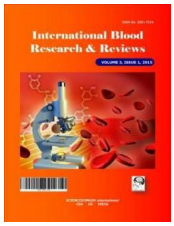

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In vivo Studies on the Possible Haematological Changes in Rats Administered *Hibiscus sabdariffa* Aqueous Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author PEA designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author PEJ managed the literature searches, analyses of the study while author CO managed the experimental process and identified the species of the plant. All authors read and approved the final manuscript.

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ABSTRACT

The aqueous extract of *Hibiscus sabdariffa* leaves is popularly consumed in the form of "Zobo" drink in Nigeria and it is assumed to have haematological benefits. The present study was conducted to evaluate the effects of aqueous extract of *H. sabdariffa* leaves on possible changes in haematological parameters using rat model. Twenty (20) adult wistar albino rats were used for the study and were assigned into four groups of five (5) rats per group. The rats in groups 2-4 were orally administered aqueous extract of *H. sabdariffa* in increasing doses (200,400 and 600 mg/ kg b.wt.) for 21 days while group 1 rats received distilled water at 10 ml/kg to serve as the normal control. On the day 21, blood samples for the haematological analyses were collected into clean sample bottles containing ethylenediamine tetra acetic acid (EDTA) at 1 mg/ml of blood. The results indicated that there were significant increases ($p<0.05$) in the Red Blood Cell (RBC) count, Packed Cell Volume (PCV) and Haemoglobin (Hb) concentration of groups 2-4 rats administered 200, 400,

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600 mg/kg b. w. of the extract respectively compared with that of the normal control rats. Non significant increases (p>0.05) were observed across the test groups in the total white blood cell (WBC), neutrophils, and lymphocytes counts compared with that of the normal control rats. The results suggest that consumption of the aqueous extract of *H. sadbariffa* leaf extract improves erythrocytic profile with insignificant effects on the leukocytic indices.

Keywords: Hibiscus sabdariffa; haematological parameters; wistar rats.

1. INTRODUCTION

H. sabdariffa is an annual or perennial herb or woody-based shrub, which is grown in all parts of the world and it is taken as a common local drink popularly known as "zobo" in Nigeria. *H. sabdariffa* is a species of hibiscus native to old world tropics. It can be found in almost all warm countries such as India, Saudi Arabia, Malaysia, Nigeria, Indonesia, Thailand, Philippians, Egypt and Mexico [1,2,3,4].

Many parts of the plant are also claimed to have various medicinal values. It is used as an infusion and considered to have anti hypertensive properties [5,6]. It has been reported that aqueous extract of H*. sabdariffa* has a blood pressure–lowering effect in rats [5,7] and in humans [8]. In Europe, the calyces have been used in herbal medicine for treating high blood pressure, heart diseases, nerve diseases, calcified arteries [5,7],arthritis, arteriosclerosis [9]. A lotion made from rosella leaves is used on sores and wounds [10]. Hibiscus species is used in many folk medicines and are widely believed to be effective in the treatment of a variety of ailments including obesity, hyperlipidaemia, and diabetes [11,12].

The leaves of *H. sabdariffa* are one of the best sources of vitamin C and anthocyanins [13,14,15]. The dried calyces contain flavonoids [16] which act as antioxidants. Despite all the medicinal uses of *H. sabdariffa*, there is dearth of information on the haematological changes associated with its subchronic administration.

The study was therefore tailored to investigate possible haematological changes associated with subchronic administration of *H. sabdariffa* in adult albino wistar rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

Fresh leaves of *Hibiscus sabdariffa* were purchased from Ogige market, Nsukka, Enugu State of Nigeria on March, 2014 and were identified by a botanist of the herbarium, Botany Department, University of Nigeria, Nsukka with herbarium number-UNH 232b. The leaves were air-dried separately at room temperature, pulverized and extracted by cold maceration method.

2.1.2 Animals

Adult male wistar albino rats of 10 to 16 weeks and average weight of 160±15 g were obtained from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were acclimatised for duration of 7 days under standard environmental conditions with a 12 hour light/dark cycle maintained on a regular feed (vital feed) and water *ad libitum*.

