Effect of Chloroform Leaf Extracts of *Portulaca oleracea* Linn. (Purslane) on Haematological Parameters in Albino Wistar Rats

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**Authors’ contributions**

This study was carried out in collaboration between all authors. Author VCO designed and carried out the study, performed the statistical analysis and wrote the manuscript. Authors HDK and GOA supervised the study, managed the analyses of the study and proofread the manuscript. All authors read and approved the final manuscript.

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**Original Research Article**

**ABSTRACT**

*Portulaca oleracea*, Linn. (Purslane), a widely eaten leafy vegetable in some parts of the world, is known to be rich in omega-3 fatty acids, vitamin E and C, and beta-carotene. It is frequently used in ethnomedicinal treatment of various ailments including anaemia. The dearth of information on the haematological properties of *Portulaca oleracea* which is used as staple vegetable and remedy for anaemia prompted this study. The study investigated the effect of the oral administration of chloroform leaf extracts of *Portulaca oleracea* (CLEPO) on haematological parameters in male albino wistar rats. Experimental animals were randomly divided into 4 groups of 16 rats each. Group A(Control) received 0.5 ml/kg bw of 20% Tween 80 (vehicle), Groups B, C & D received 125, 250 &
1. INTRODUCTION

The use of plants and herbs as food supplements and medicinal additives is fast gaining ground and recognition globally [1]. Medicinal plants are currently being used in various parts of the world especially in the tropics for the treatment of various forms of anaemia [2]. Fruits and vegetables are associated with the management of anaemia because they are rich in vitamins and minerals [3]. Consumption of green leafy vegetables is a major source of vitamins and micronutrients. Most vegetables and plants have been found to contain haematinich agents such as folic acid, vitamin B6, iron which could stimulate the erythropoietic pathway [4]. *Portulaca oleracea* has been reported to be used in the treatment of anaemia in humans [5].

*Portulaca oleracea* Lin. commonly known as purslane, a member of the family, Portulacaceae, is an important plant naturally found as a weed in farms and lawns [6]. It is a warm climate weed that is extensively distributed in the different parts of the world and can easily be referred to as a cosmopolitan weed. It is usually seen around wastelands, wayside, by the water bodies and in cultivated farms. It produces optimally in a well-drained moist soil but can be easily destroyed by continuous heavy rainfall and flood. Purslane has obovate leaves, small yellow flowers that open only in the morning, and branched succulent stems which are decumbent near the base and ascending near the top to a height of 15–30 cm [7]. It has different names in various ethnic groups in Nigeria. It is known as “Ntioke”, or “Idiridi” in Hausa and “Idiridi” in Yoruba; “Esan omode” or “Papasan” in Yoruba; “Babbajibi” or “Halshen saniya” in Hausa and “Eferemakara” in Efik [8,9].

In Nigeria, purslane is considered a weed but is widely accepted in some parts of the globe as a staple leafy vegetable where it is eaten fresh as salad, stir-fried, or cooked as spinach. It is one of the oldest leafy vegetables, used from Europe to Japan, Australia and the Americas [10]. It is eaten in many African countries, e.g. Côte d’Ivoire, Benin, Cameroon, Kenya, Uganda, Angola and South Africa. It is especially popular in Sudan and Egypt, where it is known in Arabic as ‘rigla’ [10]. It is popular as a “potherb” in many areas of Europe, Asia, and the Mediterranea region [6]. The Chinese folklore described it as “vegetable for long life” [11]. Purslane contains more omega-3 fatty acids, alpha-linolenic acid in particular than any other leafy vegetable [12]. It contains high levels of vitamin E and C, and beta carotene [13]. It has been described as a power food due to its high nutritive and antioxidant properties [14].

Despite the popular use of purslane as vegetable in different parts of the world, information on its haematological properties remains unknown. This study was therefore designed to investigate the haematological properties of chloroform leaf extracts of *Portulaca oleracea* (purslane) with a view to justifying or refuting its use in the treatment of anaemia.

