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# Comparative study of the effects of ethanolic leave extracts of *Gnetum africanum* (Okazi) and *Solanum macrocarpon* (Garden egg leaf) on the hematological parameters and antioxidant activities of male Wistar rats

Chinyere Salome Ihezuruoha [1, *], Chukwudi Joseph Anumudu [1], Chisom Victor Obichi [2], Sylvester Ifeanyichukwu Okafor [3], Ogochukwu Fidelis Okoye [1], Maduabuchi Cornelius Nwozor [1] and Ndukwe Francis Oguwike [1]

[1] *Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria.*
[2] *Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria.*
[3] *Department of Medicine and Surgery, Faculty of Clinical Medicine, Chukwuemeka Odumegwu Ojukwu University, Awka, Anambra State, Nigeria.*

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## Abstract

The aim of this study was to compare the effects of ethanolic extracts of leaves *of Gnetium africanum* (*G. africanum*) and *Solanum marcrocarpon* (*S. marcroarpon*) on the haematological parameters and antioxidant activities of male Wistar rats. Thirty (30) male rats were randomly divided into five groups of six (6) each and treated for four weeks as follows: Group 1: Low dose of *G. africanum* (500 mg/kg), Group 2: High dose of *G. africanum* (1000 mg/kg), Group 3: Low dose of *S. marcrocarpon* (500 mg/kg), Group 4: High dose of *S. marcrocarpon* (1000 mg/kg) and Group 5: Control. The criterion for statistical significance was p≤0.05. The result of Total Antioxidant Capacity (TAC) showed significant increase in high dose of *G. africanum* and *S. marcrocarpon* 987.27±108.07umol/L and 915.43±24.40umol/L respectively and significant decrease in TAC in low dose of *G. africanum* and *S. marcrocarpon* 787.83±15.35umol/L and 796.53±131.49umol/L compared to Control 805.43±11.04umol/L. There was a significant increase in RBC Count in high dose *of G. africanum* and *S. marcrocarpon* 7.74±0.50×1012/L and 7.94±0.34×1012/L compared to Control 7.56±0.29×1012/L. The result revealed significant increase in; Haemoglobin Count in high dose of *G. africanum* and *S. marcrocarpon* 16.33±1.91g/dl and 17.70±0.70g/dl, compared to Control 15.00±0.82g/dl, and mean levels of PCV count in high dose of *G. africanum* and *S. marcrocarpon* 48.67±5.51% and 55.00±1.00% compared to Control 44.67±2.89%. The results of this study showed that the two extracts possessed antioxidant and haematinic properties and that *G. africanum* has greater antioxidant activities than *S. marcrocarpon* while *S. marcrocarpon* has more haematinic properties.

**Keywords:** *Gnetum africanum; Solanum marcrocarpon*; Antioxidant; haematinic; High dose and ethanolic extract

## 1. Introduction

For many years, plants have served as a source of medicine, and today, over 80% of the population in developing nations receive their primary healthcare from medicinal plants Enenebeaku *et al*., [1]. Plants have served as the primary providers of essential human necessities such as food, clothing, and shelter as well as providing natural cures for human health Enenebeaku *et al*., [1]. According to research by Ufelle *et al*. [2], *Gnetium africanum* leaves extract had a substantial impact on Wistar rats' haematological parameters, including haemoglobin, haematocrit, total white blood

* Corresponding author: Chinyere Salome Ihezuruoha

cells, and red blood cells. Consuming this extract may therefore aid in body defense to fend off and safeguard against intruders. The observed rise in red blood cell count suggests that the kidney and/or bone marrow are stimulating the production of erythropoietin for haematopoiesis (erythropoiesis) Ufelle *et al*, [2]. Some of the phytochemical components of the *Gnetum africanum* leaves extract, such as flavonoids, tannins, terpenoids, saponins, proteins, and steroids, may be responsible for the observed haematological effects Ufelle *et al*., [2].

### 1.1. *Solanum macrocarpon*

*Solanum macrocarpon* leaf contains vital components including vitamins and minerals in a sufficient amount that are needed for enhancing human health Finelib.com, [3]. *S. macrocarpon* leaves or African eggplant leaves provide calcium, potassium, vitamin B, and C, all of which are good for humans Finelib.com, [3]. *S. macrocarpon* is commonly called in Igbo; Akwukwo Anyara/Añara, Yoruba; Efo-Igbo, and English Language; African eggplant leaf/ Garden egg leaf Finelib.com, [3].

