Haematological effects of *Gnetum africanum* leave extract in wistar rats

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ABSTRACT

**Background:** *Gnetum africanum* possess rich phytochemicals and numerous medicinal properties. Due to paucity of data on haematological effects, this study was designed. **Aim:** The aim was to investigate the haematological effects of *Gnetum africanum* leaves extract in Wistar rats. **Methods:** Acute toxicity test and phytochemical analysis of the extract was done using standard methods. Wistar rats (n=28), aged 2 to 3 months, weighing 150 to 200 grams were acclimatized for 2 weeks and grouped into 4 (A to D). Groups A, B, and C orally received graded-doses of the extract: (A=100, B=200 and C=300 mg/kg bodyweight) daily for 30 days. Group D served as control. Rat’s blood samples (3ml) were collected into ethylene diamine tetra acetic acid containers and analyzed using haematological auto analyzer (Sysmex KX-21N) following manufacturers guideline. **Results:** Acute toxicity test revealed an LD₅₀ of 3000 mg/kg bodyweight. Phytochemical analysis revealed flavonoids +++, alkaloids +, saponins +, tannins +, proteins ++, carbohydrates +, reducing sugars +, steroids ++, terpenoids +. Haematological analysis revealed significant increase (p<0.05) in group B: (Hb: 12.1±0.7g/dL, Hct: 0.36±0.02L/L, TWBC: 8.8 6±0.85 x 10⁹/L, RBC: 4.6±0.30 x 10¹²/L) and group C: (Hb: 12.6±1.5g/dL, Hct: 0.38±0.02L/L, TWBC: 9.48±1.5 x 10⁹/L, RBC: 4.95±0.31 x 10¹²/L) compared to group D (control): (Hb: 11.5±0.44g/dL, Hct: 0.34±0.08 L/L, TWBC: 5.8±0.35 x 10⁹/L, RBC: 4.02±0.43 x 10¹²/L). **Conclusion:** The results revealed dose-dependent significant increase in Hb, Hct, RBC and TWBC compared to control. These indicate possible stimulatory potentials of the extract for erythropoietin synthesis in the kidney to cause haemopoiesis.

**Key words:** *Gnetum africanum*, *G. buchholzianum*, erythropoietin, haematopoiesis, haematological parameters, phytochemicals

INTRODUCTION

Medicinal plants have remained a significant alternative source of drugs for majority of populations that experience inadequate contacts with orthodox healthcare facilities.[¹] This dependence on botanicals with medicinal activities is particularly common in developing countries where modern western medicines...
Gnetum africanum is a popular wild green leafy vegetable throughout tropics. It is seen in South Eastern Nigeria as an exotic plant. It belongs to the family of Gnetaceae and the order Gnetales.\[3\] Gnetum africanum can also be found in Angola, Congo, Gabon and Cameroon.\[5\] Gnetum africanum plant is a genus of about 30 species in the family Gnetaceae. “There are two species of Gnetum in Africa, G. africanum and G. buchholzianum, distributed in the humid tropical forests”.\[6\] These species are similar and are differentiated by the leaf shape and the character of the male reproductive part.\[6\] Leaves ovate-oblong or elliptical-oblong, opposite and simple, more rarely lanceolate, attenuate at base, abruptly acuminate, 10-13 cm long and 3.5-5 cm broad.\[6\]

Gnetum africanum is an edible plant and are locally useful in the management of splenomegaly, sore throat, childbirth pain, snake bites.\[6\] Some of the phytochemical constituents of the Gnetum africanum leaves extract include C-glycosylflavone, 2”O- rhamnoliosisoswertisin and apigenin-7-hesperidoside.\[7\]

Previous studies with other extracts revealed that Cryptolepis sanguinolenta stem ethanolic extract presents haematological challenges on white blood cells and platelets. It showed localized systemic toxicity by selectively stimulating the bone marrow\[8\] Chronic administration of Momordica charantia showed an improvement in the oral glucose tolerance curve.\[9\] Watermelon juice with sufficient consumption favour adequate bodyweight, deliver appropriate body nutrients: suggesting possibility of combating both infectious and non-infectious diseases.\[10\]

There is paucity of data on the haematological effects of leaves extract of Gnetum africanum. This present study adopted the experimental design to investigate the haematological effects of Gnetum africanum in Wistar rats. The specific objectives were to determine the acute toxicity (LD 50) of the leaves extract of Gnetum africanum in Wistar rats, the phytochemical constituents of the leaves extract of Gnetum africanum and the haematological parameters (complete blood count) of Wistar rats after oral administration of graded-doses of the leaves extract of Gnetum africanum.

**METHODOLOGY**

Collection of plant materials

The leaves of Gnetum africanum were obtained from the forest in Ajalli town, Orumba North Local Government Area, Anambra State, Nigeria for the study. It was authenticated by a taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka Campus. Specimen sample was kept in the herbarium for future reference with voucher number of UNH No 3\[3\]. Note: UNH means University of Nigeria Herbarium.

