Haematopoietic potentials of methanolic extract of *Tetrapleura tetraptera* pods in male Albino rats

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ABSTRACT

Anaemia constitutes a major challenge in the livestock industry worldwide. This study was carried out to determine the haematopoietic potential of methanol extract of *Tetrapleura tetraptera* (*T. tetraptera*) in male albino rats. Twenty five rats weighing between 147 and 166 g were used for the study. Anaemia was induced by daily removal of 2ml of blood per 100g body weight for ten days. The extraction was by cold maceration. Thereafter, the animals were administered with the extract for seven days. The rats were treated with graded doses of the extract, 50mg/kg, 100mg/kg and 200mg/kg for groups A, B and C respectively while groups D (negative control) and E (positive control) received 5 ml/kg of water. Results showed successful induction of anaemia. There was significant (p<0.05) increases in the erythrocytic parameters and plasma protein after administration of the methanolic extract of *T. tetraptera*. The concentration dependent increase in the PCV, HBC, RBC, MCH and MCHC in the groups treated with the extract when compared with the untreated groups indicates haematopoietic potentials of *T. Tetraptera*.

Keywords: Anaemia, haematopoietic, rats, *Tetrapleura tetraptera*.

INTRODUCTION

Anaemia is a decrease below normal of the erythrocyte count and/or haemoglobin concentration. It is not a disease in itself, but a sign of disease (Coles, 1986; Fry & Mc Gavin, 2012). Such diseases may be infectious such as trypanosomosis and babesiosis or non-infectious due to starvation, trauma and neoplasia. The daily production of erythrocytes in healthy individuals equals their destruction with the result that circulating red blood cell (RBC) values are always within the normal range for the species. Anaemia occurs due to inability to maintain this equilibrium as a result of excessive destruction or loss (Coles, 1986; Mills, 2000; Fry & Mc Gavin, 2012). Anaemia is one of the major syndromes commonly encountered by veterinarians in practice especially in Africa due to the abundance of the causative agents in the environment, climatic factors that favour proliferation and survival of these agents and poor management practices that enable wider spread of infectious disease agents.

Clinical manifestations of anaemia include pallor of the mucus membranes of the mouth and pharynx, the conjunctivae and lips as well as cyanosis and dilation of peripheral vessels as occur in shock. Other signs include tachycardia and polypnea, mainly after exercise. There is also weakness and poor stamina as well as fever if caused by infectious agents especially bacteria (Coles, 1986; Mills, 2000). The severity of the anaemia is determined by the nature of blood loss. It is acute if a large volume of blood is lost within a short period of time as occurs in trauma and chronic when small amount of blood is lost over a long period of time due mainly to gastro-intestinal parasites, ectoparasites, ulcerations of the gastrointestinal tract and neoplasm. Also, anaemia can result from destruction of erythrocytes by protozoan organisms including trypanosomes, *babesia* organisms or reduced erythrocyte lifespan (Coles, 1986; Mills, 2000; Fry & Mc Gavin, 2012).

Destruction of red blood cells can be due to toxins,
chemicals, haemoparasites or immune reactions while decreased red blood cell production may be due to nutritional causes such as iron deficiency, lack of vitamin B12 and bone narrow neoplasm (Stone, 2000; Kerr, 2002).

In managing anaemia, it is important that the nature of each case of anaemia is ascertained and then characterized to enable a successful treatment. The characterization of anaemia depends on some basic data including haematologic data such as Packed cell volume (PCV), haemoglobin concentration (HBC), erythrocyte count, erythrocyte sedimentation rate, reticulocyte count, leucocyte and platelet counts and examination of stained blood films for such features as alterations of red cell morphology and presence of causative agents (Mills, 2000). Also, evaluation of bone marrow functions usually via biopsies, to ascertain whether the anaemia is due to bone marrow hypoplasia and toxicity, as well as its response to the anaemia and serum analysis to access the level of red blood cell destruction via determination of bilirubin concentration (Coles, 1986). Anaemia is classified based on the morphology of red blood cells in to macrocytic, microcytic and normocytic while aetiologic or pathogenetic classification is based on the cause of the anaemia whether through blood cell loss or destruction. The last classification is responsiveness and is based on the response of the bone marrow to anaemia or presence of immature red blood cells indicating capacity of the bone marrow to produce red blood cells. Herbal medicines are presently becoming popular in treatment of diseases because conventional medicines are expensive and associated with side-effects such as headaches, nausea, vomiting, diarrhoea and allergic reactions (Atawodi et al., 2014). Several plants have been found to exhibits haematinic effects (Muriithi et al., 2015; Jimmy & Ekpo, 2016).

Among such plants is *T. tetrapeta*. *Tetrapleura tetraptera* is a medicinal plant of the *Fabaceae* family. It is generally found in low land forest areas of tropical Africa. The fruits consist of a fleshy pulp with small brownish black seeds. It is used traditionally as spice and in management of convulsions, leprosy, inflammation and rheumatism (Ojewole & Adesina, 1983; Nwawu & Akali, 1986; Okwu, 2003; Aladesanmi, 2007). It also exhibited antibacterial activities as well as good wound healing effects (Ekwenny & Okorie, 2010; Effiong et al., 2014; Ogouma et al., 2015). It has also been shown to be very potent against larvae of *Anopheles gambiae* (Aina et al., 2009). The plant is rich in crude protein, iron, fat, crude fibre, energy, phosphorus, calcium, sodium, magnesium, manganese, copper and zinc, as well as certain amino acids (Jimmy & Ekpo, 2016).