### 1.2. *Gnetum africanum*

*Gnetum africanum* is commonly called in Ibibio; Afang, Igbo; Okazi, English; Wild vegetable. The leaves can be eaten and used to cure conditions such as an enlarged spleen, boils, nausea, sore throat, labour pain, snake poisoning, diabetes mellitus, cataracts, and worm expelling Ogbonnaya *et al*, [4]. Because of the importance of *G. africanum* in the southern and eastern regions of Nigeria, particularly in the states of Akwa Ibom, Cross River, and Abia, the vegetable is prepared as a soup (where it is added in hot boiling water that contains all the needed ingredients of the desired soup and allowed to steam for approximately 5 min) Ogbonnaya *et al,* [4]. The leaves are renowned as naturally heart-healthy foods, especially when consumed fresh (Finelib.com, [3]. Garden egg leaves or African eggplant leaves provide calcium, potassium, vitamin B, and C, all of which are good for humans Finelib.com, [3]. Leaves of *Gnetum africanum* and *Solanum marcrocarpon* are local herbs used daily as remedies for certain ailments due to their antioxidant, anticarcinogenic, anti-inflammatory and haematinic properties Olusola *et al*, [5]. Following the rising demand for natural antioxidants by food industries Komlaga *et al,* [6] and daily consumption of these leaves as medicinal herbs, it is pertinent to find out which of these leaves has greater impact on antioxidant activities and haematological parameters of male Wistar rats.

### *Aim of the Study*

The aim of this study was to compare the effects of ethanolic extracts of leaves of *Gnetium africanum* and *Solanum marcrocarpon* on the haematological parameters and antioxidant activities of male Wistar rats.

### *Objectives*

Specific Objectives of the Study

- To evaluate the effect of ethanolic extract of leaves of *Gnetium africanum* and *Solanum marcrocarpon* on the haematological parameters of male Wistar rats.
- To determine the effect of ethanolic extract of leaves of *Gnetium africanum* and *Solanum marcrocarpon* on the antioxidant activities of male Wistar rats.
- To compare the effects of ethanolic extracts of leaves of *Gnetium africanum* and *Solanum marcrocarpon* on the haematological paraameters and antioxidant activities of male Wistar rats.

## 2. Methods

### 2.1. Study Area

This study was conducted at the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.

### 2.2. Animal Source

Forty-eight (48) male rats of 6-8 weeks old having an average weight of 100g were obtained from the Animal House, Department of Human Physiology, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria. The animals were apparently healthy. The rats were randomly divided into five (5) groups of 6 each. The rats were housed in well ventilated wooden cages measuring (60cm x 45cm x 30cm) under hygienic conditions and allowed to acclimatize for 14 days before commencement of experiments. Animals were fed with normal rat chow (Finisher Mash for Rats, Chikun Feed, Ibadan, Oyo State, Nigeria), and water *ad libitum*. Left over feeds and water were discarded and the cages properly cleaned with antiseptic solution such as chlorhexidine every 12 hours. Artificial light was provided by fluorescent lamp (Philips,

Holland; 18 watts) and light-dark cycle of 12-12 hours maintained. Administration of Extract followed after the acclimatization and the average weight of the animals was 130g. All experiments were carried out in accordance with International Guidelines and as stipulated by the Ethical Committee of the College of Medicine, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Amaku.

### 2.3. Sample Size Determination

Sample size of 30 rats at 95% power to detect a difference between means of 2.5 at a significant level (alpha) of 0.05 (two tailed) was chosen using the formula for the calculation of sample size for laboratory animals experiments National Research Council, [7].

$$N = 1+2C[s/d]^2$$ Ughachukwu *et al*, [8].

Where, C = a constant (7.8) at 0.05 level of significance; s = 2.75 (standard deviation from a similar previous study) (Youm *et al*, [9]; d = difference between means desired in present study.

### 2.4. Collection of Plant Leaves

Fresh leaves of *Gnetum africanum* and *Solanum marcrocarpon* were obtained from a local farm at Uli Town in Ihiala Local Government Area of Anambra State, Nigeria and were authenticated by a taxonomist at the Department of Biological Sciences, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Anambra State, Nigeria. Specimen samples of *Gnetum africanum* and *Solanum marcrocarpon* were kept in the Herbarium for future reference with voucher numbers of **COOU/BS/HERB/22/014** and **COOU/BS/HERB/22/015** respectively, Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.

### 2.5. Preparation of Ethanolic Leave Extract of *Gnetum africanum*

The leaves of *Gnetum africanum* were washed clean in tap water and allowed to dry overnight at room temperature of 25°C. The leaves were shade dried for two (2) weeks. The dried leaves of the plant were sliced off into bits and ground into powder using an electrical blender (SharkNinja, BL660, China). The powdered *G. africanum* leaves were subjected to ethanol extraction. The ethanol extract was prepared by soaking 200g of powdered *G. africanum* leaves in 750ml of ethanol at room temperature for 48hrs. The mixture was shaken at intervals. The mixture was filtered with Muslim cloth and the filtrate was concentrated by evaporation to dryness. The extract obtained was stored in airtight container until when needed for the investigation (Ebenyi *et al*, [10].

