

Hematological Profiles of Naturally Infected Pigs Treated with *Bridelia ferruginea* Leaf Extracts

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Authors' contributions

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

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ABSTRACT

Bridelia ferruginea (Benth) Phyllanthaceae called "Kizni" in Hausa, "Iralodan" in Yoruba and "Ola" in Igbo languages are widely used as food and as medicines in traditional medical practice. They are widely found in the Savannah regions especially in the moister regions. This study explores the hematological profiles of pigs naturally infected with gastrointestinal nematodes treated with N-hexane leaf extract of *Bridelia ferruginea* in three divided doses (125 mg/kg, 250 mg/kg and 500 mg/kg body weight) for twelve days. The hematological assay was carried out using mind Ray hematology auto analyzer (BC 5300). Results showed that leaf extract of *B. ferruginea* exhibited significant increase ($p < 0.05$) in packed cell volume, total leukocyte, hemoglobin, neutrophil, lymphocyte, monocyte and eosinophil counts. However, no significant difference ($p < 0.05$) was observed on the total erythrocyte and platelet counts of the animals. The results in our findings demonstrated that the leaf extract of *B. ferruginea* is capable of stimulating blood cell formation and act as active phagocytic agent against foreign compounds, and therefore can be used to formulate new plant-based phyto-medicine in a bid to improve human and animal health.

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1. INTRODUCTION

The use of herbal medicine as alternative to clinical therapy in recent times is on the increase and thus leads to their greater demand [1]. The selective use of herbal drugs in rural communities prepared and dispensed by herbalist with no formal training for the treatment of disease remains popular. Scientific investigation is therefore required for evaluation of the claimed activity and to ascertain the safety and efficiency of these herbal products as well as to establish their active constituent [2]. Findings on some medicinal herbs have shown that they have well-defined action on nervous, circulatory, respiratory, digestive and renal systems; as well as sexual organs, the skin, vision, hearing and taste [3]. Africa as a continent is blessed with vast number of medicinal herbs, which play an important role in the health care of her citizens. One of these plants is *Bridelia ferruginea*.

Bridelia ferruginea (Benth) Phyllanthaceae is a woody shrub that grows in the Savannah or rain forests of Africa [4]. In Nigeria, it is popularly called 'Kizni' (Hausa), 'Iralodan' (Yoruba) and 'Ola' (Igbo). Traditionally, the plant has been reported to poses anti-diabetic activity [5]. Wasagu et al. [6] reported its antimicrobial activities as well as anti-inflammatory activities [4]. Other reported activities of the plant include its water treatment potentials as reported by Kolawole et al. [7] as well as its potential in the treatment of various skin diseases [8]. Researchers at different times have linked the activities of this plant to the presence of divers' kind of phytochemicals such as flavonoids, triterpenoids, glucosides, bioflavonoids, phenols and tannins in various morphological parts of the plant [5,9,10]. Anaemia is a medical ailment or disease in which the red blood cell count or haemoglobin is less than normal. Normal range of haemoglobin varies in males and females. The normal haemoglobin level for men is 13.5 g/100 ml while in women it is 12.0 g/100 ml [11]. Haemoglobin levels less than the normal is medically called anaemia. Haemolytic anaemia is known to be produced by excessive destruction or shortened erythrocyte lifespan [12]. In chemically induced anaemia, the primary alteration is a marked reduction in the number of leukocytes and erythrocytes [13]. Blood constituents change in relation to the physiological status of an animal and thus act as

a pathological reflector of the status of the animals [14]. There are many blood disorders which can affect the quality as well as the functionality of blood cells or proteins in the blood clothing system or immune system [15]. In Nigeria, leaf of *Bridelia ferruginea* is used traditionally to cure many ailments but reports are scanty on its effect on the hematological parameters. Hence this study is aimed at expolring the effect of the leaf extracts of *Bridelia ferruginea* on hematological parameters in naturally infected pigs with gastrointestinal nematodes.

