Abstract

Blood and blood components play vital role in maintenance of homeostasis. However, small alteration in standard levels of blood components may lead to severe diseases or disorders. In present study we explore dose dependent effects of *Bridelia ferruginea* on hematological parameters. The hematological responses of albino rats fed with aqueous extract of *Bridelia ferruginea* stem bark was investigated at end of experiment by invitro method. Sixteen albino rats were divided into four groups, with different dosage and control group. Increasing doses (100, 200 and 400 mg kg\(^{-1}\) body weight) of the extract were administered orally to the rats for a period of two weeks. The result noted in terms of significant decreases in the level of hemoglobin (Hb), packed cell volume and percent monocyte counts were noted, whereas significant increases were observed in percent neutrophil and lymphocyte counts of the *Bridelia ferruginea* treated animals. Thus the dose dependent effects of *Bridelia ferruginea* were observed in various groups. Therefore, administration of high dose of *Bridelia ferruginea* may be destructive to blood components, whereas at low dose it is not much toxic.

**Keywords:** *Bridelia ferruginea*, hematologic study, hemoglobin, monocyte

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1. Introduction

*Brwedelia ferruginea* belongs to the family Euphorbiaceae which is commonly found in Savannah regions [1]. It is usually a gnarled shrub which sometimes reaches the size of a tree in suitable condition. Its common names are Kizni (Hausa), Marehi (Fulani), Iralodan (Yoruba), Ola (Igbo); and Kensange Abia (Boki). Its habitat is the Savannah, especially in the moister regions extending from Guinea to Zaire and Angola. The tree is 6 - 15 m high, up to 1.5 m in girth and bole crooked branching low down. The bark is dark grey, rough and often marked scaly [2]. A
decoction of the leaves has been used to treat diabetes. It is also used as purgative and a vermifuge [3]. The bark extract has been used for the coagulation of milk and also lime juice for the formulation of a traditional gargle “gun efu” [4]. It is also reported of having potential for water treatment [5]. In Togo, the roots of the plant are used as chewing sticks and the root bark is used for intestinal and bladder disorder remedies as well as skin diseases [6]. Other reported activities of the bark extract include typanocidal [1], molluscidal [8], antimicrobial [9] and antiinflammatory [10]. Antimicrobial properties of stem bark of B. ferruginea against facultative Gram negative rods have been reported by [11].

The plant was found to contain Alkaloids, Tannins, Terpenoids, Glycosides, Flavonoids, Saponins, Anthraquinones and Steroids. The activities of the methanol, petroleum ether and chloroform bark extracts of the B. ferruginea against some potential pathogenic organisms have been extensively investigated [7], [8], [9]. Bridelia ferruginea has a great antioxidant potential which can be used to protect the body against damage caused by free radicals which is regularly produced in vivo and oxidative stress induce these free radicals [12].

Erythrocytes (red blood cell) which are anucleated are packed with the oxygen carrying proteins hemoglobin. The normal concentration of erythrocytes in blood is approximately 3.9-5.5 million per micro liter in women and 4.1-6 million per micro liter in men. Human erythrocytes survive in the circulation for about 120 days, worn out erythrocytes are removed from the circulation by macrophages of the spleen and bone marrows. The signal for removal seems to be the appearance of defective complex oligosaccharides attached to integral membrane protein of the plasmalemma [13]. Leukocytes (white blood cells) migrate to the tissue, where they perform multiple functions and most die by apoptosis. Leukocytes are involved in the cellular and humoral defense of the organism against foreign material. The number of leukocytes in the blood varies according to age, sex and physiological conditions. In normal adults they are roughly 6,000-10,000 Leukocytes per micro liter of blood. Blood platelets (thrombocytes) are non-nucleated disk like cell fragments 2-4μm in diameter. Platelets originate from the fragmentation of giant polypoid megakaryocytes that reside in the bone marrow. Platelets count range from 200,000 to 400,000 per micro liter of blood. Platelets have a life span of about 10 days. Platelets function: the role of platelets in controlling hemorrhage can be summarized as primary aggregation, secondary aggregation, blood coagulation, clot retraction and clot removal.

Due to the widespread consumption of Bridelia ferruginea, it is necessary to study its effect on blood, the tissue that transports substances in the body [13]. This study was therefore designed to evaluate the effects of aqueous extracts of Bridelia ferruginea stem bark on the haematological responses of albino rats.