2. MATERIALS AND METHODS

2.1 Plant Material and Authentication

Fresh leaves of *Portulaca oleracea* Linn. (Plate 1) were collected from Alakahia axis of Port Harcourt, Nigeria, from the months of December, 2017 to February, 2018. The plant was identified by Dr. Chimezie Ekeke of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria, and a
sample was deposited at the University of Port Harcourt Herbarium with the voucher number, UPH/V/1,302.

Plate 1. *Portulaca oleracea* Linn

2.2 Preparation of Plant Extract

After collection of the plant, the leaves were shade-dried at room temperature to constant weight over a period of six weeks. The dried leaves of *P. oleracea* were weighed and ground to fine powder. Successive solvent extraction by cold maceration was done for 72 hours as described by Harborne [15] using Chloroform of analytical grade. A 500 g portion of the pulverinized leaves of *P. oleracea* was macerated by soaking in 1.5 Litres of chloroform for 72 hours, with fresh replacement of solvent every 24 hours. The combined filtrate obtained by filtration with Whatman's No. 1 filter paper, was concentrated with rotary evaporator (Model No: RE-52A) at 45°C in vacuo and later transferred to an evaporating dish and dried over a water bath (Digital thermostatic water bath, Jinotech instruments) at 45°C. The dried chloroform leaf extract of *Portulaca oleracea* (CLEPO) obtained was stored in a desiccator.

2.3 Acute Toxicity Study

The acute toxicity of the extracts was evaluated according to the method of Lorke [16].

2.4 Animals

Sixty four (64) sexually mature male wistar rats weighing an average of 200 g, procured from the Animal House of Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria were used for the study. The rats were acclimatized for two (2) weeks before commencing the study. They were fed *ad libitum* with commercially sourced feed (Top Feeds Nigeria Limited) and supplied with clean drinking water all through the study.

2.5 Ethical Approval

The study protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt with the Ref. No: UPH/CEREMAD/REC/04. The rats for the study were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

Experimental Procedure

Following acclimatization, the animals were randomly assigned to four (4) groups of sixteen (16) animals each for treatment as follows:

- **Group A** : (Control) received 0.5 ml/kg body weight of 20% Tween 80 (vehicle).
- **Group B** : Received 125 mg/kg body weight of extract
- **Group C** : Received 250 mg/kg body weight of extract
- **Group D** : Received 500 mg/kg body weight of extract

Administration of extract and vehicle was by oral gavage daily for 60 days. Animals' weights were taken weekly and the dose adjusted accordingly. On days 14, 28, 42 and 60; four rats from each group were anaesthetized and blood samples were collected by cardiac puncture into EDTA bottles. The collected blood samples were used for the estimation of haematological parameters such as haematocrit, haemoglobin concentration, erythrocyte, leucocyte, platelets count and differential cell count according to Cheesbrough [17]. The erythrocyte, leucocyte and platelets counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according using the cyanomethaemoglobin technique. The haematocrit was determined by the microhaematocrit method. Differential leucocyte count was used to determine the distribution of the various white blood cells in the circulating blood.

2.6 Statistical Analysis

Statistical analysis was done using SPSS 21. All values were expressed as mean ± SEM and data were analysed by one-way ANOVA followed by
the Tukey post-hoc test. The significance level was set at p<0.05

3. RESULTS

3.1 Acute Toxicity Study

Acute toxicity test did not show any mortality, morbidity or other apparent signs of toxicity at the doses used. This showed that the extract was well tolerated at the maximum dose of 5000 mg/kg.

3.2 Effect of Portulaca oleracea Linn. on Haematological Parameters

Tables 1-4 show the haematological changes produced by different doses of CLEPO. The extract had no significant (p>0.05) variation in the haematocrit and haemoglobin levels during the 60-day study period with the exception of the group of rats treated with 500 mg/kg (group D) which showed a significant (p<0.05) decrease in the mean haematocrit level on day 28 relative to the control (Tables 1-2).