2. MATERIALS AND METHODS

2.1 Collection of Plant and Preparation of Plant Extracts

Fresh green leaves of *Bridelia ferruginea* was collected from a local farm in the suburb of Ado Ekiti, Ekiti State, Nigeria. Identification and authentication of the plant was carried out at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria and a voucher specimen number (UHAE.2017/065) was deposited at the herbarium of the Department for future references.

2.2 Sample Preparation

The plant material were shredded with a knife and air-dried at room temperature for 45 days to get rid of its water content. The air-dried leaves were pulverized using a laboratory mechanical grinder and the fine powders obtained (800 g) stored until further use. The powdered sample was extracted with solvent combination (ethanol:water) (via maceration) of 70% ethanol for 48hrs. The mixture was decanted and filtered using sterile Whatman paper No 1. The filtrate was evaporated to dryness using a freeze dryer to obtain ethanolic residue (100 g). The yield of extracts was calculated according to the method of Nkafamiya et al. [16] using the formular below:

$$\text{Percentage yield} = \frac{\text{Mass of Extract after rotary evaporation (g)}}{\text{Mass of crude extract (g)}} \times 100.$$

The crude extract was later subjected to further extraction processes to obtain n-hexane extract which was used for further bioassay studies.

2.3 Selection and Grouping of Experimental Animals

The study was conducted at a private farm in Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. Thirty male pigs (young stock ≤ 6 months), weighing 8-10 kg, naturally parasitized with gastrointestinal nematodes (GINs) (*Strongyloides ransomi*) were selected. The experimental animals were vaccinated against enterotoxemia and pleuropneumonia vaccines. Nematode infection and eggs per gram of feces was confirmed before the beginning of study following the standard parasitological procedures of fecal examination [17]. Coproculture was carried out to ascertain the nematode species composition and identification of larvae using standard description of MAFF [18]. The experimental animals were penned singly by treatment and no physical contact was made possible between the animals from different treatment groups; Pigs were kept on plastered floor and fed with standard pig feed and water *ad libitum*.

Accordingly, the selected animals were randomly divided into five (5) groups of six animals each. Group 2, 3 and 4 served as the treatment group and received dosage of 125 mg/kg, 250 mg/kg and 500 mg/kg body weight of n-hexane leaf extract of *B. ferruginea* respectively. Group 5 served as the positive control and received a standard drug (levamisole) of 10 mg/kg body weight. Group 1 served as the negative control. Hence, received distilled water. Single dose of treatment was administered *per os* and blood samples were collected and analyzed in triplicate immediately at three (3) days interval (day 0, 3, 6, 9 and 12) for twelve (12) post treatment days and analyzed using mind Ray hematology auto analyzer (BC 5300). All the experimental procedures and protocols used for this study were in accordance with the guidelines and principles of Laboratory Animal Care of the National Society of Medical Research [19].

2.4 Data Analysis

The results were expressed as Mean \pm S.E.M. One way analysis of variance (ANOVA) was carried out on the results and significance was accepted at $p < 0.05$. The graphical analyses were carried out using Graph-Pad Prism5 Program (Graph-Pad Software, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

Groups with asteric (*) shows significant difference at $p < 0.05$.

The evaluation of hematological parameters is useful information in determining the effect of foreign substances including plant extracts *in vivo*. They are used to ascertain any possible change in the levels of biomolecules such as enzymes, metabolic products, hematology, normal functioning and histopathology of the organs [20]. In this study, the hematological parameters in naturally infected pigs treated with n-hexane leaf extract of *B. ferruginea* were evaluated. The results (Figs. 1-9) showed the effect of different doses of n-hexane leaf extract of *B. ferruginea* on the hematological parameters of the helminth infected animals. Nine hematological parameters, which include packed cell volume (PCV), Total Leukocyte Count (TLC), Total Erythrocyte Count (TEC), Hemoglobin, Platelets, Neutrophil, Lymphocytes, Monocytes, and Eosinophils were analyzed using hematology auto analyzer. The results of the study showed that n-hexane leaf extract of *B. ferruginea* administered at different doses and for the duration of the experiment regulate the haemopoetic system. There was a significant increase ($p < 0.05$) in the PCV level of group 3 and 4 animals (Fig. 1). This is similar to the findings of Okon et al. [13] and in agreement with the reports of Nwankpa et al. [21]. Packed Cell Volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in blood. According to Isaac et al. [22], PCV is involved in the transport of oxygen and absorbed nutrients. Hence, Increased PCV levels in the treated animals shows a better transportation of oxygen and absorbed nutrients in these animals and consequently enhance their health status.

