Effects of *Justicia carnea* Leave on Hematological Parameters in Albino Mice Carried Out in Mbingo Annex Hospital Laboratory in Bamenda, North West Region, Cameroon

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Authors’ contributions

This work was carried out in collaboration among all authors. Author ANA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NC managed the analyses of the study. Author NDM managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

This study is design to ascertain the hematological status in albino mice treated with *Justicia carnea* leave, which could aid in the treatment of anemia. The hematological parameters investigated include Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Platelets (PLTS), Hemoglobin (Hb), Red Blood Cells (RBCs), Pack Cell Volume (PCV) and White Blood Cells (WBCs). Nine (9) male albino mice of approximately the same weight 300 g were grouped into 3 groups that’s Negative Control (NC) Positive Control (PC) and
Test Control (TC), each made up of 3 mice. Negative control mice were given normal feed moistened with water. Positive control received diluted iron folate with normal feed, finally Test control mice were administered powdered leaves of Justicia carnea with normal feed for 7 days, after which blood samples were collected using EDTA tubes by exsanguination and run for hematological parameters using auto-hematological analyzer. The results of various groups were found to be; as there was a significant increase in RBCs, Hb, & PCV ($P=0.05$), between the groups. No significant change was observed on the MCV, MCH, and MCHC. Lymphocytosis was observed in all groups with mark difference in granulocytosis. TC had granulocytosis, NC showed normal granulocyte scores and the PC show granulocytopenia with a statistical difference of $P=0.0017$. A significant difference was seen in platelets between the groups $P=0.02$. This study shows that J. carnea leaves possess anti-anemic potential, lending credence to the use of these plant leaves in folk medicine for the management of hemolytic anemia would be helpful. Further research on the various phytochemicals of the plant should be done and also its toxicological aspects.

Keywords: Hematological parameters; albino mice; and justicia caenea.

1. INTRODUCTION

Anemia can be define as a decrease in the ability of the blood to carry oxygen due to a decrease in the total number of erythrocytes, a diminish concentration of Hb per erythrocyte or a condition of both [1]. Anemia is the most common blood disorders affecting about a third of the global population. Anemia increases the cost of medical care and lowers a person’s productivity through a decrease ability to work. When anemia comes on slowly, the symptoms are often vague and may include feeling tired, weakness, shortness of breath or poor ability to exercise. Anemia that comes quickly often has greater symptoms which may include confusion, feeling like one is going to pass out, loss of consciousness, or increase thirst. It’s classified based on the size of RBCs and the amount of hemoglobin in each cell. If the cells are small, it is microcytic anemia while if large, it is macrocytic anemia while if they are normal size, it is normocytic anemia [2]. Hematology refers to the study of the number and morphology of the cellular elements of the blood, the Red blood cells (erythrocytes), white blood cells (leucocytes), and platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease [3]. It has been reported that, ingestion of drugs can alter the hematological parameters [4]. They are good indicators of the physiological status of animals [5]. Therefore hematological parameters could be an important tool in the assessment of effect of drugs [6]. Medicinal plants have been documented as having beneficial properties used for the management of various ailments because they have been discovered to contain bioactive compounds called phytochemicals and secondary metabolites that protect humans against diseases, example of some documented plants used in the treatment of anemia condition include Coco nucifera, leaves extracts of Tectona grandis etc. The availability of some synthetic drugs used in the treatment of a specific disease is common but because of the high cost and side effects associated with their use, attention is currently been focused on the use of medicinal plant products in the management or prevention of various diseases. The genus Justicia, name after the 18 century by a Scottish botanist James Justice, belongs to the large family of Acanthaceae consisting of about 600 species of herbs and shrubs native to the tropics and subtropics. Justicia carnea is a flowering plant, widely distributed in various parts of Africa [1]. In Cameroon the shrubs of Justicia carnea are grown around homes which easily grow and propagate from stems cutting by pushing the stems 1-2 inches into the soil. A survey among the Bakweri population in Cameroon revealed that, the plant under study is locally called” Ewolamajia” meaning a plant that gives blood. The deep colour juice from the leaves of this plant is extracted either by soaking or boiling in water which can be drank as tea. This study will aid to establish whether individuals using these leaves as blood show improved hematological profile which can aid in the treatment of anemia. This study is design to ascertain its hematological status in albino mice treated with Justicia carnea leave.