CLEPO had no significant (p>0.05) effect on the erythrocyte count, leucocyte count and differential leucocyte count of treated rats in comparison with the control on day 14, 28, 42 and 60 (Tables 3-6). The platelet count increased in all the treatment groups throughout the 60 days of treatment especially in 125 and 500 mg/kg treated rats (group B and D respectively) on day 60 where the increase was significant (p<0.05) relative to the control (Table 7) and (Fig. 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Haematocrit (%)</th>
<th>Duration</th>
<th>14 days</th>
<th>28 days</th>
<th>42 days</th>
<th>60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A (control)</td>
<td>42.75±1.80</td>
<td>40.25±1.03</td>
<td>39.75±1.03</td>
<td>44.25±0.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B (125 mg/kg)</td>
<td>41.75±0.25</td>
<td>38.75±1.11</td>
<td>37.55±2.93</td>
<td>45.50±0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C (250 mg/kg)</td>
<td>43.75±0.48</td>
<td>40.50±0.65</td>
<td>38.00±2.42</td>
<td>43.25±1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D (500 mg/kg)</td>
<td>40.00±1.58</td>
<td>34.75±1.97</td>
<td>40.00±0.82</td>
<td>43.24±1.65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a' indicates significant difference at p<0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Erythrocyte (X10^12/L)</th>
<th>Duration</th>
<th>14 days</th>
<th>28 days</th>
<th>42 days</th>
<th>60 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A (control)</td>
<td>7.00±0.41</td>
<td>6.00±0.00</td>
<td>6.00±0.00</td>
<td>6.25±0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B (125 mg/kg)</td>
<td>7.00±0.00</td>
<td>5.75±0.25</td>
<td>5.25±0.75</td>
<td>6.50±0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C (250 mg/kg)</td>
<td>7.25±0.25</td>
<td>6.00±0.00</td>
<td>5.50±0.50</td>
<td>6.00±0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D (500 mg/kg)</td>
<td>6.25±0.25</td>
<td>5.25±0.48</td>
<td>6.25±0.25</td>
<td>6.25±0.25</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a' indicates significant difference at p<0.05.
Table 4. Effect of chloroform leaf extract of *P. oleracea* (CLEPO) on leucocyte count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Duration</th>
<th>Leucocyte (×10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14 days</td>
<td>28 days</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>5.00±0.41</td>
<td>5.75±0.25</td>
</tr>
<tr>
<td>Group B</td>
<td>7.25±0.48</td>
<td>6.50±0.96</td>
</tr>
<tr>
<td>Group C</td>
<td>6.25±0.48</td>
<td>7.00±0.71</td>
</tr>
<tr>
<td>Group D</td>
<td>6.50±0.96</td>
<td>7.75±0.85</td>
</tr>
</tbody>
</table>

* Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript ‘a’ indicates significant difference at p<0.05

4. DISCUSSION

From the results of this study, *P. oleracea* leaf extract had no significant effect on the erythrocyte, leucocyte and differential leucocyte count as well as the haemoglobin level. No significant variation was observed in the haematocrit values in all the test groups throughout the 60-day *P. oleracea* leaf extract treatment period except on day 28 at the dose of 500 mg/kg (group D) where a significant decrease occurred. This decrease in haematocrit seems to be an incidental finding as the haematocrit value of this group on day 60 with the value of 44.25%, was 2nd to the highest among all the test groups. It is a known fact that a significant decrease in haematocrit with no significant effect on erythrocyte count and haemoglobin level is rather abnormal. It is worthy to note that red blood cells serve as carriers of haemoglobin which is the oxygen carrying pigment of the body and haematocrit is the measure of volume of red blood cells to the total blood volume. According to Schlam et al. [18], there is a direct correlation among haematocrit, haemoglobin level and erythrocyte count. Chineke et al. [19] had opined that elevation in haemoglobin value is an indication of elevated RBC count with a corresponding decline in circulating plasma volume. Thus, haematocrit, haemoglobin level and RBC count are used to screen for anaemia [20]. This result therefore shows that leaf extracts of *P. oleracea* had no significant effect on erythropoiesis and leucopoiesis. This finding agrees with an earlier study by Oyedeji and Bolarinwa [21] who reported that methanol extract of aerial parts of *P. oleracea* at the doses of 25, 50 and 75 mg/kg when administered to rats for 30 days, had no significant variation in the haematological parameters. Although there is variation in both the extracting solvents and plant parts used in the two studies, the results are similar. In the present study, elongation of the exposure period did not also affect the blood parameters which is an indication of the safety of *P. oleracea* as an edible plant.

