Haematinic and haematopoietic potentials of methanolic extract of Citrullus lanatus (Watermelon) seeds in experimental rats

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Abstract

Anaemia is thought to afflict 30% of women between the ages of 15 and 49, 40% of pregnant women, and 37% of children aged 6 to 59 months worldwide. Haematinic and haematopoietic effects of Citrullus lanatus seeds extract (CLSE) was investigated in albino rats. Twenty-five rats were grouped into 5, labeled A to E. Naïve groups (C and D) were not induced for anaemia while anaemic groups (A and B) were intra-peritoneally induced for anaemia with 0.1 mg/kg of phenylhydrazine for one week before commencement of extract administration. Groups (A–B) and (C–D) were orally administered with graded-doses of CLSE (250–500 mg/kg) respectively for 14 days. Group E served as normal control. Blood sample (5 mL) was collected from each rat on days 8 and 15, 3.0 mL dispensed into ethylene diamine tetraacetic acid containers for estimation of haematological parameters. Day 8 revealed a significant decrease and increase in haemoglobin (Hb), haematocrit (Hct), red blood cells (RBC) of group A and group D respectively, when compared to group E (p<0.05) respectively. The neutrophil of groups A and B decreased significantly when compared to the control (p<0.05). The lymphocytes of groups A and B increased significantly when compared to the control (p<0.05). The monocyte and eosinophil of group A increased significantly when compared to the control (p<0.05). The lymphocytes of group A significantly increased when compared with the control (p<0.05). The seed extracts of Citrullus lanatus demonstrated dosage and time-dependent haematopoietic and haematinic potentials in normal and anaemic albino rats.

Keywords

Citrullus lanatus seeds, haematinic, anaemia, haematological parameters, red blood cells, white blood cells.
1. Introduction
Blood is a very important tissue in the human body and plays a vital role in human physiology. It is lifesaving and made up of different components which include red blood cells, white blood cells, platelets and plasma [1]. These hematological parameters include red blood cells (RBC) count, white blood cell (WBC) count, mean cell volume (MCV), mean cell hemoglobin (MCH), packed cell volume (PCV), hemoglobin (Hb) concentration, mean cell hemoglobin concentration (MCHC) and platelets counts [2]. They serve as different clinical indicators for health and disease conditions.

Anaemia is one of the most common clinical conditions characterized by a decrease in the level of circulating hemoglobin, less than 13g/dl in males and 12g/dl in females. Hematinic involve essential substance needed for the normal process of erythropoiesis and include iron, copper, vitamins and so on. Deficiency and lack of these substances are often associated with anemia and defective erythropoiesis [3].

Over the years, plants whether herbs, shrubs or trees, in parts or a whole, have been used or involved in the treatment and management of several diseases and disorders [4]. Due to poverty, lack of information and under-development in Nigeria and other African countries, the use of medicinal plants and herbs have played a major and leading role in the various therapeutic needs of the people and this has often led to the indiscriminate use and application of these local plants or herbs in treatment and management of ailments, even without adequate and sufficient clinical and laboratory experiments to assess this plants in case of their toxicity or ineffectiveness [5].

Watermelon (Citrullus lanatus) is an important and popular cucurbit crop that is grown and cultivated globally but with its origin in southern Africa (Fig. 1). They belong to the family Cucurbitaceae and have three subspecies namely C.lanatus lanatus (citron watermelon), C.lanatus mucospermus (egusi watermelon) and C.lanatus vulgaris (dessert watermelon), and provide nutritional compounds and health promoting amino acids [6]. Citrullus lanatus seed was reported to play a major and vital role in anti-inflammatory, antimicrobial, anti-prostate hyperplasia, anti-diabetic activities and so on [7].

Anaemia has greatly contributed to increase in mortality and morbidity among children and pregnant women. It involves insufficient red blood cells that aid in the transportation of oxygen in the body. It is a good sign of most basic pathological conditions and is prevalent in developing countries like Nigeria and Africa in general. Herbal medicine is a very good alternative source of medicine and contributes to the health needs of people in rural areas. In different parts of the world today, plants are becoming a vital and/or alternative source of drugs or therapeutic remedies for people who lack access to orthodox healthcare facilities and care [8]. Infectious diseases account for majority of deaths in low and medium income countries. Citrullus lanatus is a popular crop that contains bioactive compounds that have been reported to play a vital role in curing numerous sickness and ailments. Nevertheless, the lack of sufficient scientific and laboratory research and experiments to assess and manage the side-effects and toxicity attributed to these plant extracts provide room for more research work. The study aimed to investigate the haematinic and haematopoietic potentials of methanol extract of Citrullus lanatus seeds in albino rats.