As depicted in Fig. 2, there is a significant difference ($p < 0.05$) in all the treatment groups when compared with the control group. Similar reports were reported by Nwankpa et al. [21] and also supported with the observation made by Ahumibe and Braide [23] who suggested that increase in leukocyte counts helps to stimulate cytokine erythropoietin. *B. ferruginea* extract may plausibly stimulate cytokine erythropoietin to stimulate blood cell synthesis. Leukocytes are essential for the protection of animals against foreign invaders, and their elevation is indicative of response to an immunological challenge. Fig. 3. presents the erythrocyte counts of the experimental animals. There was no significant difference ($p < 0.05$) in the erythrocyte count of all the tested groups. This is in contrast to the findings of Esenowo et al. [24] and Ogbonna et

al [25] in their separate studies. Erythrocyte functions primarily in picking up inhaled oxygen from the lungs and transport it to the body's

tissues, and also pick up about 24% carbon dioxide waste at the tissues and transport it to the lungs for exhalation.

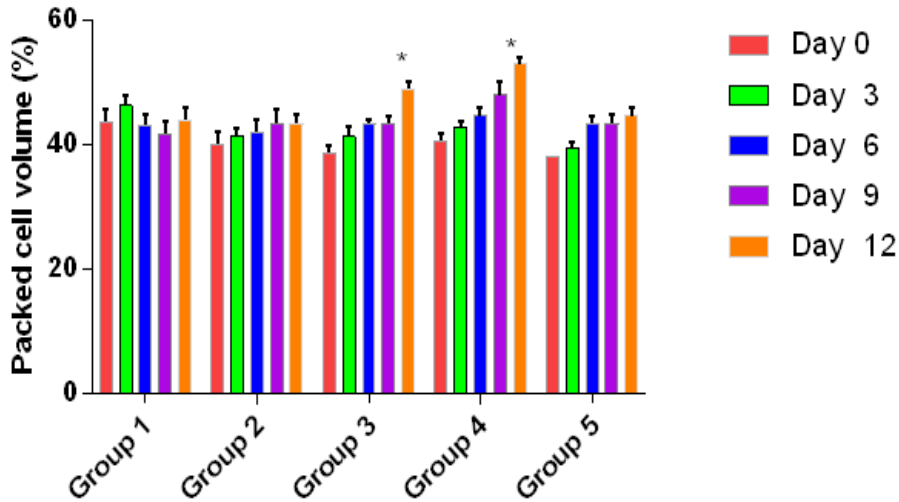


Fig. 1. Packed cell volume (PCV) of the helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*

Legend:

Group 1: Served as the negative control group, hence, no treatment was administered.

Group 2: Received 125 mg/kg b.wt. N-hexane leaf extract of *B. ferruginea*

Group 3: Received 250 mg/kg b.wt. N-hexane leaf extract of *B. ferruginea*

Group 4: Received 500 mg/kg b.wt. N-hexane leaf extract of *B. ferruginea*

Group 5: Served as the positive control group, hence, they received standard drug (levamisole) (10 mg/kg b.wt.)