Percentage yield of extract was determined using the formula:

$$\text{Percentage yield} = \frac{Final\ weight\ of\ extract}{Weight\ of\ dry\ plant\ leaf} \times 100$$

### 2.6. Preparation of Ethanolic Leave Extract of *Solanum marcrocarpon*

Leaves of *Solanum marcrocarpon* were shade-dried and powdered using electrical blender (SharkNinja, BL660, China); 200g of the blended leaves were soaked in 750mL of ethanol (85% ethanol and 15% water) for three days and then filtered. After filtration, the filtrate was heated at a temperature of 40°C for evaporation to occur Olusoji *et al*, [11].

Percentage yield of extract was determined using the formula:

$$\text{Percentage yield} = \frac{Final\ weight\ of\ extract}{Weight\ of\ dry\ plant\ leaf} \times 100$$

### 2.7. Acute Toxicity Studies

#### *2.7.1. Acute Toxicity Evaluation (Median lethal dose, $LD_{50}$) of G. africanum*

Acute toxicity evaluation of the test substance was done following the Lorke's method Lorke, [12]. For each test substance, two stages of tests were involved. In the first stage, nine (9) wistar rats were assigned to 3 groups (A, B and C) of three (3) rats each and were treated with 10, 100 and 1000 mg/kg of the *G. africanum* extract respectively. The animals were thereafter monitored for the manifestations of toxicity signs and deaths within 24 hours. With zero mortality recorded, the study proceeded to the second phase which also involved the use of three (3) rats assigned to three(3) groups (A-C) of one(1) each. Single treatment doses assigned to the groups were 1600, 2900 and 5000 mg/kg of the *G. africanum* extracts respectively. The animals were again monitored for toxicity signs and deaths within 24

hours. The acute toxicity test ($LD_{50}$) of the ethanolic leave extract of *G. africanum* on Wistar rats showed no death was recorded among the rats, even at 5000mg/kg. Signifying no symptoms of toxicity during the investigation. The $LD_{50}$ of ethanolic leave extract of *G. africanum* was found to be >5000mg/kg. The acute toxicity test ($LD_{50}$) of the ethanolic leaves extract of *G. africanum* on Wistar rats showed no mortality or toxicity at 5000mg/kg. The $LD_{50}$ of ethanolic leaves extract of *G. africanum* was found to be >5000mg/kg. The $LD_{50}$ was calculated using the formula;

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where,

- $D_0$ = highest non-lethal dose;
- $D_{100}$ = lowest lethal dose

*2.7.2. Acute Toxicity Evaluation (Median lethal dose, $LD_{50}$) of S. marcrocarpon*

Acute toxicity evaluation of the test substance was done following the Lorke's method Lorke, [12]. For each test substance, two stages of tests were involved. In the first stage, nine (9) wistar rats were assigned to 3 groups (A, B and C) of three (3) rats each and were treated with 10, 100 and 1000 mg/kg of the *S. marcrocarpon* extract respectively. The animals were thereafter monitored for the manifestations of toxicity signs and deaths within 24 hours. With zero mortality recorded, the study proceeded to the second phase which also involved the use of three (3) rats assigned to three(3) groups (A-C) of 0ne(1) each. Single treatment doses assigned to the groups were 1600, 2900 and 5000 mg/kg of the *S. marcrocarpon* extracts respectively. The animals were again monitored for toxicity signs and deaths within 24 hours.

The acute toxicity test ($LD_{50}$) of the ethanolic leave extract of *S. marcrocarpon* on Wistar rats showed no death was recorded among the rats, even at 5000mg/kg. Signifying no symptoms of toxicity during the investigation. The $LD_{50}$ of ethanolic leave extract *S. marcrocarpon* was found to be >5000mg/kg. The acute toxicity test ($LD_{50}$) of the ethanolic leaves extract of *S. marcrocarpon* on Wistar rats showed no motality or toxicity at 5000mg/kg. The $LD_{50}$ of ethanolic leave extract of *S. marcrocarpon* was found to be >5000mg/kg. The $LD_{50}$ was calculated using the formula;

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where,

- $D_0$ = highest non-lethal dose;
- $D_{100}$ = lowest lethal dose.

### 2.8. Experimental Design

A total of forty-eight(48) adult male rats with an average weight of 150g were obtained for this study, eighteen(18) of the animals were randomly selected and used for the acute toxicity studies and the remaining Thirty(30) male rats were randomly divided into five groups of six(6) each and treated as follows:

- **Group 1:** Low dose of *Gnetum africanum* (500 mg/kg).
- **Group 2:** High dose of *Gnetum africanum* (1000 mg/kg)
- **Group 3:** Low dose of *Solanum marcrocarpon* (500 mg/kg)
- **Group 4:** High dose of *Solanum marcrocarpon* (1000 mg/kg)
- **Group 5:** Negative Control

Administration was via the oro-gastric route using oral gavage needle and a 2ml syringe. The administration process was carried out in accordance with the University of California, San Francisco's research office's explanation of the Institutional Animal Care and Use Committee's standard procedure for oral dosing UCSF, [13] and administration of extracts lasted for twenty-eight(28) days. Body weights were taken on weekly bases.