Figure 1: Citrullus lanatus fruit and its seeds

2. Materials and methods
2.1 Collection of plant materials
The seeds of Citrullus lanatus were obtained from the fruits purchased at Ogbete market, Enugu North Local Government Area, Enugu State, Nigeria between the month of November and December 2021. The seeds were authenticated by a taxonomist at the Department of Plant Science and Biotechnology;
University of Nigeria Nsukka and a voucher specimen were kept in the herbarium for future reference. The herbarium reference Number of Citrullus lanatus is UNN/PSB/Consult/2017/2721-03.

2.2 Reagents and Chemicals
Drabkin’s solution, Turk’s Solution, Ammonium oxalate solution., Formal citrate Solution., Leishman type of Romanowsky stain, Phenylhydrazine solution, and Methanol were purchased from Alpha Pharmaceuticals in Enugu, Nigeria.

2.3 Animal housing
Twenty five (25) albino rats weighing between 150 to 200g, aged 2 to 3 months, were purchased and housed in the Animal House of the College of Medicine, University of Nigeria Enugu Campus. They were allowed to aclimatize for two weeks and fed with standard pellets (Guinea feed® Nigeria PLC). They had access to water and feed *ad libitum*. The rats were kept at a temperature of 28-32°C with dark light (13:11) cycle. They were kept in stainless steel wire mesh cages which were perforated beneath for the exit of the rats’ feaces to prevent coprophagy. All the rats were handled in the study according to International guidelines for handling experimental animals by American Physiological Society (APS). Ethical approval for the study was given by the Ethics Committee of the Faculty of Veterinary Medicine, University of Nigeria (Approval number: UNN/eTC/14/654672).

2.4 Preparation of extracts (methanol extraction)
Three hundred grams (300g) of the ground, shade-dried, powdered seeds of Citrullus lanatus were soaked in 2.5 litres of methanol for 48 hours with two-hourly vigorous shaking. The mixture was filtered through Whatman No.1 filter paper and evaporated to dryness on a rotary evaporator (Model 349/2 Carting Ltd). The concentrate (6.5% yields) was stored at 4°C until needed.

2.5 Phytochemical analysis
This was done in the Department of Pharmacognosy, University of Nigeria, Nsukka, Nigeria, according to standard and previously described methods [8-11].

2.6 Acute toxicity test: (median lethal dose, LD₅₀)
This was performed on mice according to the procedure described by Lorke, [12].

2.7 Study design
The study adopted the experimental design. Albino rats (n=25) were used in this study to examine the haemopoietic activity of methanol extracts of Citrullus lanatus seeds. Phytochemical analysis was also done on the methanol extract to reveal the phytochemical constituents. Albino rats (n=25) were divided into 5 groups of 5 rats per group, labelled A to E. Groups (A–B) were orally-induced for anaemia with 10 mg/kg of phenyl hydrazine for 7 days before oral administration of the extracts. Groups (A–B) and (C–D) were orally administered with graded-doses (250–500) mg/kg of the extract for 2 weeks while group E served as control without extract. (Fig. 2).

2.8 Sample collection
Blood sample (2.0 mL) was collected from each rat via the retro-bulber plexus of the medial canthus vein on days 8 and 15 into K₂EDTA anticoagulant bottles for estimation of haematological parameters (complete blood count).

2.9 Analytic methods
Haemoglobin estimation was done using cyanmethaemoglobin Method [13]. The haematocrit (PCV) values were estimated by the microhaematocrit method [13].

2.10 White blood cell counts (Visual method)
White blood cell count was done by the method described by Dacie and Lewis [13].

2.11 Differential leucocyte count
The differential count of the leucocytes was done using a Romanowsky-type stain, the Leishman method as described by Dacie and Lewis [13].