2. Materials and Methods

2.1 Extraction of plant material: Fresh stem bark peelings of Bridelia ferruginea were collected at a farm in the suburbs of Ado Ekiti, Nigeria. The plant was identified and authenticated by a plant scientist in the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria and a voucher specimen was deposited accordingly at the herbarium of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria.

2.2 Extraction: The fresh bark peelings of the plant were air-dried, pulverized and extracted exhaustively in distilled water. The filtrate was concentrated and evaporated to dryness at 60°C, using rotary evaporator (Stuart Barloworld, Model RE 300). The yield was calculated and the dry extract was stored in a refrigerator at -4°C until use for the experiments.
2.3 Animals: A total number of 16 albino rats weighing between 100-190 g were used in this study. The animals were obtained from the animal house of the Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria. The animals were randomly distributed into cages and allowed to acclimatize for 14 days in a well ventilated room at a room temperature of 28.0±2.0°C under natural lighting condition. The animals were allowed free access to standard mouse chow (Topfeeds Ltd., Sapele, Nigeria) and tap water ad libitum. All animals used in this study were handled in accordance with the international, national and institutional guidelines for Care and Use of Laboratory Animals as promulgated by the Canadian Council of Animal Care (2009).

2.4 Experimental protocol: Animals were divided into four groups- A, B, C and D, representing group 1, 2, 3 and control, respectively.

Group A was given single daily doses of 100 mg kg⁻¹ of BF for 14 days.

Group B received single daily doses of 200 mg kg⁻¹ of BF for 14 days.

Group C was given single daily doses of 400 mg kg⁻¹ of BF for 14 days.

Group D (control group), containing four animals, was given only distilled water daily for 14 days. BF was administered orally using a calibrated 1 mL syringe with attached polythene cannula. At the end of the treatment period, the animals were sacrificed.

2.5 Hematological assays: Whole blood was collected from the animals into EDTA bottle and assayed for PCV, hemoglobin, WBC and differential cell counts using standard techniques.

2.6 Statistical analysis: Data were expressed as Mean±SE of mean. Comparisons between control values and values of treated groups of albino rats were performed with one-way Analysis of Variance (ANOVA). Statistical significance was set at p<0.05.

3. Results

3.1 Hematological parameters results: The results of the effect of different doses of aqueous extract of Bridelia ferruginea on the hematological parameters are shown in Figs 1. The machine analyzed blood samples to give about seven parameters, which include Hemoglobin, PCV, WBC, Monocytes, Eosinophils, Neutrophil and Lymphocytes.

Table 1:
Haematological parameters of albino rats administered aqueous extracts of Bridelia ferruginea stem bark

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Control Group</th>
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</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>150.50 ± 6.99b</td>
<td>148.50 ± 3.61b</td>
<td>131.00 ± 3.71b</td>
<td>161.50 ± 5.17b</td>
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<tr>
<td>PCV (%)</td>
<td>31.50 ± 2.62a</td>
<td>33.00 ± 1.95ab</td>
<td>32.25 ± 1.65ab</td>
<td>38.25 ± 1.37b</td>
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<tr>
<td>WBC (x 10³ mm⁻³)</td>
<td>4.20 ± 70.71b</td>
<td>4.65 ± 253.31bc</td>
<td>2.90 ± 147.19b</td>
<td>5.27 ± 319.83c</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>41.00 ± 2.97a</td>
<td>49.25 ± 1.49b</td>
<td>48.75 ± 2.42b</td>
<td>43.00 ± 1.73ab</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>41.50 ± 2.17a</td>
<td>42.25 ± 1.03a</td>
<td>47.75 ± 1.65b</td>
<td>45.50 ± 0.95ab</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.75 ± 0.25a</td>
<td>2.00 ± 0.40a</td>
<td>2.75 ± 0.47a</td>
<td>2.00 ± 0.00a</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>7.00 ± 0.57a</td>
<td>7.75 ± 0.75a</td>
<td>6.25 ± 0.62a</td>
<td>7.25 ± 0.47a</td>
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</table>

abc Means within a row with different superscripts are significantly (P<0.05) different.
4. Discussion

This work tested the effect of aqueous extract of Bridelia ferruginea on hematological parameters of albino rats. The results of the study show that, the stem bark extract of Bridelia ferruginea administered at the dosages used and for the duration of the experiment suppress the haemopoetic system.