2.1.3 Chemicals/reagents/samples

All chemicals used were of the analytical grade from May and Baker, England, BDH, England or Merck, Darmstadt, Germany.

2.1.4 Instruments/Equipment

Instrument/equipment include Rotary evaporator (Model Modulyo 4K, Edward, England), Chemical Balance (Gallenkamp, England), Conical Flasks (Pyrex, England), Spectrophotometer, Haemocytometer, (PIC, England), Adjustable Micropipette (Perfect, USA.), Refrigerator (Kelvinator, Germany), Microscope (Motic USA) Microhaematocrit centrifuge and reader (Hawksley, England).

2.2 Methods

2.2.1 Experimental design

Twenty (20) male albino wistar rats were acclimatized at the same conditions of temperature and pressure, and the same animal feeds were used for all the rats. The rats were assigned into four (4) groups of five (5) rats each as shown below:

Group 1	Normal Control.
Group 2	200 mg/kg b.w. of aqueous leaf extract of *H. sabdariffa.*

Group 3 400 mg/kg b.w. of aqueous leaf extract of *H. sabdariffa.*
Group 4 600 mg/kg b.w. of aqueous leaf extract of *H. sabdariffa*.

2.2.2 Aqueous extraction of the leaves of *H. sabdariffa*

The leaves of *H. sabdariffa* were air-dried separately at room temperature, then pulverized. The ground samples were extracted with aqueous solvent, using cold maceration techniques. The samples were filtered using Whatman filter paper. The filtrates were concentrated to solid matter using rotary evaporators, which then become the stock sample of the aqueous leaf extract. These extracts were stored in the refrigerator compartment till the time of use.

2.2.3 Blood collection

Blood samples were collected from the animals into EDTA bottle using orbital techniques for haematological determination. Blood samples were collected from the retrobulbar plexus of the median canthus of the eye of the rats.

2.2.4 Haematological determinations

2.2.4.1 Determination of packed cell volume

The packed cell volume (PCV) was determined by the microhaematocrit method [17]. Micro-capillary tubes were almost filled with the anti-coagulated blood samples and one end sealed with plasticine. The filled tubes were centrifuged at 10,000 revolutions per minute for 5 minutes using a microhaematocrit centrifuge. The PCV was read as a percentage on the microhaematocrit reader [17].

2.2.4.2 Determination of haemoglobin concentration

The haemoglobin concentration (Hb) was determined by the cyanmethaemoglobin method [18]. The blood sample (0.02 ml) was added to 5 ml of Drabkins reagent in a clean test tube. This was mixed gently and kept at room temperature for 20 minutes for colour development. The absorbances of both sample and standard were read, against a working reagent blank at a wavelength of 540 nm using a spectrophotometer. The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with the factor derived from the absorbance and concentration of the standard.

2.2.4.3 Erythrocyte count

The erythrocyte count was determined by the haemocytometer method [17]. Blood sample (0.02 ml) was added to 4 ml of red blood cell diluting fluid (sodium citrate, formaldehyde solution and distilled water) in a clean test tube, to make a 1:200 dilution. A drop of the diluted blood was charged onto the Neubaeur counting chamber and allowed to settle for 2-3 minutes. The high dry objective (x 40) of the light microscope was used in carrying out the erythrocyte count, in the five groups of 16 small squares. The number of erythrocytes enumerated for each sample was multiplied by 10,000 to obtain the erythrocyte count per microlitre of blood [17]

2.2.4.4 Total leukocyte count

The total leukocyte count was determined by the haemocytometer method [17]. Blood sample (0.02 ml) was added to 0.38 ml of white blood cell diluting fluid (glacial acetic acid tinged with gential violet) in a clean test tube, to make a 1:20 dilution. A drop of the diluted blood was charged onto the Neubaeur chamber and allowed to settle for 2 minutes. The x10 objective lens of the light microscope was used in making a total count of white blood cells on the four corner squares. The number of cells counted for each blood sample was multiplied by 50 to obtain the total leukocyte count per microlitre of blood [17].

