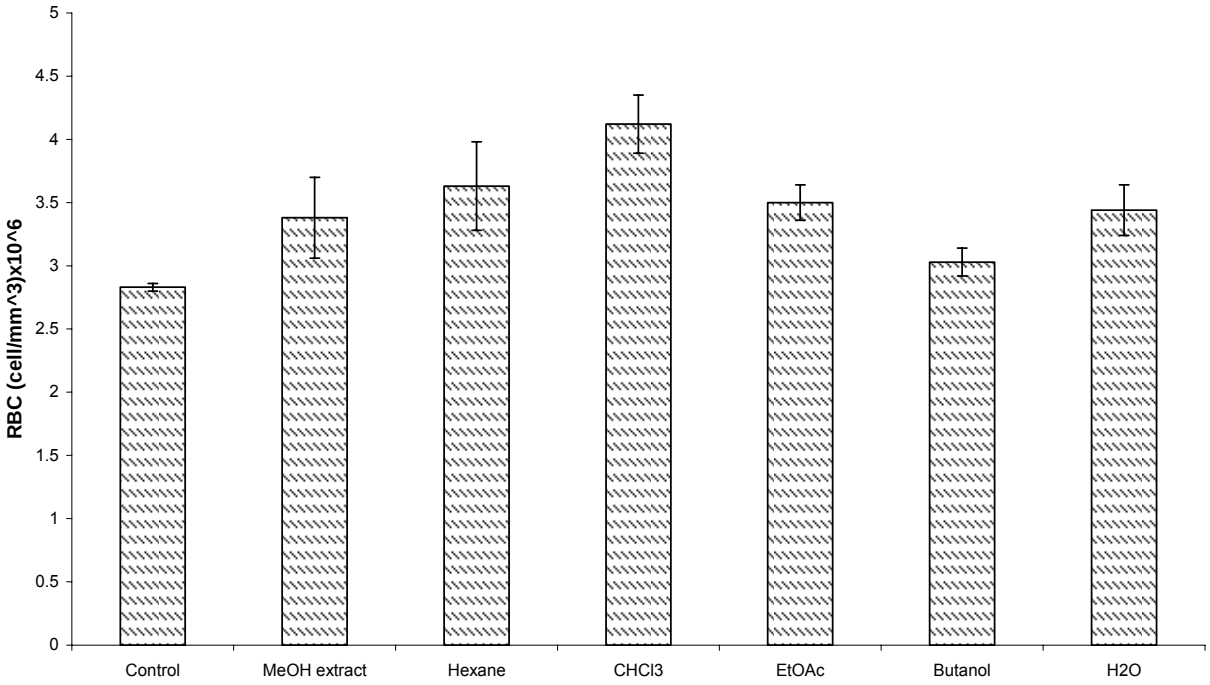

Figure 2: The effect of fractions of *G. braunii* on RBC in albino rats

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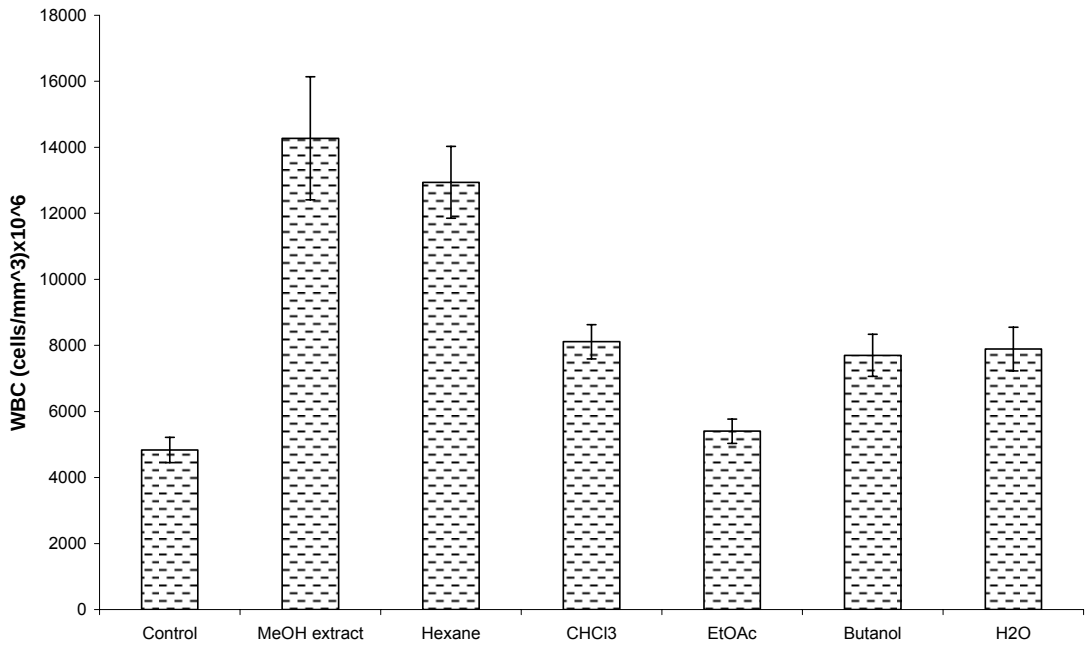

Figure 3: The effect of fractions of *G. braunii* on WBC in albino rats

The direct bilirubin levels significantly elevated ($p<0.05$) in the methanol, hexane and ethyl acetate fractions while total bilirubin significantly increased in the hexane, ethyl acetate and water fractions only (Table 2). However, both hexane and ethyl acetate fractions showed significant increases in both total and direct bilirubin levels.