Animal housing

Male Wistar rats (n=28) aged 2 to 3 months and weighing 150 to 200 grams were purchased and housed in the Animal House of College of Medicine, University of Nigeria Enugu Campus. The rats were acclimatized for two weeks, fed with standard rat chow and had access to water ad libitum. Adult male mice (n=13) were also purchased for acute toxicity study. All the rats and mice were handled in the study according to International guidelines for handling experimental animals by American Physiological Society (APS).

Preparation of extract

Two hundred (200) grams of the grinded shade dried leaves of Gnetum africanum was extracted with methanol and the mixture sieved. The remaining methanol in the extract was evaporated to achieve a concentrated crude extract. Ten (10) grams of the concentrated crude extract was dissolved in 100 mL of distilled water to get a concentration of 100 mg/mL for use.

Acute toxicity test (Median lethal dose, LD 50)

This was performed on mice according to the procedure described by Lorke.\[11\] The LD 50 was performed in two stages. In the first stage, 3 groups with 3 mice each were treated with 10, 100 and 1000 mg/kg bodyweight of the extract and observed for 24 hours for the number of deaths. In the second stage, 4 mice were treated with 1500, 2000, 2500 and 3000 mg/kg bodyweight of extract based on the percentage survival rates. The LD 50 was calculated as the geometric mean of the...
highest non-lethal and the lowest lethal doses.\[11\]

**Phytochemical analysis**

Phytochemical analysis of the crude methanolic extract of *Gnetum africanum* leaves were done in the Department of Pharmacognosy, University of Nigeria, Nsukka, Nigeria with the method described by Ioan, Ciulei.\[12\]

**Experimental design**

Wistar rats (n=28) were divided into 4 groups of 7 rats per group, labeled A to D. Groups A, B and C were orally administered with graded-doses of the crude extract (100, 200 and 300) mg/kg bodyweight respectively daily for 30 days. Group D served as control and orally received only feed and water *ad libitum*. On day 31, 3.0 ml of blood samples were collected from each rat through the retro-obital plexus of the median canthus of the eyes with capillary tube into tri-potassium ethylene diamine tetra acetic acid (K3-EDTA) anticoagulant containers for the haematological analysis using haematological auto analyzer (Sysmex KX-21N) following manufacturers guideline.

**Statistical analysis**

The statistical package for social science computer software (SPSS) version 20 was used for data analysis. Analysis of variance (ANOVA) and student’s t-test were done at 95% confidence interval with p-value of ≤0.05 being considered as significant.

**RESULTS**

The acute toxicity test of the leaves extract of *Gnetum africanum* revealed an oral LD50 of 3000 mg/kg bodyweight. The phytochemical analysis of the leaves extract of *Gnetum africanum* was as shown in table 1. The results revealed flavonoids ++++, alkaloids +, saponins +, tannins +, proteins ++, carbohydrates +, reducing sugars +, steroids ++ and terpenoids +. The mean and standard deviation of haematological parameters of test and control Wistar rats after oral administration of graded doses of crude methanolic extract of *Gnetum africanum* leaves were as shown in table 2. Wistar rats in group A revealed no significant difference (p > 0.05) in haematological parameters when compared with group D (controls). Groups B and C revealed dose dependent significant increase (p < 0.05) in haemoglobin, haematocrit, total white blood cell and red blood cell count when compared with group D (controls). The differential WBC counts were not significant when compared with group D (controls).

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Antraquinone glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Anthracene glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
</tr>
<tr>
<td>Proteins</td>
<td>++</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis test for glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Cyano-genetic glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Fat and oils</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: (-) Absent, (+) Present, (++), Moderately present, (+++) Abundantly present
Table 2: The mean and standard deviation of haematological parameters of test and control Wistar rats after oral administration of graded-doses of crude methanolic extract of *Gnetum africanum* leaves