2.2.4.5 Differential leukocyte count

Smears for differential leukocyte counts were prepared on clean slides and stained by the Leishman technique [17]. The differential leukocyte count was enumerated by the battlement counting method. The x100 (oil immersion) objective lens of the light microscope was used in making a differential leukocyte count and the different cells of the leukocytic series were identified and scored using the differential cell counter [17].

2.3 Statistical Analyses

Data obtained were analyzed using One-way Analysis of Variance (ANOVA). Variant means were separated using Duncans Multiple range Post hoc Test. Results were presented as Mean ± Standard Error of the Mean (Mean ±SEM).

3. RESULTS

Dose-dependent significant increase ($p<0.05$) was observed on the RBC count across the test groups (rats administered 200, 400 and 600 mg/kg b.w. of aqueous extract of *H. sabdariffa* leaves) when compared to that of the normal control rats.

There was significant increase ($p<0.05$) in the PCV of all test groups (Group 2-4) administered 200, 400 and 600 mg/kg b.w. of the extract compared with the PCV of the normal control (Group 1). Relative increase was observed in the PCV of group 2 rats compared with the PCV of group 3 and group 4 administered middle dose (400 mg/kg b.w.) and high dose (600 mg/kg b.w.) of the extract respectively; however, such an increase was found to be non significant ($p>0.05$). The PCV of group 3 rats administered middle dose (400 mg/kg b.w.) showed non-significant increase ($p>0.05$) compared with the PCV of group 4 rats administered high dose (600 mg/kg b.w.) of the extract. The PCV of the treated groups was significantly ($p<0.05$) higher than that of the normal control.

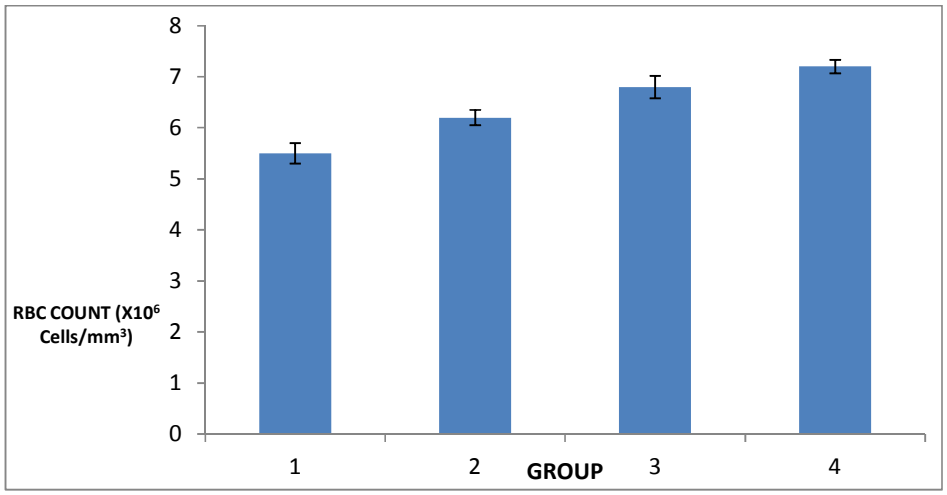

Fig. 1. Effect of aqueous extract of *H. sabdariffa* leaves on Red Blood Cell Count (RBC) in wistar albino rats