2.12 Platelet count
Platelet count of whole blood was done according to the method of Lewis et al [13].

2.13. Red blood cell count
Red blood cell count was done through visual method according to Dacie and Lewis [13].

2.14 Absolute red blood cell indices: calculation [13]
MCHC = Hb/PCV x100 (g/dL or %)
MCH = Hb/RBC (pg)
MCV = PCV/RBC (fL)

2.15 Statistical analysis
Data were subjected to inferential statistics and analysed using student’s t-test and one-way analysis of variance at 95% confidence interval. Bonferron’s Multiple Comparison Test was done to determine patterns of significance on group-to-group bases. Probability value less than or equal to 0.05 was considered statistically significant.
3. Results

The acute toxicity test revealed oral LD50 of 4950mg/kg body weight.

The phytochemical analysis of *Citrullus lanatus* seeds revealed the following phytochemical constituents: alkaloids, steroids, glycosides, saponins, flavonoids, terpenoids and tannins (Table 1).

Table 1. Phytochemical constituents of seeds extract of *Citrullus lanatus*

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Inference</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Present</td>
<td>+++</td>
</tr>
<tr>
<td>Steroids</td>
<td>Present</td>
<td>++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Present</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Present</td>
<td>+++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Present</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
<td>++</td>
</tr>
</tbody>
</table>

Key: +++ = present in high concentration; ++ = present in moderate concentration; + = present in low concentration.

The mean ± standard deviation of haematological parameters of albino rats after oral administration of graded doses of crude methanolic extract of *Citrullus lanatus* seeds on day 8 revealed: a significant increase in haemoglobin (Hb) of non-induced group D (12.3 ± 0.5 g/dL) when compared to group E control (11.0 ± 0.6 g/dL) (p < 0.05), a significant increase in haematocrit (Hct) of non-induced group D (0.37 ± 0.01 L/L) when compared to group E control (0.35 ± 0.02 L/L) (p < 0.05), a significant increase in red blood cells (RBC) of non-induced group D (3.55 ± 0.11 x10^{12}/L) when compared to group E control (3.20 ± 0.41 x10^{12}/L) (p < 0.05). A significant decrease in anaemia-induced groups A of haemoglobin (Hb) (10.5 ± 0.5), haematocrit (Hct) (0.32 ± 0.01), and RBC (3.01 ± 0.66), when compared to their control group E (11.0 ± 0.6), (0.35 ± 0.02), (3.20 ± 0.41), respectively. The neutrophil of anaemia-induced groups A (49 ± 2 %) and B (51 ± 4 %) decreased significantly; when compared to the control (59 ± 3 %) (p < 0.05). The lymphocytes of anaemia-induced group A (54 ± 6 %) and B (52 ± 3 %) increased significantly; when compared to the control (38 ± 3 %) (p < 0.05). The monocyte and eosinophil of anaemia-induced group A (2 ± 1 %) and (3 ± 4 %) increased significantly; when compared to the control (1 ± 0.5 %) and (2 ± 0.5 %) (p < 0.05) respectively, (Table 2).

The mean ± standard deviation of haematological parameters of albino rats after continued oral administration of graded doses of crude methanolic extract of *Citrullus lanatus* seeds on day 15 revealed: a significant increase (p < 0.05) in haemoglobin (Hb) of non-induced groups C (12.6 ± 0.3 g/dL) and D (13.8 ± 0.5 g/dL); when compared to the control group E (11.0 ± 0.6 g/dL), a significant increase (p < 0.05) in haematocrit (Hct) of non-induced groups C (0.37 ± 0.01 L/L) and D (0.39 ± 0.01 L/L); when compared to
the control group E (0.35 ± 0.02 L/L), a significant increase (p < 0.05) in red blood cells (RBC) of non-induced groups C (3.55 ± 0.14 x10^12/L) and D (4.02 ± 0.11 x10^12/L); when compared to the control group E (3.21 ± 0.41 x10^12/L). Total white blood cell (TWBC) of non-induced group D (14.2 ± 0.5 x10^9/L) increased significantly; when compared with the control (12.1 ± 4.06 x10^9/L) (p < 0.05). Lymphocyte of anaemic group A (44 ± 3 %) significantly increased (p < 0.05); when compared with the control (38 ± 3 %), (Table 3).