However, while some reports recorded good haematopoietic potentials (Muriithi et al., 2015; Jimmy & Ekpo, 2016), others claimed that the plant is haemolytic (Odesanmi et al., 2010).

This study was therefore conducted to further evaluate the effects of the methanolic pod extract of *T. tetrapeta* on haemorrhagic anaemia in rats.

**MATERIALS AND METHODS**

Twenty five (25) male albino rats were used for the study. They were obtained from a laboratory animal house in Ossiosoma L.G.A. of Abia State. They were randomly divided into 5 groups of 5 rats, each. The rats were kept in metal cages in the laboratory animal house of the Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike (MOUAU). Abia State, Nigeria for two weeks, fed with Vital feed® (Nigeria) and water was provided *ad libitum*.

**ETHICAL APPROVAL**

Ethical approval for the use of animals in this research was sought and obtained from College of Veterinary Medicine Research Ethical Committee, MOUAU (MOUAU/CVM/REC/202118).

**INDUCTION OF ANAENIA**

After acclimatization, anaemia was induced by daily removal of 2ml of blood per 100g body weight for ten days (Ihedioha & Daniel-Igwe, 2014).

**COLLECTION AND IDENTIFICATION OF PLANT MATERIAL AND EXTRACTION**

Dried fruits of *T. tetrapeta* were purchased from a local market (Orie Ugba) in Umuahia, Abia State. The fruits were washed with distilled water and dried for 7 days. They were later ground into small coarse particles and stored in airtight containers prior to extraction. Extraction was by cold maceration. Ground fruit (500 grams) was soaked in one litre of absolute methanol and was agitated every 3 hours for 3 days (72 hours). It was filtered with Whatman’s filter paper no 1 into a beaker and oven dried at 39°C with a yield of 90.2g crude extract.

Yield was calculated as follows: Percentage yield = \( \frac{192g}{20.500g} \times 100 = 18.04g \)

**HAEMATOLOGICAL ANALYSES**

Haematological analyses were carried out immediately after collection of blood samples and standard procedures were followed for the assay of all parameters. Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (HBC) was determined by the Cyanomethaemoglobin method (Schalm et al., 1975; Coles, 1986). Red blood cell (RBC) and total white blood cell (WBC) counts were carried out by the haemocytometer method while the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae and the differential WBC count was...
determined by the Leishman method (Schalm et al., 1975; Coles, 1986).
The plasma protein was determined using a refractometer (VEEGEE CLX-1; Nova Tech, Culver City, USA).

**DETERMINATION OF BODY WEIGHT**
The animals were weighed using a weighing balance, Measure Tech (JYT 20, China).

**DATA ANALYSIS**
Data generated were subjected to analysis of variance (ANOVA) and variant means were separated using the least significant difference (LSD) method using SPSS statistical package version 16.0. Significance was accepted at P< 0.05.

**RESULTS**
The result of haematological parameters of rats after induction of anaemia is presented in Table I. After induction of anaemia, the mean PCV, HBC and RBC count of groups A, B, C and D were significantly lower (p< 0.05) when compared with group E (control). The mean corpuscular volume (MCV) of groups A - D were significantly higher (p< 0.05) when compared with group E (control). The mean corpuscular haemoglobin concentration (MCHC) of groups A, B and D were significantly lower (p< 0.05) when compared with groups C and E (control) and no significant difference (p> 0.05) between groups C and E. There were no significant (p> 0.05) variations in all the white blood cell values in all the groups. Also, there were no significant (p> 0.05) variations in the mean values of the plasma protein except group C that was significantly higher (p< 0.05) when compared with groups B and E (control). There were also no significant (p> 0.05) variations in the mean body weights of all the groups. After administration of the extract, the means PCV of all the groups were higher when compared with group D but only group A was significant (p< 0.05). The mean HBC values of groups C (14.87 ± 0.25) and D (14.69 ± 0.26) were lower when compared with other groups but were only significant (p< 0.05) when compared with groups A (15.95 ± 0.38) and B (15.09 ± 0.39). The means red blood cell count (RBC) of all the groups were higher when compared with control group E which was (7.46 ± 0.67). However, only group D

### Table I. Haematological profile and body weights of the rats after induction of anaemia.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean ± Standard error</th>
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<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>25.50 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HBC (g/dl)</td>
<td>8.88 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RBC (10&lt;sup&gt;6&lt;/sup&gt;/µl)</td>
<td>3.47 ± 0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>73.59 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>25.65 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.94 ± 0.88&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WBC (10&lt;sup&gt;3&lt;/sup&gt;/µl)</td>
<td>12.50 ± 0.49</td>
</tr>
<tr>
<td>Differential WBC count (10&lt;sup&gt;3&lt;/sup&gt;/µl)</td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>2.42 ± 0.25</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>9.86 ± 0.51</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>Basophil</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Protein (g/dl)</td>
<td>8.04 ± 0.21</td>
</tr>
<tr>
<td>Bodyweight (g)</td>
<td>153.16 ± 9.63</td>
</tr>
</tbody>
</table>