Table 2: Effect of fractions of *G. Braunii* on serum bilirubin concentration (µmol/L) in rats

Groups	Direct bilirubin (µmol/L)	Total bilirubin (µmol/L)
Control	0.58 ± 0.03	1.32 ± 0.004
Crude	0.85 ± 0.06*	1.70 ± 0.14
Hexane	0.67 ± 0.02*	1.49 ± 0.06*
$CHCl_3$	0.63 ± 0.03	1.36 ± 0.07
ETOAc	1.16 ± 0.04*	2.37 ± 0.13*
Butanol	0.60 ± 0.04	1.39 ± 0.04
H_2O	0.59 ± 0.02	1.46 ± 0.034*

* significant at $p<0.05$

The assay of the hepatic enzymes AST and ALT in Figure 4, revealed that serum AST and ALT decreased ($p<0.05$) in chloroform fraction while ALT activity increased ($p<0.05$) in hexane, EtOAc, butanol and H_2O fractions. AST activity also increased ($p<0.05$) in all the fractions except butanol. There was no significant change ($p>0.05$) in the activities of these enzymes in the methanol fraction.

DISCUSSION

Different solvent systems are routinely used to identify different bioactive components from plant extracts. The results of this investigation demonstrate that hydrophobic and hydrophilic solvents isolated different active principles which exhibited different hematologic and biochemical effects in rats.

The ability of some fractions ($CHCl_3$, EtOAc and H_2O) to increase the PCV level in albino rats is an indication of hemoconcentration and may be due to increased RBC mass. Increased RBC is suggestive of polycythemia and a positive erythropoetic effect. Thus, it is likely that these fractions enhanced the oxygen carrying capacity of the animals. This is supported by other workers that reported similar result on ethanol stem-bark extract study of *Mangifera indica L.* and *Garcinia cambogia* (Oluyemi et al., 2007; Nwinuka et al., 2008). Furthermore, the increased WBC count indicates that these fractions to an extent, affected the defense mechanism of treated rats (Oluyemi et al., 2007).

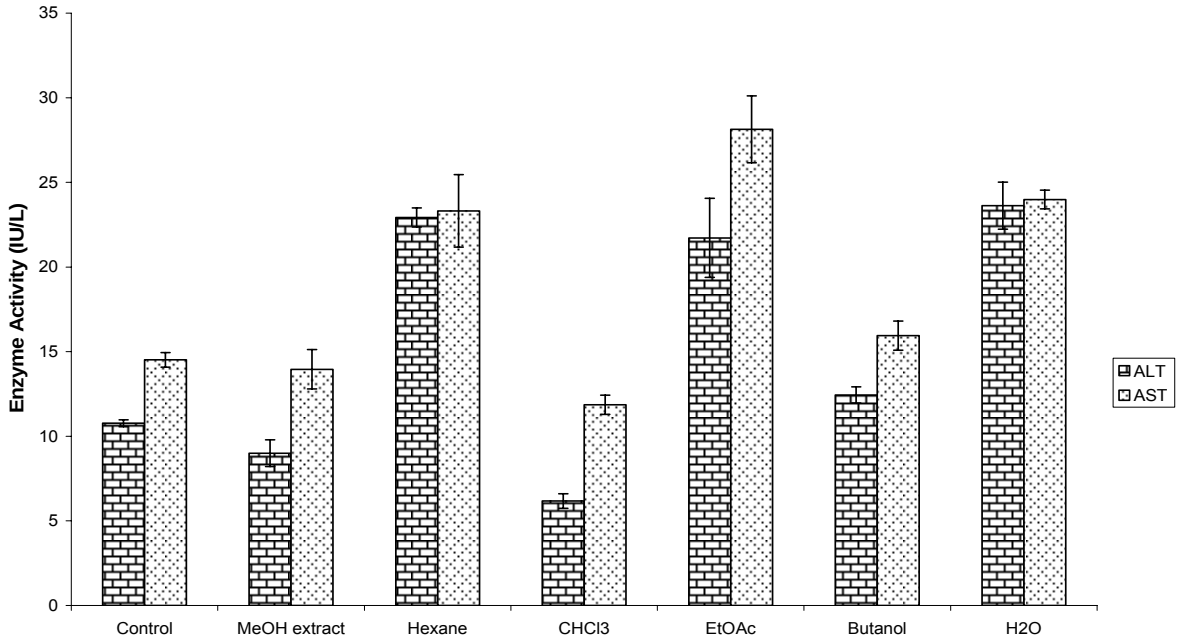

Figure 4: The effect of fractions of *G. braunii* on ALT and AST activity in albino rats

The elevated bilirubin levels may suggest lyses of red cells in EtOAc fraction-treated rats, and agrees with our earlier studies of this fraction of *G. braunii* where increased MDA levels were recorded as a result of elevated lipid peroxidation (Okpuzor et al., 2009). This is likely due to loss of functional integrity of the cellular membrane of the red cells in the EtOAC fraction-treated rats (Iweala & Okeke, 2005). The marked difference in the effect of the *G. braunii* fractions on liver enzymes ALT and AST, support the view of additive and supra-additive action of a plant's multiple constituents as stated by Spinella (2002). This synergy is demonstrated in the crude fraction of *G. braunii* which exhibited no significant change in ALT and AST levels while the reduction in the levels of these enzymes in the chloroform fraction may indicate the healing potential and non toxicity (Scott Luper, 1998; Smith et al., 2002). However, the increased ALT and AST activities in the other fractions may suggest that this dose is toxic and damaging to the liver. This is supported by other workers who have reported that some plant extracts are hepatotoxic (Gathumbi et al., 2000; Shahraki et al., 2007).

In conclusion, this study demonstrates that the chloroform fraction contains bioactive agents that influence hematologic parameters and levels of liver enzymes in rats.

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