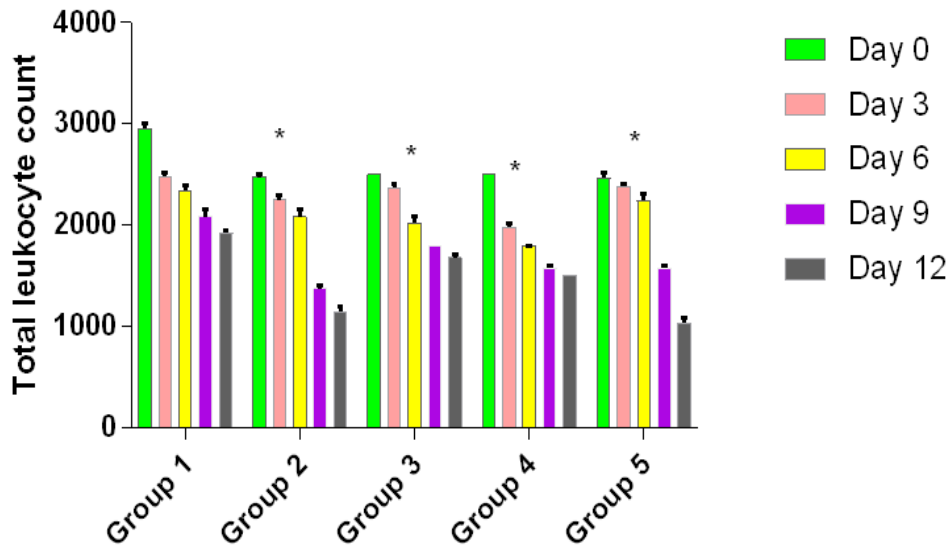


Fig. 2. Total leukocyte count (TLC) in the helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*

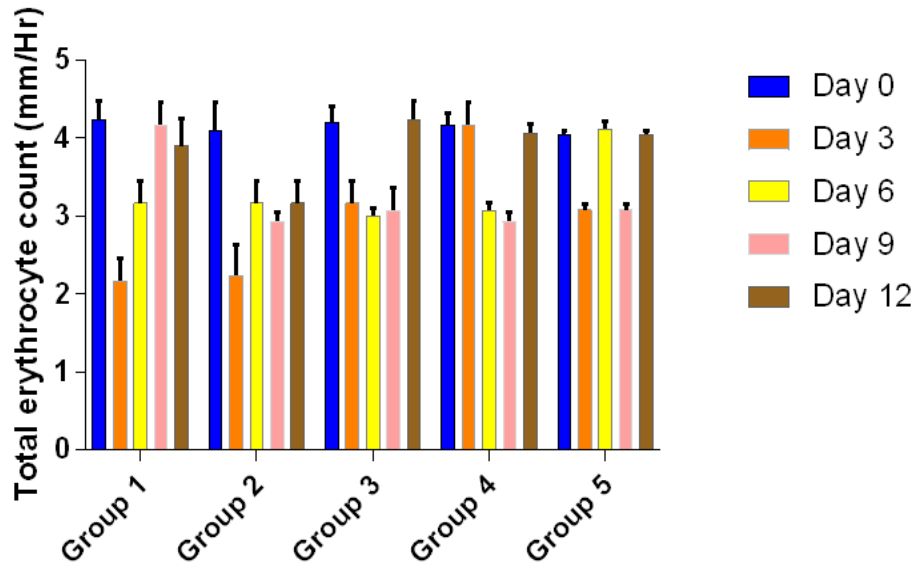


Fig. 3. Total erythrocyte count (TEC) of helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*