<sup>a</sup>Different superscripts in a row indicate significant difference between the groups (p<0.05).
The decreased values of PCV, HBC, RBC and MCHC indicate a macrocytic normochromic anaemia. The significant increase in the PCV, HBC, RBC, MCH and MCHC in the groups treated with the extract when compared with the untreated group (D) suggests that the extract possesses some haematopoietic potentials. The increase in the plasma protein content in the treated groups may be due to increase in food intake. The extract have some properties including zinc and amino acids which stimulate appetite as most drugs used in managing anaemia have been found to improve appetite in patients. That may also explain why the plant is used in postpartum medication among some locals in Nigeria.

**DISCUSSION**

The significant reduction in PCV, HBC and RBC in all the groups bled indicated that anaemia was successfully induced. This is in agreement with Ihedioha and Daniel-Igwe (2014) who reported daily removal of 2 ml of blood per 100g for 10 days for successful induction of anaemia with minimal or no mortality. The increase in MCV values after induction of anaemia indicates presence of macrocytes which are mainly reticulocytes (Coles, 1986; Kerr, 2002). This also shows bone marrow response to the anoxic condition created by the continual removal of blood (Chulilla, 2009). The decreased values of PCV, HBC, RBC and MCHC and increased MCV indicate a macrocytic normochromic anaemia. The significant increase in plasma protein among groups bled indicated that anaemia was successfully induced. The decreased bone marrow response to the anoxic condition created by the reticulocytes (Coles, 1986; Kerr, 2002). This also shows anaemia indicates presence of macrocytes. This is in agreement with Okorie-Kanu et al., (2017) that anaemia was successfully induced. The significant reduction in PCV, HBC and RBC in all the control. The MCV values of groups A - D were all higher when compared with group E but only group D was significant (p<0.05). There was also a significant increase in the mean plasma protein value when compared to the control. There were no significant (p> 0.05) variations in the MCH and MCHC, white blood cell. Also, there were no significant (p> 0.05) variations in the mean body weights of all the groups (Table II).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Group E</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>42.50 ± 0.57ab</td>
<td>42.00 ± 1.70ab</td>
<td>39.00 ± 1.00ab</td>
<td>38.30 ± 1.91b</td>
<td>39.25 ± 0.95ab</td>
</tr>
<tr>
<td>HBC (g/dl)</td>
<td>15.95 ± 0.38a</td>
<td>15.09 ± 0.39ac</td>
<td>14.87 ± 0.25b</td>
<td>14.69 ± 0.26b</td>
<td>15.52 ± 0.37bc</td>
</tr>
<tr>
<td>RBC (10⁶/µl)</td>
<td>6.70 ± 0.68ab</td>
<td>6.81 ± 0.38ab</td>
<td>6.68 ± 0.40ab</td>
<td>5.53 ± 0.22b</td>
<td>7.46 ± 0.67a</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>65.35 ± 6.03ab</td>
<td>62.09 ± 3.06ab</td>
<td>58.72 ± 2.04ab</td>
<td>69.56 ± 4.08a</td>
<td>53.53 ± 3.56b</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>26.22 ± 2.90</td>
<td>23.86 ± 1.32</td>
<td>22.50 ± 1.40</td>
<td>26.72 ± 1.18</td>
<td>28.35 ± 2.10</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>39.95 ± 1.14</td>
<td>38.44 ± 1.10</td>
<td>38.23 ± 1.37</td>
<td>38.65 ± 1.53</td>
<td>39.62 ± 1.44</td>
</tr>
<tr>
<td>WBC (10³/µl)</td>
<td>14.60 ± 1.52</td>
<td>15.05 ± 1.24</td>
<td>13.46 ± 0.43</td>
<td>13.53 ± 1.49</td>
<td>13.86 ± 0.95</td>
</tr>
</tbody>
</table>

**Table II. Haematological profile and body weight of the rats after 1 week administration of methanolic extract of Tetrapleura tetraptera pods.**

*Different superscripts in a row indicate significant difference between the groups (p<0.05). Legends: PCV - Packed cell volume; HBC - Haemoglobin concentration; RBC - Red blood cell; MCV - Mean corpuscular volume; MCH - Mean corpuscular haemoglobin; MCHC - Mean corpuscular haemoglobin concentration, WBC- White blood cell.*

(5.53 ± 0.22) was significantly (p< 0.05) lower than the control. The MCV values of groups A - D were all higher when compared with group E but only group D was significant (p< 0.05). There was also a significant increase in the mean plasma protein value when compared to the control. There were no significant (p> 0.05) variations in the MCH and MCHC, white blood cell. Also, there were no significant (p> 0.05) variations in the mean body weights of all the groups (Table II).
REFERENCES


Article history: Received: July 27, 2021, Revised: August 12, 2021 Accepted: August 13, 2021