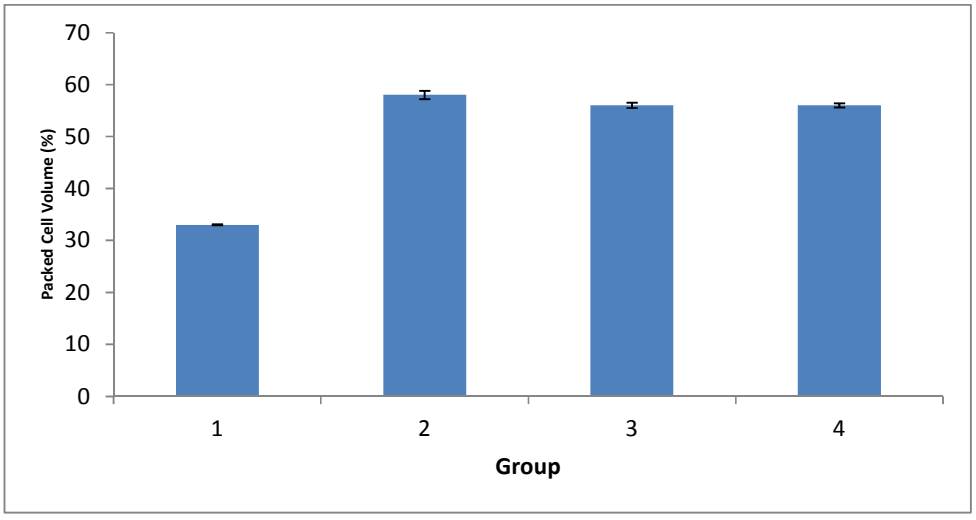

Fig. 2. Effect of aqueous extract of *H. sabdariffa* leaves on Packed Cell Volume (PCV) in wistar albino rats

The results indicate that the haemoglobin concentrations of rats in groups 2-4 (Treated groups) were significantly ($p<0.05$) higher than that of the normal control rats (Group 1). The haemoglobin concentrations of the treated groupd did not vary significantly among one another.

There were no significant ($p > 0.05$) changes in the total white blood cell count of the treated groups when compared with that of the normal control rats.

No significant change ($p>0.05$) was observed in the neutrophil count across the test groups of rats administered 200, 400 and 600 mg/kg b.w. of the aqueous extract of *H. sabdariffa* leaves when compared with that of the normal control rats.

Fig. 6 shows non-significant increase ($p>0.05$) on the lymphocyte count of all test groups (group 2, 3 and 4) administered 200,400 and 600 mg/kg b.w. of the extract compared with the lymphocyte count of the normal control

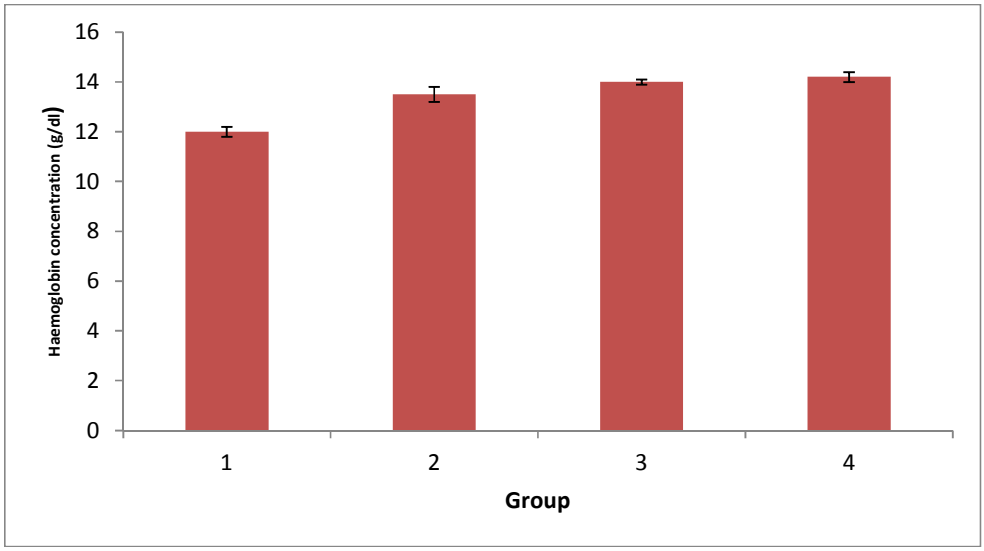

Fig. 3. Effect of subchronic administration of *H. sabdariffa* on the haemoglobin concentration of albino wistar rats