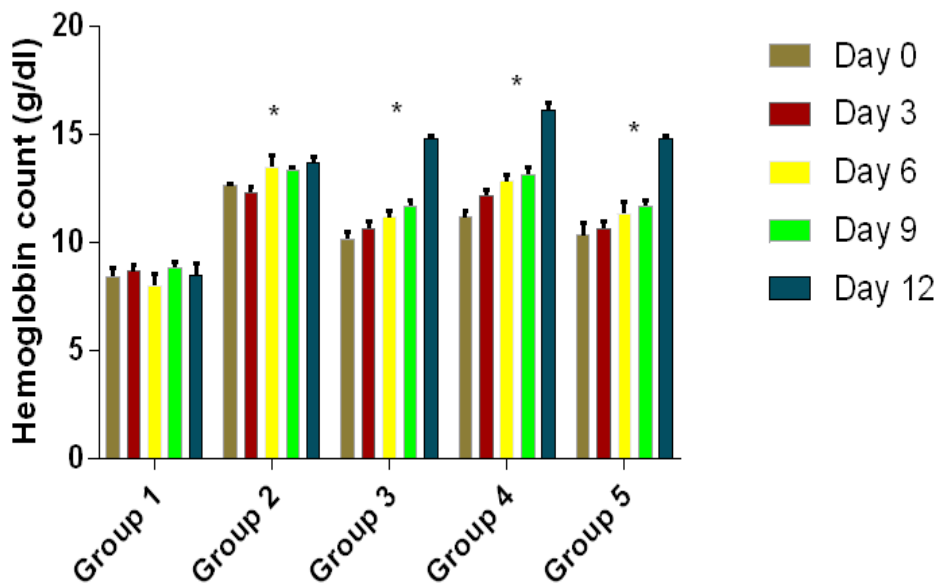


Fig. 4. Hemoglobin count (Hb) of the helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*

The hemoglobin count at the end of the experiment is shown in Fig. 4. A significant increase ($p < 0.05$) was observed in all the treatment groups when compared to group 1 animals. This is in concord with the reports of

Okon et al. [13]. Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of almost all vertebrates as well as the tissues of some invertebrates. They function primarily in the circulation of oxygen

from the lungs to the rest of the body. The platelet count as depicted in Fig. 5. showed no significant difference in all the treatment groups including the control group. Fig. 6 and 7 represent the neutrophil and lymphocyte counts. There is a significant difference ($p < 0.05$) in group 4 and 5 animals when compared to the control group in the neutrophil count whereas no

significant difference ($p < 0.05$) across all the groups in its lymphocyte count. Nwankpa et al. [21] reported similar observations. Neutrophils and lymphocytes as well as other hematological parameters are measurable indices of the blood, which can be used to evaluate hematopoietic function [26]. Neutrophil are important phagocytic cells normally elevated in the early inflammatory

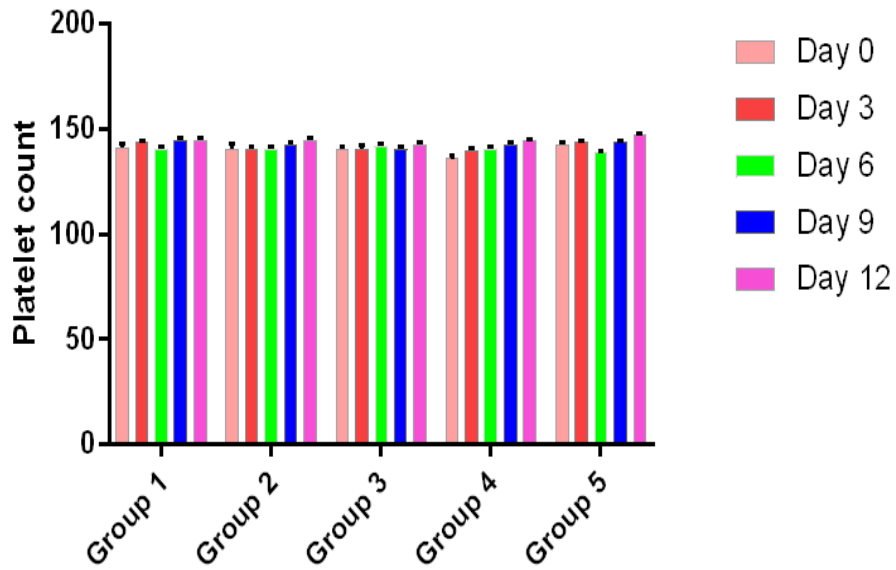


Fig. 5. Platelet count of the helminth-infected pigs treated with n-hexane leaf fraction of *Bridelia ferruginea*