4. Discussion
The acute toxicity studies, which were designed to determine the dose that will produce mortality or serious toxicological effects when given once or over a few administrations over a short period were carried out. The test revealed high acute toxicity (LD50 of greater than 4959 mg/kg) which indicates that Citrullus lanatus is not toxic, non-poisonous and is suitable for consumption. Other researchers got similar results of the acute toxicity during their own research, for instance, according to Omoboyowa, et al. [14], in their research of protective effects of methanol extract of Citrullus lanatus on paracetamol-induced hepatotoxicity in adult Wistar rats, found the acute toxicity of Citrullus lanatus to be 5000mg/kg, agreeing to its edibility.

A phytochemical examination was carried out on the extract to reveal the non-nutritional bioactive compounds. We observed a significant number of

Table 2. Haematological parameters of albino rats on day 8

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>A (250 mg/kg)</th>
<th>B (500mg/kg)</th>
<th>C (250mg/kg)</th>
<th>D (500mg/kg)</th>
<th>E Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaemic</td>
<td>10.5 ± 0.5*</td>
<td>11.4 ± 0.6</td>
<td>11.6 ± 0.7</td>
<td>12.3 ± 0.5*</td>
<td>11.0 ± 0.6</td>
</tr>
<tr>
<td>Non-anaemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct (L/L)</td>
<td>0.32 ± 0.01*</td>
<td>0.34 ± 0.01</td>
<td>0.35 ± 0.01</td>
<td>0.37 ± 0.01*</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>RBC (x10^12/L)</td>
<td>3.01 ± 0.66*</td>
<td>3.22 ± 0.31</td>
<td>3.25 ± 0.14</td>
<td>3.55 ± 0.11*</td>
<td>3.20 ± 0.41</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>36.61 ± 0.55</td>
<td>33.10 ± 0.90</td>
<td>34.27 ± 0.35</td>
<td>33.13 ± 0.36</td>
<td>34.27 ± 0.35</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>35.77 ± 3</td>
<td>35.56 ± 2</td>
<td>31.43 ± 1.3</td>
<td>31.30 ± 2</td>
<td>31.43 ± 1.3</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>101.17 ± 20</td>
<td>93.10 ± 15</td>
<td>100.93 ± 25</td>
<td>95.99 ± 25</td>
<td>109.03 ± 25</td>
</tr>
<tr>
<td>Platelet (x 10^9/L)</td>
<td>257 ± 38</td>
<td>245 ± 54</td>
<td>265 ± 21</td>
<td>215 ± 42</td>
<td>261 ± 21</td>
</tr>
<tr>
<td>TWBC (x 10^9/L)</td>
<td>9.5 ± 2.0</td>
<td>9.6 ± 2.9</td>
<td>10.± 4.1</td>
<td>10.8 ± 2.0</td>
<td>12.1 ± 4.06</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>49 ± 2*</td>
<td>51 ± 4*</td>
<td>56 ± 3</td>
<td>57 ± 4</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>54 ± 6*</td>
<td>52 ± 3*</td>
<td>41 ± 3</td>
<td>42 ± 2</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>2 ± 1*</td>
<td>1 ± 0.5</td>
<td>1 ± 0.6</td>
<td>1 ± 0.8</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>3 ± 4*</td>
<td>2 ± 0.1</td>
<td>1 ± 1</td>
<td>2 ± 0.5</td>
<td>2 ± 0.5</td>
</tr>
</tbody>
</table>

Key: * (p < 0.05), b.wt (bodyweight), Hb (haemoglobin), Hct (haematocrit), RBC (red blood cells), TWBC (total white blood cells), MCH (mean cell haemoglobin), MCHC (mean cell haemoglobin concentration), MCV (mean cell volume).