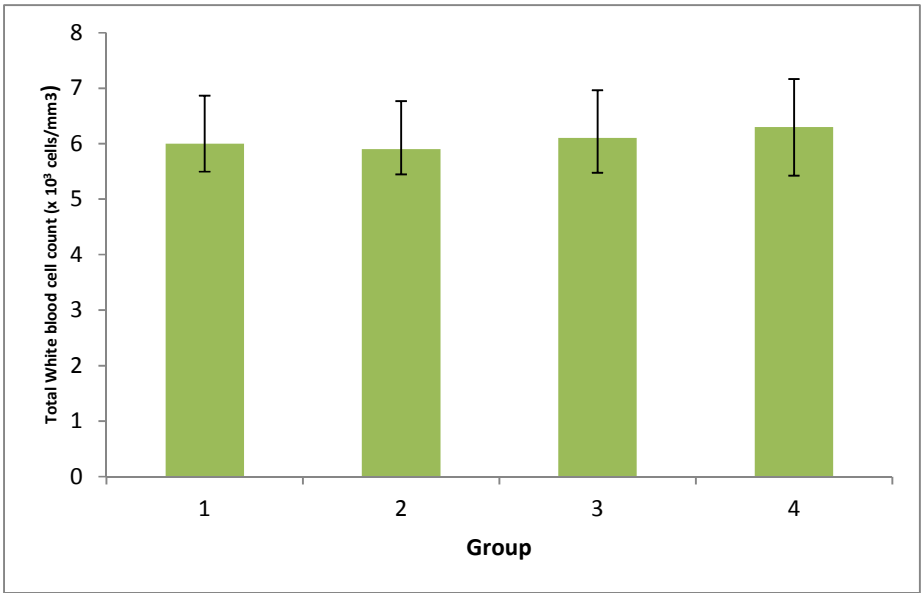

Fig. 4. Effects of aqueous extract of *H. sabdariffa* on the total white blood cell count of albino wistar rats

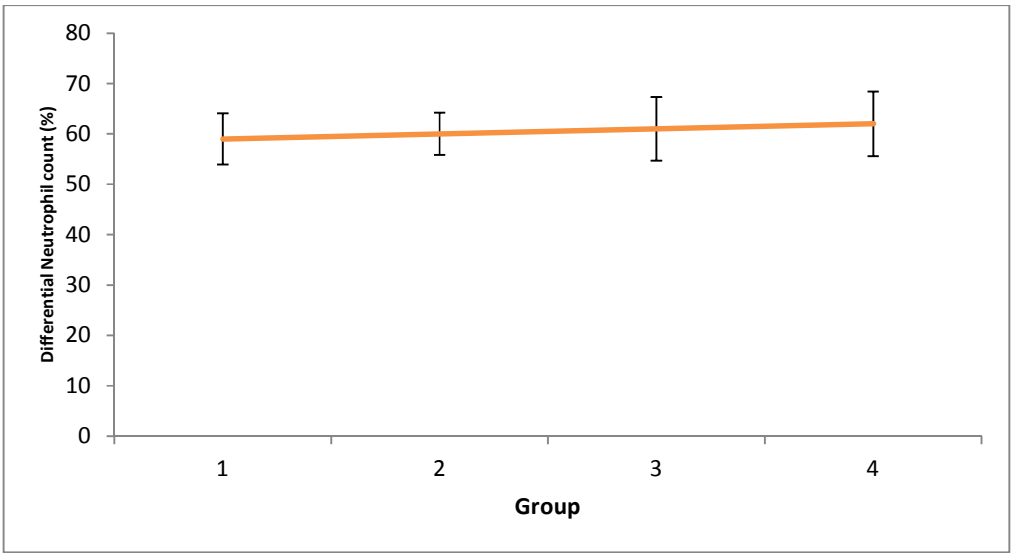

Fig. 5. Effect of aqueous extract of *H. sabdariffa* leaves on differential neutrophil Count in wistar albino rats