<table>
<thead>
<tr>
<th>Group/Variables</th>
<th>A 100mg/kg&lt;sup&gt;bd&lt;/sup&gt; wt</th>
<th>B 200mg/kg&lt;sup&gt;bd&lt;/sup&gt; wt</th>
<th>C 300mg/kg&lt;sup&gt;bd&lt;/sup&gt; wt</th>
<th>D Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>11.7 ± 1.2</td>
<td>12.1 ± 0.7 *</td>
<td>12.6 ± 1.5 *</td>
<td>11.5 ± 0.44</td>
</tr>
<tr>
<td>Hct (L/L)</td>
<td>0.35 ± 0.01</td>
<td>0.36 ± 0.02 *</td>
<td>0.38 ± 0.02 *</td>
<td>0.34 ± 0.08</td>
</tr>
<tr>
<td>RBC (x 10&lt;sup&gt;12&lt;/sup&gt;/L)</td>
<td>4.38 ± 0.20</td>
<td>4.6 ± 0.30 *</td>
<td>4.95 ± 0.31 *</td>
<td>4.02 ± 0.43</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.43 ± 1.7</td>
<td>33.61 ± 0.5</td>
<td>33.16 ± 0.4</td>
<td>33.82 ± 0.8</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>26.71 ± 0.6</td>
<td>26.30 ± 0.3</td>
<td>25.45 ± 0.5</td>
<td>28.61 ± 0.2</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>79.91 ± 1.5</td>
<td>78.26 ± 2.1</td>
<td>76.77 ± 1.8</td>
<td>84.58 ± 1.2</td>
</tr>
<tr>
<td>TWBC (x10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>6.25 ± 1.3</td>
<td>8.86 ± 0.85 *</td>
<td>9.48 ± 1.5 *</td>
<td>5.8 ± 0.35</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>50 ± 3.0</td>
<td>53 ± 3.0</td>
<td>56 ± 2.0</td>
<td>55 ± 3.0</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>47 ± 1.0</td>
<td>45 ± 2.0</td>
<td>40 ± 2.0</td>
<td>42 ± 2.0</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>2 ± 1.0</td>
<td>1 ± 1.0</td>
<td>2 ± 1.0</td>
<td>2 ± 1.0</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>1 ± 1.0</td>
<td>1 ± 1.0</td>
<td>2 ± 1.0</td>
<td>1 ± 0.5</td>
</tr>
</tbody>
</table>

Key * - p < 0.05 (Significant)

Abbreviations: Hb: Haemoglobin, Hct: Haematocrit, RBC: Red Blood Cell, MCHC: Mean Cell Haemoglobin Concentration, MCH: Mean Cell Haemoglobin, MCV: Mean Cell Volume, TWBC: Total White Blood Cell, g/dL: Gram per decilitre, L/L: Litre per Litre, Pg: Picogram, FL: Femtolitre

**DISCUSSION**

*Gnetum africanum* is an edible plant that possesses numerous medicinal properties. Available literature reveals that both the leaves and the seeds extract of *Gnetum africanum* have shown medicinal potentials in the management of splenomegaly, sore throat,Labour pain, snake bites and diabetes.[13,14,15] Little or no haematological effects have been documented on *Gnetum africanum* collected from our locality. Due to this paucity of data on haematological effects of *Gnetum africanum*, the present study was designed.

The acute toxicity test of the leaves extract of *Gnetum africanum* revealed an oral LD<sub>50</sub> of 3000 mg/kg bodyweight. This high LD<sub>50</sub> indicates that the extract is not toxic and is safe for consumption. The revealed phytochemical constituents suggest that the extract possess numerous medicinal properties.[16]

Some of the haematological parameters (haemoglobin, haematocrit, total white blood cell and red blood cell) were significantly increased in groups B and C Wistar rats when compared with the control. The observed haematological effects were dose-dependent since it occurs at increasing dose of the extract. The observed increase in haemoglobin and haematocrit indicates that the extract of *Gnetum africanum* may possess properties that mimic haematinic actions thus can be used to correct anaemia.[17] The observed increase in total white blood cell (leucocytosis) indicates stimulatory action on the immune system by the leaves extract of *Gnetum africanum*. Hence the consumption of this extract may be helpful in body defence to fight and protect against invaders. The observed increase in red blood cells count indicates stimulatory action on erythropoietin synthesis by the kidney and/or bone marrow for haematopoiesis (erythropoiesis).[18] The observed haematological effects may be attributed to some of the phytochemical constituents of the *Gnetum africanum* leaves extract like flavonoids, tannins, terpenoids, saponins, proteins and steroids.

“Flavonoids and tannins are phenolic compounds, and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.”[20] “Similarly, terpenoids, as vitamins, act as regulators of metabolism and play a protective role as antioxidants.”[21] The antioxidant activity of the botanical has made it useful in the management of various diseases. Antioxidants are important in the prevention of oxidative stress. “They terminate chain reactions triggered by free radicals by removing free radical intermediates and inhibit other oxidation reactions.”[22] There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. There...
is evidence of the presence of saponins in traditional medicine preparations.\textsuperscript{[23]} Tannins have been reported to be used in the management of poisons from poison oak or bee stings.\textsuperscript{[24]} They have also shown potential antiviral, anti-bacterial and anti-parasitic effects.\textsuperscript{[25]}

**CONCLUSION**

The results revealed dose-dependent significant increase in Hb, Hct, RBC and TWBC when compared with the control. This may be attributed to some of the phytochemical constituents of the extract that have antioxidant effects and can support cell growth through protein synthesis. The observed effects indicate that the extract may possess stimulatory potential on erythropoietin synthesis in the kidney to cause haematopoiesis. Further studies such as fractionation of the extract using gas chromatography-mass spectrometry (GC-MS)\textsuperscript{[26]} is recommended in order to characterize the leaves extracts and determine the active components.

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**REFERENCES**


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