![Graphical representation of platelet count of rats treated with 125, 250 and 500 mg/kg doses of Chloroform Leaf extract of *P. oleracea* (CLEPO) for 60 days](image)

* Experimental groups are compared with Group A (control). Superscript ‘a’ indicates significant difference at p<0.05
Table 5. Effect of chloroform leaf extract of *P. oleracea* (CLEPO) on lymphocyte and eosinophil count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lymphocyte (%)</th>
<th>Eosinophil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration</td>
<td>14 days</td>
</tr>
<tr>
<td>Group A (control)</td>
<td></td>
<td>65.00±3.00</td>
</tr>
<tr>
<td>Group B (125 mg/kg)</td>
<td></td>
<td>71.50±3.97</td>
</tr>
<tr>
<td>Group C (250 mg/kg)</td>
<td></td>
<td>68.25±3.84</td>
</tr>
<tr>
<td>Group D (500 mg/kg)</td>
<td></td>
<td>68.25±3.50</td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a' indicates significant difference at *p* < 0.05.*

Table 6. Effect of chloroform leaf extract of *P. oleracea* (CLEPO) on neutrophil and monocyte count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Neutrophil (%)</th>
<th>Monocyte (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration</td>
<td>14 days</td>
</tr>
<tr>
<td>Group A (control)</td>
<td></td>
<td>32.00±2.86</td>
</tr>
<tr>
<td>Group B (125 mg/kg)</td>
<td></td>
<td>26.00±3.34</td>
</tr>
<tr>
<td>Group C (250 mg/kg)</td>
<td></td>
<td>30.50±3.30</td>
</tr>
<tr>
<td>Group D (500 mg/kg)</td>
<td></td>
<td>31.00±4.02</td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript 'a' indicates significant difference at *p* < 0.05.*
Table 7. Effect of chloroform leaf extract of *P. oleracea* (CLEPO) on platelet count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Platelet (X10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duration</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Group A (control)</td>
<td></td>
</tr>
<tr>
<td>Group C (250 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>Group D (500 mg/kg)</td>
<td></td>
</tr>
</tbody>
</table>

*Results are given as mean ± SEM for 4 rats in each group. Experimental groups are compared with Group A (control). Superscript ‘a’ indicates significant difference at p < 0.05*.

The increased platelet count following the treatment with *P. oleracea* leaf extract in this study is unknown. Low platelet count is an indicator for abnormal skin and mucosal bleeding. This result therefore suggests that the extract did not cause any bleeding in the treated rats rather it stimulated the increased production of platelets from the bone marrow. The physiological and therapeutic actions of *P. oleracea* is attributed to the presence of the numerous bioactive substances such as flavonoids, alkaloids, coumarins, anthraquinone glycoside, cardiac glycoside, and omega-3 fatty acids.

5. CONCLUSION

Based on the findings of this study, it is concluded that *P. oleracea* leaf extracts as used in this study had no significant effect on haematological parameters; therefore may not be a good remedy for the treatment of anaemia. The absence of deleterious effect on the haematological parameters over a 60 day period seems to justify the use of *Portulaca oleracea* as a staple vegetable. It is recommended that further research should be carried out to explore the mechanism for the increased platelet production associated with *P. oleracea*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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