### 2.9. Calculation of Extracts Doses

The concentration in ml of the extract administered to the animals was calculated using the formula:

$$\text{Dose in ml} = \frac{\text{dose in mg/kg} \times \text{weight of rats in kg}}{\text{Stock concentration in mg/ml}}$$

The same calculation was done for other groups and corresponding dose of extract was administered to the rats.

### 2.10. Collection of Blood Samples

The rats were anesthetized, one at a time using Chloroform Ughachukwu *et al*, [8]. Thereafter, blood samples were collected using the method described by Hoff [14]. Briefly, the skin over the jugular vein was cleaned with methylated spirit-soaked cotton wool and 1.0-1.5mls of whole blood withdrawn through the jugular vein using a 25G hypodermic needle fitted unto a 2ml syringe. The withdrawn blood samples were transferred into plain bottles containing anticoagulant (Ethylenediamine tetraacetic acid bottles) to help prevent blood sample coagulation. The tests to determine the haematological parameters and antioxidant activities.

### 2.11. Determination of Haematological Parameters

Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the procedure carried as specified by the producer. The parameters which were evaluated included: Red Blood Cells Count(RBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelets (PLT), Total Leukocytes Count (TLC) and Differential Leukocytes Count(WBC) counts were obtained at once for each blood sample.

#### *2.11.1. Principles of Haematological Analyzer*

The haematological parameters were determined using the automated haematology analyzer according to the methods by Dacie and Lewis [15], modified by Chhabra [16] and the procedure outlined by the producer.

Procedure

To analyze a whole blood sample, the sample was presented to the diluent dispenser and the [DILUENT] key was pressed to aspirate 20µL of the sample into the dispenser. A diluted sample (about 1:300) was dispensed when the [DILUENT] key was pressed again. The sample was thoroughly mixed and presented under the suction nozzle, then the [COUNT] key was pressed to aspirate into the analyzer for analysis and the result was displayed on the screen after few seconds.

### 2.12. Determination of Antioxidant Activities

#### *2.12.1. Determination of SOD activity*

SOD was assayed by colorimetric method of Misra and Fredovich [17].

Principle

The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion ($O_2$) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion introduced increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

Procedure

To 80 µl of sample/blank in a clean test tube was add 1000 µl of carbonate buffer (pH 10.2). The resulting solution was mixed thoroughly, and allowed to equilibrate by incubating at 37 °C for 5 minutes. Thereafter, 600 µl of freshly prepared epinephrine was added and the reaction mixture was read at 30 seconds interval for 150 seconds at 480 nm. The blank was treated the same way except that 80 ul of distilled water was used instead of plasma. The changes in absorbances of both test and blank were determined. The % inhibition of auto oxidation of epinephrine by SOD was calculated and the plasma SOD activity was expressed as U/ml. One unit of SOD activity was equivalent to the amount of SOD that can cause 50% inhibition of epinephrine.

$$\text{Calculation: \% inhibition} = (\Delta OD_{blank} - \Delta OD_{test} / \Delta OD_{blank)} \text{ X } 100$$

$$\text{Enzyme Unit (U/ml)} = (\text{\% inhibition}/50) \text{ X dilution factor.}$$

*2.12.2. Determination of MDA level*

MDA level was determined by the colorimetric method of Gutteridge and Wilkins, [18].

Principle: Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, it forms a pink coloured product, which has absorption maximum at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample.

Procedure

To 0.1 ml of sample in test tube was added 1 ml of 1% Thiobarbituric acid dissolved in alkaline medium (sodium hydroxide). The mixture was mixed thoroughly, and 1 ml of glacial acetic acid was added to the mixture. The reaction mixture was also shaken thoroughly and incubated in boiling water (100 °C) for 15 minutes. It was allowed to cool and the turbidity removed by centrifugation at 3000 rpm for 10 minutes. Thereafter, the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was added to the blank instead of plasma. The level of MDA in the serum is expressed as nmol/ml using the molar extinction coefficient for MDA (1.56x105 M-1cm-1).

*2.12.3. Calculation*

$$\text{MDA (nmol/ml)} = (\text{OD X } 1000000)/ E_{532}$$

Where

$E_{532}$ = Molar extinction coefficient for MDA ($1.56x10^5$ $M^{-1}cm^{-1}$)

*2.12.4. Estimation of total antioxidant capacity*

Total antioxidant activity was estimated by Ferric Reducing Ability of Plasma (FRAP) method by Benzie and Strain, [19].