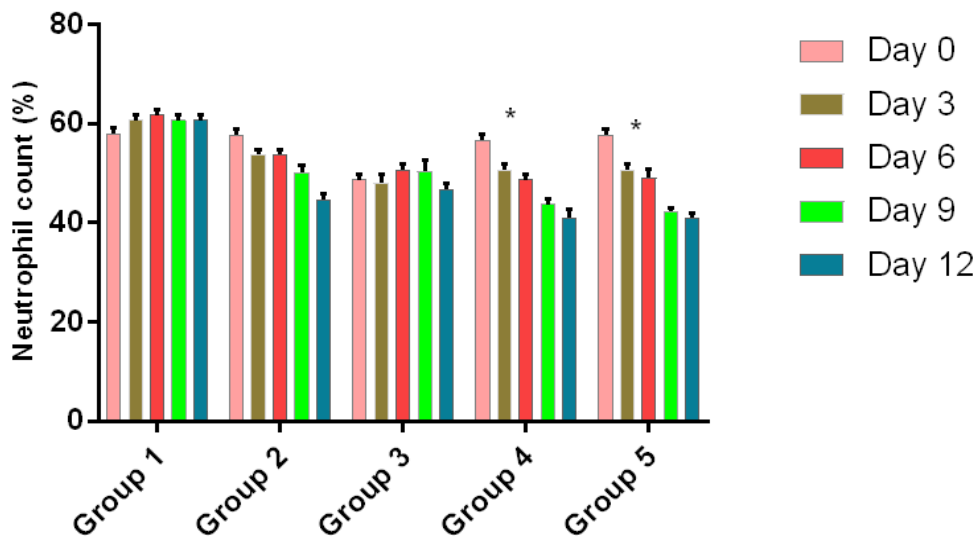


Fig. 6. Neutrophil count of the helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*

response [27,28], while lymphocytes are sub types of leucocytes critically essential for providing cell mediated immunity. Group 4 and 5 showed a significant difference ($p < 0.05$) in the monocyte count compared to the control group. Monocytes are the largest type of leukocyte and can differentiate into macrophages and myeloid lineage dendritic cells. They help fight bacteria,

viruses, and other infections in the body. Hence, influencing the process of adaptive immunity. Eosinophil is a specialized cell of the immune system saddle with the responsibility of combating multicellular parasites and certain infections in vertebrates. As depicted in Fig. 9, there are variations in all the treatment groups including the control.

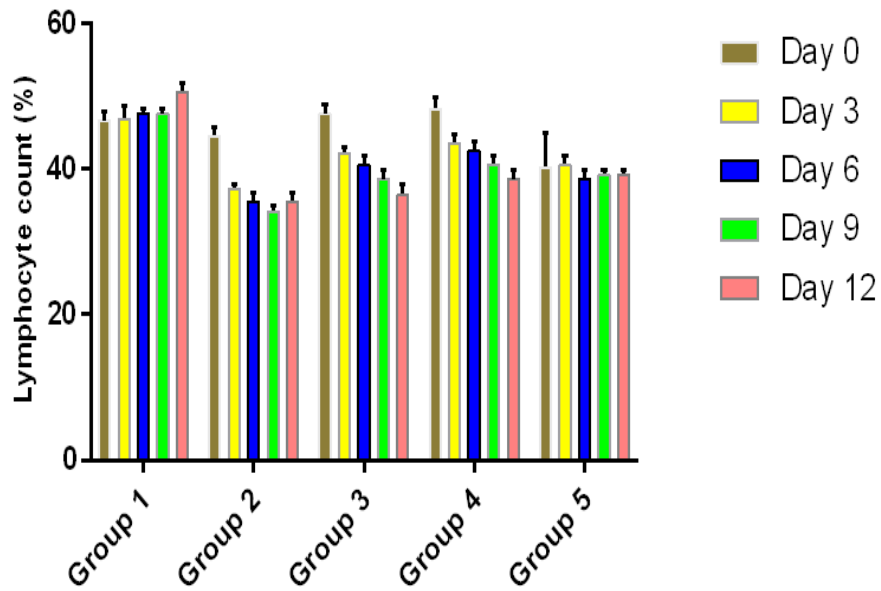


Fig. 7. Lymphocyte count of the helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*