2. MATERIALS AND METHODS

2.1 Study Area

Samples and specimens obtain were analyzed at Bingo Annex Hospital Bamenda Cameroon.
2.2 Plants Material

Fresh leaves of *Justicia carnea* was harvested from the Bambui locality (Tubah Sub Division, North West Region Cameroon). The leaves were removed from the stems, rinse in clean water and then dried in shadow for weeks. After drying, the leaves were grind to powder with the aid of a blender to obtain approximately 30 g. The powered leaves were stored in airtight container until use.

2.3 Animal Handling and Grouping

A total of nine (9) male albino mice of approximately the same weight 300 g were used in this research. All experimented animals were obtained from the research Biopham Centre Dschang. The animals were house in a plastic bowl rapped with net and were acclimatized. The animals were allowed free access to food and water. After acclimatization, the animals were grouped into 3, that’s Negative Control, Positive control and Test Control, each made up of 3 mice, using a random sampling technic. The Negative control mice were given 25 g of normal feed (that’s grind corn mixed with soya bean, fish & vitamin), moisten with water. Positive control mice received 4ml of diluted iron folate mixed with 25 g normal feed. Lastly, Test control mice were been administered 1-2 preparation of powered leaves of *Justicia carnea* and normal feed, of which 25 g of it were administered orally for 7 days.

3. HEMATOLOGICAL ANALYSIS

Hemoglobin (Hb), Red Blood Cells (RBCs), Pack Cell Volume (PCV), Platelets (PLTs), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and White Blood Cell (WBC) were analyzer and results printed by the aid of auto-hematological analyzer.

3.1 Collection of Blood Sample

After 7 days of administration, blood samples were collected by exsanguination and placed in EDTA tubes for Hematological studies.

3.2 Body Weight and Relative Organ Weight Determination

The percentage change in the body weight of the experimental animals was calculated as:

\[
\frac{\text{Final body weight} - \text{initial body weight}}{\text{Final body weight}} \times 100.
\]

Similarly after exsanguin, the liver and the heart were closely observed for goose pathological changes and then weighted by the aid of a balance and the relative organ weight were calculated as; (Relative organ weight= Organ weight [g] / Body weight [g]).

3.3 Statistical Analysis

The data collected were analyzed using SPSS (Statistical Package of Social Science) version 21. Differences in means were done using the least significant difference (LSD) at P= 0.05. The results were expressed as mean +- standard error of mean (S.E.M.).

The mean distribution of RBCs and Hb within the 3 study groups is presented in Fig. 1.

The mean distribution values of PCVs within the 3 study groups are also presented in Fig. 2.

The mean distribution values of PLTs within the 3 study groups are also presented in Fig. 4.

4. RESULTS

In this study *Justicia carnea* leaves induced changes in erythrocytes and related parameter profiles such as Hb in the study albino mice (Table 1 or fig. 1). Seven days after administration of the leave at the dose levels of approximately 25g, there were significant increase in erythrocytes and Hb levels. When comparing the test (T) to the negative control (NC) group, there was no statistical significance between their RBC values, despite the fact that their Hb levels significantly differed. When compared with the positive control (PC), the RBC and Hb values between the T and PC group were significantly different. Furthermore, With regards to PCV, there were statistical significant (\(P < 0.05\)) differences between the T group, negative and positive control groups (Table 2 or Fig. 2). In addition, Despite the randomization in occurrence of the MCV, MCH and MCHC values (as seen in Fig. 3 above), there was no statistical significant difference between the Mean ±standard deviation values within and between the three groups in this study (\(P > 0.05\)). The study also revealed that although there were significant increase in the WBC count above normal in the Test group (leucocytosis).
compared to the NC and PC groups; there was no statistically significant difference between the mean ± standard deviations between the three groups. The same was also observed in the lymphocyte percentage scores within the study groups. All the three groups had lymphocytosis. The ANOVA output found no statistical difference between the groups ($P = 0.062$). But with respect to the granulocyte percentage, the negative control group had normal granulocyte percentage scores. The positive control rather had granulocytosis while the test group had granulocytosis (Table 3). The differences between the mean ± standard deviation scores in the three groups was statistically significant at 0.05 significance level ($P = 0.0017$). To conclude, no group was observed with thrombocytopenia or thrombocytosis but test group had higher thrombocyte counts (which were still within normal limits) compared to the negative and positive control groups. There was a statistically significant difference between the three study groups ($P = 0.002$). This is presented in Table 4 above.