At low pH, Antioxidant power causes the reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) that can be monitored by measuring the change in absorption at 593nm. FRAP values are obtained by comparing the absorbance change at 593 nm in mixture (test), with those containing ferrous ion in known concentration (Standard).

Procedure

Initially, a working reagent comprising acetate buffer (pH 3.6), ferric chloride and tripyridyltriazine in the ratio of 10:1:1 respectively was prepared. To 60 µl of sample or standard or blank in a clean test tube, 1.8 ml of working reagent was added. The reaction mixture was mixed thoroughly, and incubated at 37 °C for 10 minutes. The resulting blue coloured solution developed was then read at 593 nm. The blank was treated the same way except that 60 ul of distilled water was added instead of plasma. The standard solution contains 1000 µmol/l of ferrous sulphate.

Calculation

$$\text{Total Antioxidant Capacity (µmol/l)} = \frac{\text{OD TEST}}{\text{OD STD}} \text{x standard concentration (1000)}$$

### 2.13. Statistical Analysis

The data generated in this study was statistically analyzed using the SPSS version 23 software. Means and standard errors of mean were calculated. Statistical differences between the experimental and control groups were determined using ANOVA and values were considered significant at $p \leq 0.05$.

## 3. Results

**Table 1** The levels of Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Total Antioxidant Capacity (TAC) in the various groups

| GROUPS | MDA (nmol/mL) | SOD (U/L) | TAC (umol/L) |
|---|---|---|---|
| Low dose *G. africanum* | 2.28±0.70 | 24.62±2.00 | 787.83±15.35* |

| | | | |
|---|---|---|---|
| High dose *G. africanum* | 2.97±0.12 | 25.89±2.34 | 987.27±108.07* |
| Low dose *S. marcrocarpon* | 2.87±0.78 | 24.26±1.67 | 796.53±131.49* |
| High dose *S. marcrocarpon* | 2.96±0.69 | 25.75±0.69 | 915.43±24.40* |
| Control group | 2.96±0.50 | 24.41±1.14 | 805.43±11.04 |
| f-value | 0.725 | 0.636 | 3.944 |
| p-value | 0.595 | 0.648 | 0.036 |

= $p \leq 0.05$.

**Table 2** The levels of some White Blood Cell (WBC) and its Differentials and Platelet in the various groups

| **GROUPS** | **WBC($\times 10^{12}$/L)** | **LYMP(%)** | **GRAN(%)** | **PLT(%)** |
|---|---|---|---|---|
| Low dose *G. africanum* | 5.66±0.76 | 62.73±3.30 | 32.37±3.72 | 252.33±34.53 |
| High dose *G. africanum* | 5.94±0.01 | 63.167±7.38 | 31.97±7.29 | 276.33±18.15 |
| Low dose *S. marcrocarpon* | 4.38±0.48 | 65.27±6.92 | 30.27±6.99 | 273.67±19.22 |
| High dose *S. marcrocarpon* | 6.32±1.93 | 68.30±8.55 | 27.30±7.69 | 248.67±77.84 |
| Control group | 5.93±1.11 | 65.73±3.06 | 29.03±4.19 | 238.67±24.01 |
| f-value | 1.442 | 0.383 | 0.343 | 0.473 |
| p-value | 0.290 | 0.816 | 0.843 | 0.755 |

= $p \leq 0.05$.

**Table 3** The Levels of some Red Blood Cell(RBC), Haemoglobin(HB), Packed Cell Volume(PCV), Mean Corpuscular Haemoglobin(MCH), Mean Corpuscular Volume(MCV) and Mean Corpuscular Haemoglobin Concentration(MCHC) in the various groups

| **GROUPS** | **RBC($\times 10^{12}$/L)** | **HB(g/dl)** | **PCV(%)** | **MCH(pg)** | **MCV(FL)** | **MCHC(g/dL)** |
|---|---|---|---|---|---|---|
| Low dose *G. africanum* | 6.63±1.14* | 14.63±1.10 | 43.00±3.00 | 22.34±2.49 | 56.00±0.69* | 34.02±0.24* |
| High dose *G. africanum* | 7.74±0.50 | 16.33±1.91* | 48.67±5.51* | 21.15±2.68 | 56.93±0.23* | 33.55±0.81 |
| Low dose *S. marcrocarpon* | 5.96±0.40* | 14.23±0.90 | 42.00±3.00* | 23.88±0.94 | 57.00±2.78* | 33.90±0.30 |
| High dose *S. marcrocarpon* | 7.94±0.34 | 17.70±0.70* | 55.00±1.00* | 22.31±0.38 | 31.10±18.66* | 32.17±0.75* |
| Control group | 7.56±0.29 | 15.00±0.82 | 44.67±2.89 | 19.84±0.45 | 54.33±0.15 | 33.60±0.71 |
| f-value | 5.457 | 4.439 | 7.327 | 2.337 | 5.298 | 4.364 |
| p-value | 0.014 | 0.025 | 0.005 | 0.126 | 0.015 | 0.027 |

= *$p \leq 0.05$.