Table 3: Haematological parameters of albino rats on day 15

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>A (250 mg/kg)</th>
<th>B (500mg/kg)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaemic</td>
<td>11.7 ± 0.6</td>
<td>11.9 ± 0.9</td>
<td>12.6 ± 0.3*</td>
<td>13.8 ± 0.5*</td>
<td>11.0 ± 0.6</td>
</tr>
<tr>
<td>Non-anaemic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hct (L/L)</td>
<td>0.35 ± 0.01</td>
<td>0.36 ± 0.01</td>
<td>0.37 ± 0.01*</td>
<td>0.39 ± 0.01*</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>RBC (x10^12/L)</td>
<td>3.26 ± 0.66</td>
<td>3.47 ± 0.31</td>
<td>3.55 ± 0.14*</td>
<td>4.02 ± 0.11*</td>
<td>3.21 ± 0.41</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>36.61 ± 0.55</td>
<td>33.10 ± 0.90</td>
<td>34.27 ± 0.35</td>
<td>33.13 ± 0.36</td>
<td>34.27 ± 0.35</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>35.77 ± 3</td>
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<td>11.± 4.1</td>
<td>14.2 ± 0.5*</td>
<td>12.1 ± 4.06</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>55 ± 4</td>
<td>56 ± 2</td>
<td>59 ± 2</td>
<td>60 ± 3</td>
<td>59 ± 3</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>44 ± 3*</td>
<td>37 ± 2</td>
<td>36 ± 3</td>
<td>38 ± 2</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>1 ± 0.5</td>
<td>1 ± 1</td>
<td>1 ± 0.6</td>
<td>2 ± 1</td>
<td>1 ± 0.5</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>2 ± 1</td>
<td>1 ± 1</td>
<td>2 ± 0.1</td>
<td>2 ± 0.5</td>
<td>2 ± 0.5</td>
</tr>
</tbody>
</table>

Key: * (p < 0.05), b.wt (bodyweight), Hb (haemoglobin), Hct (haematocrit), RBC (red blood cells), TWBC (total white blood cells), MCH (mean cell haemoglobin), MCHC (mean cell haemoglobin concentration), MCV (mean cell volume).
Day 15 revealed a significant increase in haemoglobin (Hb), haematocrit (Hct), and red cell blood (RBC) of non-induced group D significantly; compared to the control. This increase in haematological values suggests that *Citrullus lanatus* possesses haemopoietic potential. There was a significant decrease in the Hb, Hct and RBC of the anaemia-induced group A, when compared to their respective controls, this is due to the effect of the phenyl hydrazine (an anaemia-inducer) administered on the rats in these groups which have not been corrected. The lymphocytes of anaemia-induced groups A and B increased significantly, compared to the control. This shows the immunologic properties of this extract. The monocyte and eosinophil in anaemia-induced group A increased significantly, compared to control. The increase in eosinophil may probably indicate that the extract possesses anti-parasitic potential. On the other hand, other haematological parameters showed neither significant increase nor decrease.

Day 8 revealed increase in haemoglobin (Hb), haematocrit (Hct), and red cell blood (RBC) in non-induced group D significantly; compared to the control. This increase in haematological values suggests that *Citrullus lanatus* possesses haemopoietic potential. There was a significant decrease in the Hb, Hct and RBC of the anaemia-induced group A, when compared to their respective controls, this is due to the effect of the phenyl hydrazine (an anaemia-inducer) administered on the rats in these groups which have not been corrected. The lymphocytes of anaemia-induced groups A and B increased significantly, compared to the control. This shows the immunologic properties of this extract. The monocyte and eosinophil in anaemia-induced group A increased significantly, compared to control. The increase in eosinophil may probably indicate that the extract possesses anti-parasitic potential. On the other hand, other haematological parameters showed neither significant increase nor decrease.

Phytochemical constituents in *Citrullus lanatus* seeds extract which include alkaloids, steroids, glycosides, saponins flavonoids, terpenoids and tannins, indicating its pharmacological and medicinal properties, which show that the extract possesses medicinal properties and can be used as a herbal medicine [15-17]. Our phytochemical results showed similar patterns of presence of the phytoconstituents of methanol seed extracts of *Citrullus lanatus* in the study by Adunola et al. [18].

The study evaluated the haematinic properties of methanol extracts of the seeds of *Citrullus lanatus* in Phenylhydrazine-induced anaemic adult albino rats. The seed extracts of *Citrullus lanatus* demonstrated dosage and time-dependent haematopoietic and haematinic potentials in normal and anaemic albino rats. The presence of some phytochemicals together with the iron content in the seeds of *Citrullus lanatus* may be responsible for the observed haematinic activity of the extracts. This provides scientific justification for its use in African traditional medicine for the treatment of anaemia. The seed extracts need to be fractionated to pin-point the particular components responsible for the observed effects and then standardize the extract as a possible haematinic.

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**Conflicts of interest**
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**References**