### Table 1. RBCs and Hb distribution between the study groups

<table>
<thead>
<tr>
<th></th>
<th>RBC (cells x $10^{12}$/L)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (NC)</td>
<td>4.21±0.32</td>
<td>12.4±0.60a</td>
</tr>
<tr>
<td>Positive control (PC)</td>
<td>3.96±0.95b</td>
<td>11.73±0.94b</td>
</tr>
<tr>
<td>Test (T)</td>
<td>5.13±0.46</td>
<td>15.57±0.61</td>
</tr>
<tr>
<td>F – VALUE</td>
<td>68.21</td>
<td>89.74</td>
</tr>
<tr>
<td>P - VALUE</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*a = P < 0.05 when NC is compared with T, b = P < 0.05 when PC is compared with T*

![Fig. 1. Mean RBC and Hb distribution between the study groups](image)

### Table 2. PCV distribution between the study groups

<table>
<thead>
<tr>
<th></th>
<th>Pack cell volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCV (%)</td>
</tr>
<tr>
<td>Negative control (NC)</td>
<td>36.80±0.71a</td>
</tr>
<tr>
<td>Positive control (PC)</td>
<td>35.13±0.52b</td>
</tr>
<tr>
<td>Test (T)</td>
<td>46.70±0.46</td>
</tr>
<tr>
<td>F – VALUE</td>
<td>42.61</td>
</tr>
<tr>
<td>P - VALUE</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*a = P < 0.05 when NC is compared with T, b = P < 0.05 when PC is compared with T*
Fig. 2. Mean PCV distribution between the study groups

Table 3. Red cell indices distribution between the study groups

<table>
<thead>
<tr>
<th>Red cell indices</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (NC)</td>
<td>88.45±1.01</td>
<td>29.75±0.21</td>
<td>33.70±0.40</td>
</tr>
<tr>
<td>Positive control (PC)</td>
<td>88.80±1.41</td>
<td>29.67±0.94</td>
<td>33.40±0.23</td>
</tr>
<tr>
<td>Test (T)</td>
<td>91.00±2.47</td>
<td>30.33±1.01</td>
<td>33.33±0.26</td>
</tr>
<tr>
<td>F - VALUE</td>
<td>54.21</td>
<td>63.82</td>
<td>59.67</td>
</tr>
<tr>
<td>P - VALUE</td>
<td>0.129</td>
<td>0.184</td>
<td>0.188</td>
</tr>
</tbody>
</table>

Fig. 3. Mean red cell indices distribution between the study groups

Table 4. PLT distribution between the study groups

<table>
<thead>
<tr>
<th>Platelets</th>
<th>PLT (x10⁹/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (NC)</td>
<td>501.00±3.94</td>
</tr>
<tr>
<td>Positive control (PC)</td>
<td>484.33±2.61</td>
</tr>
<tr>
<td>Test (T)</td>
<td>632.37±3.28</td>
</tr>
<tr>
<td>F - VALUE</td>
<td>532.04</td>
</tr>
<tr>
<td>P - VALUE</td>
<td>0.002</td>
</tr>
</tbody>
</table>
5. DISCUSSION