## 4. Discussion

Leaves of *Gnetum africanum* and *Solanum marcrocarpon* are local herbs used daily as remedies for certain ailments due to their antioxidant, anticarcinogenic, anti-inflammatory and haematinic properties Saka *et al*, [20]; Olusola *et al*, [21]. The present study evaluated and compared the effect of ethanolic extracts of *Gnetum africanum* and *Solanum marcrocarpon* on the haematological parameters and antioxidant activities of male Wistar rats.

The acute toxicity study conducted for *Gnetum africanum* showed that the rats were generally active and showed no visible signs of illness or toxicity after 48 hours of administration of single dose of *Gnetum africanum* up to 5000 mg/kg. No mortalities were recorded throughout the period of the experiment. $LD_{50}$ was greater than 5,000 mg/kg body weight of the rats. The absence of mortalities and no signs of toxicity in the rats suggest that the plant was well tolerated and safe. This is in accordance with the finding of Udeh *et al*, [22] who reported absence of mortalities and no signs of toxicity.

The acute toxicity study conducted for *Solanum marcrocarpon* showed that the rats were generally active and showed no visible signs of illness or toxicity after 48 hours of administration of single dose of *Solanum marcrocarpon* up to 5000 mg/kg. No mortalities were recorded throughout the period of the experiment. $LD_{50}$ was greater than 5,000 mg/kg body weight of the rats. The absence of mortalities and no signs of toxicity in the rats suggest that the plant was well tolerated and safe. This finding supports the previous report by Mbegbu *et al*, [23].

There was a significant (p= 0.036) decrease in the mean level of total antioxidant capacity in low dose *Gnetum africanum*(Group 2) when compared to Control group (Group 1) as seen in Table 4.1. However, high dose *Gnetum africanum* (Group3) significantly (p = 0.036) increased total antioxidant capacity when compared with the control group. This positive effect of high dose *Gnetum african* was dose-dependent. This agrees with the finding of Ezekwe *et al*, [24], who reported that *Gnetum africanum* is loaded with a host of important phytochemicals and has antioxidant properties which increase in potency with increase in dose.

Low dose *Solanum marcrocarpon*(Group 4) significantly(p=0.036) decreased the total antioxidant capacity compared with Control. While high dose of *Solanum marcrocarpon*(Group 5) significantly increased the mean level of total antioxidant capacity when compared to the Control group. The present study's findings indicated that the ethanolic leaf extract of *S. macrocarpon possessed* antioxidant properties and may be effective against oxidative stress. Secondly, the increase in total antioxidant capacity indicates that the antioxidants in *S. marcrocarpon* were absorbed and it improved in vivo antioxidant defense status. The main function of antioxidants is to protect the body against the destructive effects of free radicals damage Marques *et al,* [25]. Free radicals may be generated in cells and tissues from internal (such as inflammation, diseases or metabolism) or external sources (irradiation, pollution, food, drugs), or as a consequence of decreased protective capacity Rice-Evans *et al,* [26]. In any case, an increase in free radicals production can originate oxidative damage Ghiselli *et al,* [27]. The result of this study is consistent with the reports of other studies by Adewale *et al,* [28] and Oyesola *et al*, [29].

In comparison, *Gnetum africanum* has a greater total antioxidant capacity than *Solanum marcrocarpon.*

The result revealed that low dose of *Gnetum africanum* insignificantly (p = 0.595) decreased the mean value of malondialdehyde(MDA) when compared to Control, however, at high dose, *Gnetum africanum* increased the mean value of MDA insignificantly (p = 0.595) when compared to Control. There was also an insignificant (p = 0.595) decrease in low dose of *Solanum marcrocarpon* compared to Control while at high dose, the mean level of MDA of the animals in group 4 was equal to that of the Control. This positive effect of high dose *Gnetum africanum* was dose-dependent.