The hemoglobin values at the end of the experiment were 161.50 ± 5.17 g/dl for the control group and 150.50 ± 6.99, 148.50 ± 3.61, and 131.00 ± 3.71 g/dl for the experimental groups. This shows a reduction in the hemoglobin level at the higher dose administration. The mean haemoglobin (Hb) concentration of the control group animals (161.50 ± 5.17 g/dl) were significantly (P<0.05) higher than treatment group 3(131.00 ± 3.71 g/dl) which is similar (P>0.05) but no significant difference to group 1 (150.50 ± 6.99g/dl) and group 2(148.50 ± 3.61g/dl).

The mean packed cell volume of the control group (38.25 ± 1.37%) was significantly (P<0.05) higher than treatment group 1 (31.50 ± 2.62%) but similar (P>0.05) to group 2 (33.00 ± 1.95%) and group 3(32.25 ± 1.65%). The white blood cell count gives a mean value of (5.27 ± 319.83) (x 10^3 mm^3) for the control group animals and were significantly (P<0.05) higher than treatment group 1(4.20 ± 70.71) (x 10^3mm^3) and group 3 (2.90 ± 147.19) (x 10^3mm^3) but similar (P>0.05) to group 2 (4.65 ± 253.31) (x 10^3mm^3), while treatment group 1 (4.20 ± 70.71) (x 10^3mm^3) and group 2 (4.65 ± 253.31) (x 10^3mm^3) were similar (P>0.05).

Percentage neutrophil counts of the animals for control group (43.00 ± 1.73%), group 1 (41.00 ± 2.97%), group 2 (49.25 ± 1.49%) and group 3 (48.75 ± 2.42%) were similar (P>0.05). Treatment group 2 (49.25 ± 1.49%) and group 3 (48.75 ± 2.42%) were significantly (P<0.05) higher than treatment group 1 (41.00 ± 2.97%).

The percentage lymphocytes of the animals for control group (45.50 ± 0.95%), group 1 (41.50 ± 2.17%), group 2 (42.25 ± 1.03%) and group 3 (47.75 ± 1.65%) were similar (P>0.05). Treatment group 3 (47.75 ± 1.65%) were significantly (P<0.05) higher than treatment group 1(41.50 ± 2.17%) and group 2 (42.25 ± 1.03%) lymphocyte level increased at the end of the experiment as compared to the control group.

The percentage eosinophils counts of the animals for control group (2.00 ± 0.00%), group 1 (1.75 ± 0.25 %), group 2 (2.00 ± 0.40 %) and group 3 (2.75 ± 0.47%) were similar (P>0.05). Percentage monocytes for control group (7.25 ± 0.47%), group 1 (7.00 ± 0.57 %), group 2 (7.75 ± 0.75 %) and group 3 (6.25 ± 0.62 %) were similar (P>0.05). In addition, 2 weeks administration of BF resulted in a dose-dependent increase in lymphocyte and eosinophils but an altered decrease in monocytes counts. This result suggests that B. ferruginea stem bark extracts can improve the immune level of albino rats; since WBCs and lymphocytes are involved in the development of cellular immunity [14]. The neutrophil counts of the experimental animals were significantly (P<0.05) higher at 200mg/kg administration of aqueous extract of B. ferruginea stem bark extracts. High neutrophil counts can be the result of many factors which include bacterial infection, acute inflammation, stress response effect from some drugs and splenectomy, among others [15]. Since no disease condition was observed in the animals, and also following the absence of other differentials in the experimental animals; the significant (P<0.05) increase in neutrophil count at 200 mg/kg administration of the aqueous extract of B. ferruginea stem bark extracts to the albino rats could be due to burden on the immune system of the animals.

Conclusions

The study shows that the stem bark extracts of B. ferruginea administered at the
dosages used and for the duration of the experiment suppress the haemopoetic system. The reduction may have occurred due to lysis of blood cells and probably suppression of blood cell synthesis by high saponin level found in the B. ferruginea stem bark extracts (via the foam index technique) [15]; [16]. Saponins are known to be toxic to body systems [17].

Despite the widespread application of the plant as a condiment and herbal medicine, the extract has been observed to suppress the haemopoetic system. It is therefore suggested that continuous usage of the stem bark at high dosages should be avoided.

References


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