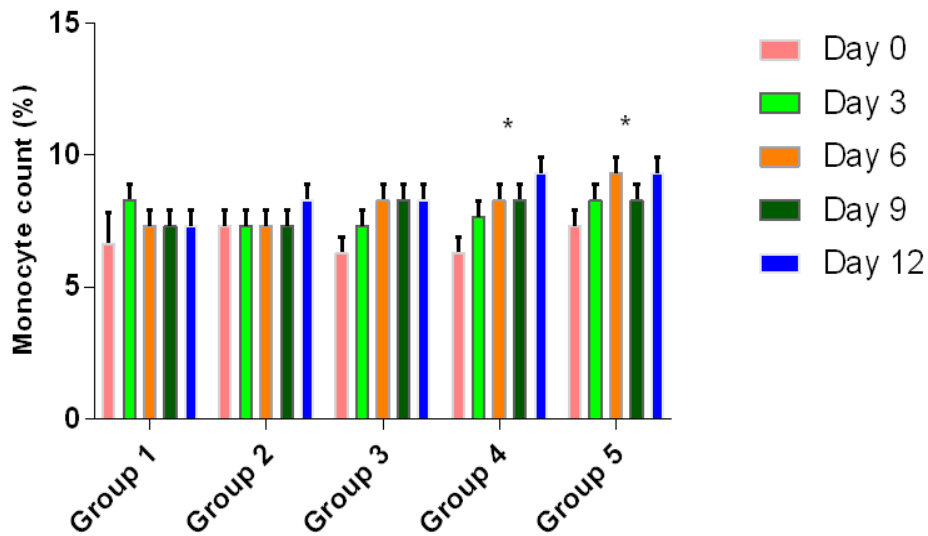


Fig. 8. Monocyte count of the helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*

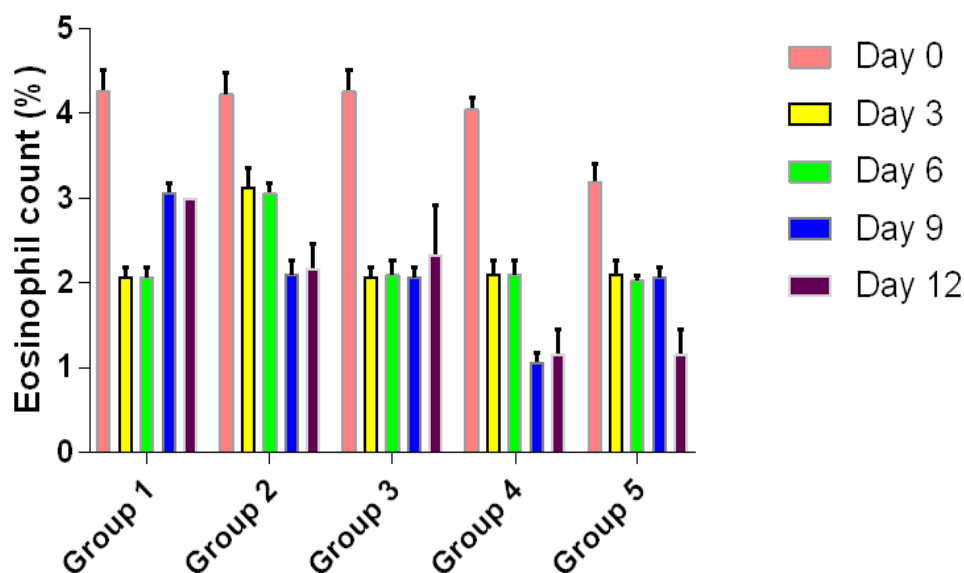


Fig. 9. Eosinophil count of the helminth-infected pigs treated with n-hexane leaf extract of *Bridelia ferruginea*.

4. CONCLUSION

This study investigated the effects of n-hexane leaf extract of *B. ferruginea* plant on hematological parameters in naturally infected pigs. The result demonstrated that the extract is capable of stimulating blood cell formation and act as active phagocytic agent against foreign compounds. It therefore supports its use as medicine for haemopoietic conditions.

CONSENT

It is not applicable

ETHICAL APPROVAL

As per international standard or university standard written animal ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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