In comparison, *Gnetum africanum* has greater effect on MDA than *Solanum marcrocarpon.* Increase in MDA suggests the occurrence of oxidative stress and lipid peroxidation as a mechanism of tissue damage. Serum Malondialdehyde (MDA) has been used as a biomarker of lipid peroxidation and has served as an indicator of free radical damage. Oxidative stress occurs when free radical production exceeds the antioxidant capacity of endogenous molecules such as glutathione, superoxide dismutase, thioreduxin and vitamin E. Lipid peroxidation results when fatty acids come into contact with reactive oxygen species (ROS), producing a series of reactive aldehydes, including MDA. While decrease in MDA as observed in low doses *Gnetum africanum* and *Solanum marcrocarpon* might be attributed to the high antioxidant activity of the extracts, mopping up free radicals in the body. This finding is in agreement with the works of Robert and Breyer-Brandwijk, [30]; Ebenyi *et al*, [10]. Antioxidants are important in the prevention of oxidative stress. "Antioxidants terminate chain reactions triggered by free radicals by removing free radical intermediates and inhibit other oxidation reactions" Nassiri and Hosseinzadeh, [31].

There was an insignificant (p = 0.648) increase in the mean value of superoxide dismutase(SOD) in low dose of *Gnetum africanum* compared to Control, however, at high dose, *Gnetum africanum* insignificantly increased the mean values of superoxide dismutase compared to Control. Whereas low dose of *Solanum marcrocarpon* insignificantly (p = 0.648) decreased mean level of superoxide dismutase and high dose of *Solanum marcrocarpon* insignificantly (p = 0.648) increased the mean value of superoxide dismutase compared to control. The effect of the extracts was observed in a dose-dependent manner.

In comparison, *Gnetum africanum* has more effect on SOD than *Solanum marcrocarpon*.

Increase in SOD indicates that the low and high dose of *Gnetum africanum* and high dose of *Solanum marcrocarpon* were capable of boosting enzyme activities i.e. increasing enzyme protein expression which suggests antioxidant capacity. This finding supports the report of Iheme *et al,* [32]. Decrease in SOD at low dose of *Solanum marcrocarpon* showed that continuous administration of the *S. marcrocarpon* at 500 mg/kg may induce oxidative stress.

There was a significant($p = 0.014$) decrease in the mean value of red blood cell of low dose of *G. africanum* when compared with Control but high dose of *G. africanum* showed a significant(0.014) increase when compared with Control. The positive effect of *G. africanum* was dose-dependent

Low dose of *S. marcrocarpon* significantly ($p=0.014$) decreased the mean level of RBC when compared to Control. However, high dose of *S. marcrocarpon* significantly ($p=0.014$) increased the mean value of RBC when compared to Control. The positive effect of *S. marcrocarpon* was dose-dependent.

Comparatively, *S. marcrocarpon* has more impact on RBC than *G. africanum.*

An increased mean value of RBC count indicates that the extracts *G. africanum* and *S. marcrocarpon* may possess haematinic properties. The result of this study is in accordance with the findings of Potterat [33]; Olajire and Azeez, [34] and Ufelle *et al,* [2] who reported significant increase in RBC count after administration of *S. marcrocarpon* extract. Excessive increase in RBC count signifies polycythaemia. Polycythaemia, also known as erythrocytosis, means having a high concentration of red blood cells in the blood. This makes the blood thicker and less able to travel through blood vessels and organs.

The significant ($p<0.05$) decrease observed in red blood cell may be due to saponins which are abundant bioactive compounds in *G. africanum a*nd *S. marcrocarpon.* As previously reported in the study of Ossamulu *et al*., [35], this may cause heamolysis of red blood cells Kar, [36]. Secondly, *G. africanum a*nd *S. marcrocarpon* have been reported to contain high amount of dietary fiber which have the ability to bind cations López and Martos, [37] such as iron. This may interfere with iron absorption which may reduce its bioavailability Reinhold *et al*., [38] thereby causing a decrease in red blood cells.

There was a significant ($p=0.025$) decrease in haemoglobin(HB) count in low dose of both *G. africanum a*nd *S. marcrocarpon.* However, there was a significant ($p=0.025$) increase in the mean value of (HB) count in high dose of both *G. africanum a*nd *S. marcrocarpon.* The observed haematological effects were dose-dependent since it occured at increasing dose of the extract.

Comparatively, *S. marcrocarpon* has greater effect on haemoglobin count than *G. africanum.*

The observed increase in haemoglobin indicates that the extract of *Gnetum africanum* and *S. marcrocarpon* may possess properties that mimic haematinic actions thus, may be used to correct anaemia Saka *et al,* [20]. The result of this study is in accordance with the finding of Ufelle *et al,* [2].

For the Packed Cell Volume (PCV), the result showed statistically significant (0.005) decrease in low dose of *G. africanum* and *S. macrocarpon* when compared to Control group and significant ($p=0.005$) increase in high dose of *G. africanum* and *S. macrocarpon* compared to Control group. The observed haematological effects were dose-dependent since it occured at increasing dose of the extracts.