The actual physiological status of organisms can be diagnosed through use of blood parameters. For normal functioning of the body of an organism, it must keep its blood composition and constituent under natural conditions. The present study showed that leaves of *Justicia carnea* demonstrated varying degrees of hematological changes at the dose levels of 25 g. The significant (p<0.05) increase in erythrocytes and hematocrit counts (i.e RBCs, Hb and PCV) after oral administration of *Justicia carnea* leaves (TC), iron folate (PC) and normal feed (NC) as shown in Table 1 and Table 2, suggests that the leaf may contain phytochemicals and compounds that stimulate the secretion or formation of erythropoietin in the stem cells of normal mice. Stimulation of stem cells in the bone marrow to produce red blood cells occurs due to the action of erythropoietin. Phytochemicals such as saponin, alkaloids, tamin, and flavonoid have been documented to have erythropoietic properties [7], which if present in the leaf explains the significant increase in RBCs, Hb and PCV. This may also suggest that the plant may cause polycythemia [8,9,10,11]. This is similar to the research carry out by Chimaraoke et al., [1] in Nigeria on the same plant in 2017 and disagrees with a study carry by Mishra and Tandon in India on *Bougainvillea spectabilis* leaves in Swiss albino mice in 2011 [12], which show a significant reduction in RBCs and Hb, and also disagrees with that conducted by Owoyele et al in Nigeria in 2011 [13], which show no significant increase in RBCs, Hb and PCV. Furthermore, the present study reveals no significant change in the Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH) and Mean Cell Hemoglobin concentration (MCHC). This could be attributed to the fact that the mice were not induce by phenyl hydrazine to become anemic. The study is in line with that carryout by Mishra and Tandon in India on extracts of ornamental plants in Swiss albino mice in 2011 [14], and disagrees with that done by Obel et al. in Kenya on *Carissa edulis* extract in 2016 [15] which revealed a significant increase in MCV, MCH and MCHC. In addition, the present study show a significant increase in WBCs most especially in the Test control with all groups having lymphocytosis but a mark difference in granulocyte showing normal granulocyte score in the Negative control, granulocytopenia in the positive control and finally granulocytosis in the Test control(P = 0.0017) Table 3. Increase WBC have been report to boost the immune system [16], the significant increase in WBC count, lymphocyte count and granulocyte count cause by the plant leaves reflect leucopoietic and possible immunomodulatory effects of the extract which augmented the production of more WBC and lymphocyte [17]. This agrees with research performed by Chimaraoke et al., on *Justicia carnea* extracts in Nigeria in 2017 [1], and disagrees with that performed by Olusegum et al., on *Ocinum gratissimum* in Wister rats in Nigeria in 2008. [18]. The study also revealed a
significant increase in platelet count, most especially in the test group when compared to the Negative and Positive control. Platelets are the blood cells involved in Coagulation. Coagulation of blood requires that the platelets should be in sufficient size, number and function. The increase in the platelet levels observed in this study may be explained by stimulatory effect on thrombopoietin. The significant increase in platelets and related parameter profiles after oral administration of *Justicia carnea* leaves suggests that the leaves contain compounds and phytochemicals that may have stimulated thrombopoietic process in the albino mice. This agrees with that conducted by Obel et al. in Kenya on Carissa edulis extract in 2016 [15] and disagrees with that carried out by Olusegum et al., [18] on Ocimum gratissimum in Wister rats in Nigeria in 2008, which showed a decrease in Platelet count.

6. CONCLUSION

The approximated values in this leaves base on the aid of automated analysis and statistical analysis were very reasonable, which suggests that the leaves of this plant can make valuable contributions to improved nutrition, blood parameters and well-being. This study shows that *J. carnea* leaves possess anti-anemic potential, lending credence to the use of these plant leaves in folk medicine for the management of hemolytic anemia is helpful. Further studies are warranted to determine the bioactive component present in *J. carnea* leaves that could be responsible for its anti-anemic effects.

COVER STATEMENTS

This is to indicate that, this article is the fruits of our original Research and that this article has not been submitted elsewhere for publication. Where ever the information has been adapted, the author has provided adequate citation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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