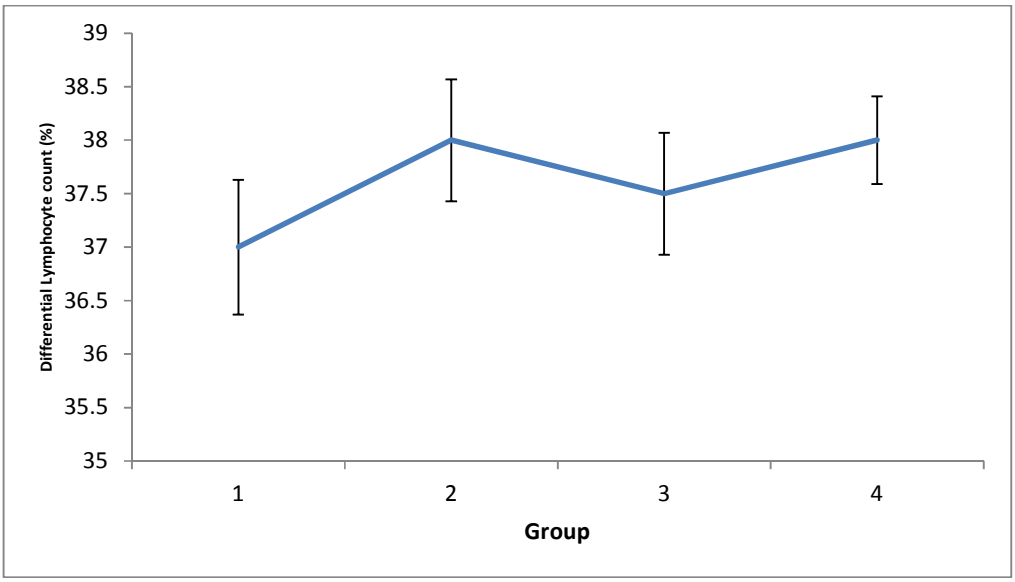

Fig. 6. Effect of aqueous extract of *H. sabdariffa* leaves on lymphocyte count in wistar albino rats

(group 1). Relative increase was observed on the lymphocyte count of group 2 rats administered low dose (200 mg/kg b.w.) compared with the lymphocyte count of groups 3 and 4 rats administered 400 and 600 mg/kg b.w of the extract, however such decreases were found to be non-significant ($p>0.05$).

4. DISCUSSION

The observed significant increases ($p<0.05$) on the RBC, PCV and Hb of groups 2-4 rats administered 200, 400 and 600 mg/kg .b.w of aqueous extract of *H. sabdariffa* leaves respectively when compared to the normal control could be attributed to reduced loss of blood cells due to lipid peroxidation and attendant lysis [19]. The antioxidants present in the extract may have probably mediated the mitigation of RBC lysis. Researchers have reported the presence of phytochemicals such as flavonoids, anthocyanins in the *H. sabdariffa* extract [13,14,15]. Flavonoids have been

reported to posses potent antioxidant effects [20]. It is suggested that these antioxidants present in the extract may have prevented oxidative red blood cell membrane peroxidation. Lipid peroxidation of erythrocytic cell membrane leads to lysis of red blood cell with attendant decrease in circulating red blood cell count (anaemia). The increase could also be due to the erythropoietic ability of antioxidant molecules of the extract [21]. Although, the erythropoietic property can be due to anthocyanin-associated increase in absorption of iron, a property that has been widely reported in ascorbate administration [22]. Iron has not only been reported to be present in significant amount in the extract of *H. sabdariffa*, but anthocyanins themselves are known to induce the synthesis and release of erythropoietin by the kidney. This is in agreement with the findings of [22,23].

The non-significant increases ($p>0.05$) observed on the administration of the extracts at varying doses across all the test groups (Group 2-4) on TWBC, lymphocyte, and neutrophil compared with that of the normal control rats indicates that the consumption of aqueous extracts of *H*. *sabdariffa* may not cause any significant effect on the white blood cell indices. The white blood cells are known for defense against invading pathogens and transforming native cells [24]. This implies that administration of this extract does not impair these functions.

5. CONCLUSION

Administration of aqueous extract of *H. sabdariffa* at varing doses for 21 days lead to improvement in erythrocytic profile without any deleterious effect on the white blood cell indices. This indicates that the extract as used in this study may be good for improvement of anaemic conditions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experimemts have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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