In comparison, *S. macrocarpon* has more effect on PCV compared to *G. africanum.* Increase in packed cell volume as seen in Group 2 and Group 4 suggests that the extracts of *G. africanum* and *S. marcrocarpon* may have haematopoietic potentials. *G. africanum and S. marcrocarpon* have been demonstrated to constitute high level of minerals ($Fe^{2+}$, $Zn^{2+}$ and $Cu^{2+}$), vitamins (A, C, E, B) and phytochemicals such as polyphenols and flavonoids which are potent antioxidants Anosike *et al*, [39]. These antioxidants may have mopped up free radicals Aduwamai *et al,* [40] and the minerals and vitamins helped to increase haematopoiesis and erythropoiesis in the bone marrow (Olajire and Azeez, [34]. Iron is known as an important integral part of haemoglobin, myoglobin and cytochrome, while zinc is RBC-SOD co-factor which plays essential role in the synthesis of haemoglobin, protects the integrity of erythrocytes and reduces oxidative stress (Fukushima *et al*, [41]. The result of this study is consistent with the report of Olajire and Azeez, [34].

Significant ($p=0.015$) increase was observed in mean corpuscular volume in low and high dose of *G. africanum* and as well as in low dose of *S. marcrocarpon* while a significant decrease was seen in high dose of *S. marcrocarpon* when compared to control. The positive effect was dose-dependent.

Comparatively, *S. macrocarpon* has more effect on MCV compared to *G. africanum.*

High mean corpuscular volume (MCV), also known as macrocytosis, is associated with vitamin B12 and folic acid deficiency, liver disease, hyperglycemia, and other pathologic conditions (Maruyama *et al*, [42].

The low and high dose of *G. africanum* and low dose of *S. marcrocarpon* showed significant (p=0.027) increase in the mean value of mean corpuscular hemoglobin concentration (MCHC) whereas high dose of *S. marcrocarpon* significantly (p=0.027) decreased the mean value of mean corpuscular hemoglobin concentration. The observed effect was dose-dependent.

In comparison, *G. africanum* has greater impact on MCHC than *S. marcrocarpon.*

Increase in mean value of mean corpuscular hemoglobin concentration implies that the animals have higher than normal concentration of hemoglobin in their red blood cells. It can also occur in conditions where red blood cells are fragile or destroyed, leading to hemoglobin being present outside of the red blood cells. Mean corpuscular hemoglobin concentration is a measure of the average concentration of hemoglobin inside a single red blood cell. Low MCHC values occur as a result of anemia due to iron deficiency. It can also indicate thalassemia. This is an inherited blood disorder in which fewer red blood cells and less hemoglobin are present in the body. The result of this study is inconsistent with the report of a previous study by Anorue *et al,* [43], who reported insignificant decrease and increase in MCHC after three weeks administration of varying doses of *S. marcrocarpon.*

The observed no significant effect of the ethanolic *S. macrocarpon* and *G. africanum* extract on the MCH was similar to those of Sodipo *et al*. [44] and Duru *et al*. [45], and implies that the incorporation of haemoglobin into RBC as well as the morphology and osmotic fragility of the RBC were not altered. Thus, in addition, the ethanolic *S. macrocarpon* and *G. africanum* extract did not seem to have the potential to cause macrocytic anaemia in the treated animals.

In comparison, *S. marcrocarpon* has more effect on the MCH than *G. africanum.*

The observed insignificant effect of white blood cell and its differentials (granulocyte and lymphocyte) is not in line with the works done by Kolodziej and Kiderlen, [46] and Ufelle *et al*, [47] who reported significant increase in total white blood cell after administration of *G. africanum.* The difference between the result of this study and those of Kolodziej and Kiderlen, [46] and Ufelle *et al*, [47] may be due to different doses of extract administered or duration of administration. Increase in total white blood cell (leucocytosis) indicates stimulatory action on the immune system by the leaves extract of *Gnetum africanum and S. marcrocarpon.*

Comparatively, *S. marcrocarpon* has more effect on WBC and lymphocyte than *Gnetum africanum.* While *Gnetum africanum* has more positive effect on granulocyte than *S. marcrocarpon.*

The observed positive effects were dose-dependent.

The non-significant result observed in platelet count is not consistent with another similar study conducted by Ekweogu *et al,* [48] who reported significant increase in platelet count in all animals fed with *S. aethiopicum.*

In comparison, *G. africanum* has more effect on patelet count than *S. marcrocarpon*

## 5. Conclusion

The results of this study have shown that ethanolic extracts of *G. africanum* and *S. marcrocarpon* possessed antioxidant and haematinic properties and may be used in the management of oxidative stress and anaemia. The result showed that *G. africanum* has greater antioxidant activities than that of *S. marcrocarpon, and S. marcrocarpon* has more haematinic properties when compared to *G. africanum.*

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

There was no conflict of interest.

*Statement of ethical approval*

Ethical approval was obtained from the Research Ethics Committee of Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.

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