



## ***Hibiscus sabdariffa* Aqueous Leaf Extract Reverses Hematological Alterations in Phenyldiazine Anemic Wistar Rats**

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### **Authors' contributions**

This work was carried out in collaboration among all authors. Author AOO designed the study. Author IW performed the statistical analysis. Author JIU wrote the protocol. Author EBU wrote the first draft of the manuscript. Authors TAK and OGA managed the analyses of the study. Author BBA managed the literature searches. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Background:** The extract of *Hibiscus sabdariffa* commonly known as Sobo is widely consumed for its nutritional and health benefits. This study investigated the effect of aqueous leaf extract of *H. sabdariffa* on anemic condition caused by phenylhydrazine in rats.

**Materials and Methods:** Thirty (30) rats used for this study were divided into three groups of 10 rats each. Group 1 received distilled water and served as control. Group 2 received phenylhydrazine (40 mg/kg, i.p.). Group 3 was treated with phenylhydrazine (40 mg/kg, i.p.) 30 minutes prior to administration of (200 mg/kg, p.o.) aqueous extract of *H. sabdariffa* (Sobo) once daily for 14 days. At the end, 2 ml blood samples were collected through cardiac puncture into

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clean sample bottles containing ethylenediamine tetra acetic acid (EDTA) for hematological analyses.

**Results:** Sobo significantly increased ( $P<0.05$ ) hemoglobin (Hb) concentration and packed cell volume (PCV) in the phenylhydrazine-treated rats. Also, Sobo significantly ( $P<0.05$ ) increased total white blood cell (TWBC) in phenylhydrazine administered rats. However, the extract did not produce any significant effect on mean corpuscular hemoglobin concentration (MCHC) relative to control and anemic groups.

**Conclusion:** The findings from this study revealed that the aqueous extract of *H. sabdariffa* demonstrates anti-anemic effect in rats treated with phenylhydrazine, suggesting its ethno-pharmacological beneficial effect in anemic conditions.

*Keywords:* Sobo; anemic condition; immune system.

## 1. INTRODUCTION

*Hibiscus sabdariffa* L. is a member of the Malvaceae family. It is a medicinal plant known worldwide and used in treating different cardiovascular diseases [1-3]. In Egypt and the Sudan, it is used as a beverage that helps to lower body temperature [4] to treat cardiac conditions and as a diuretic [5-7]. It has been reported to be traditionally used in treatment of high blood pressure [8,9]. According to [10] *H. sabdariffa* L. is used in folk medicine as a diuretic, mild laxative, and treatment for cardiac and nerve diseases. It has been documented as medicinal herb in the treatment of hypertension [11-13]. It is thought that the antioxidant and diuretic effects of hibiscus are its most important mechanisms involved in lowering blood pressure [8]. According to [14] *H. sabdariffa* is highly rich in vital minerals and nutrients such as iron, copper, calcium, magnesium and manganese required for healthy growth in humans. [14] had also suggested that plant derived medicines are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment; *H. sabdariffa* readily provide such benefits. Also documented, is that some plant chemicals present in the extract such as alkaloids, tannins, saponins, glycosides, phenols and flavonoids are the bioactive agents responsible for the medicinal benefits of *H. sabdariffa*– induced reduction in hypertension and cholesterol [14]. Previous studies have also reported its beneficial effects in conditions connected with hypercholesterolemia including lowering of total cholesterol and low density lipoprotein cholesterol and increasing high density lipoprotein cholesterol [15]. Indeed, report from the study by [16] showed decreased levels of total cholesterol, very low density lipoprotein concentration and high density lipoprotein concentration in a paracetamol abused animal subjects.

The extract of *H. sabdariffa* L. is popularly consumed with ethnomedicinal claim of hematological benefits [17], but no scientific investigations have been conducted to corroborate this claim. Hence this study, designed to evaluate the effect of aqueous leaf extract of *H. sabdariffa* L. on hematological parameters viz; hemoglobin concentration, packed cell volume, total white blood cell, and platelets in phenylhydrazine treated albino rats, with a view to determining its hematoprotective efficacy as anti-anemic agent.

## 2. MATERIALS AND METHODS

### 2.1 Plant Materials

Fresh leaves of *H. sabdariffa* were purchased from Watt market in Calabar South, Cross River State, Nigeria. The leaves were authenticated by the Chief Botanist, Department of Botany, University of Calabar, Cross River State, Nigeria.

### 2.2 Preparation of Extract

The extraction was carried out according to the method of [18] with little modification. Thirty (30) grams of dried leaves was soaked in 2 liters of water for 24 hours and filtered using Whatman's No.1 filter paper as previously described [18, 19]. The filtrate was stored in clean plastic containers and refrigerated. The extract was brought out of the refrigerator 2 hours to oral administration. Different concentration of the extract was administered orally according to body weight.

### 2.3 Experimental Animals

Approval for the study was obtained from the Animal Ethical Committee of the College of Medical Sciences with the ethical approval

number: FAREC-FBMS/13/2018. Strict adherence and compliance to the guide for the care and use of laboratory animals as stipulated by the National Research Council (2011) was maintained [20]. Thirty (30) Albino Wistar rats of both sexes weighing between 130-150 g from the start of the experiment were used for this study. They were maintained in the animal facility of the Department of Physiology, University of Calabar, Nigeria, at a temperature of  $30 \pm 2^\circ\text{C}$  and 12 h light/dark cycles with free access to food and water.

## 2.4 Experimental Design

The study was carried out as previously demonstrated by [21] with little modifications. Thirty (30) rats used for this study were divided into three groups of 10 rats each. Group 1 received distilled water and served as control. Group 2 received phenylhydrazine (40 mg/kg, i.p., (anemic group)). Group 3 was treated with phenylhydrazine (40 mg/kg, i.p.) 30 minutes prior to administration of (200 mg/kg, p.o.) aqueous extract of *H. sabdariffa* (Sobo) (anemic + Sobo group) once daily for 14 days after which blood samples were collected for hematological analyses.

## 2.5 Collection of Blood Samples

Blood samples were collected using cardiac puncture through a modified procedure described by [22] into 30 EDTA bottles.

## 2.6 Statistical Analysis

Results were expressed on the mean  $\pm$  standard deviation. Statistical analysis was carried out

using SPSS software, version 16.0 (SPSS Inc. Chicago II, USA). Differences among the groups were investigated using one-way analysis of variance (ANOVA) followed by a student's t-test.  $P < 0.05$  was considered statistically significant. Variant means were separated using multiple range Duncan's post hoc test.

## 3. RESULTS

### 3.1 Effect of Aqueous Leaf Extract of *H. sabdariffa* on Hemoglobin Concentration in Rats Treated with Phenylhydrazine

The effect of Sobo on phenylhydrazine induced alteration in hemoglobin is shown in Table 1. There was a significant decrease ( $P < 0.05$ ) in hemoglobin concentration following phenylhydrazine treatment (Anemic group) when compared with Control. However, hemoglobin concentration was significantly increased ( $P < 0.05$ ) in the Sobo + Anemic treatment group when compared with Anemic group.

### 3.2 Effect of Sobo on Packed Cell Volume in Rats Treated with Phenylhydrazine

The effect of Sobo on phenylhydrazine induced alteration in mean Packed Cell Volume (PCV) is shown in Table 2. As shown in Table 2, administration of phenylhydrazine caused a significant decrease in PCV when compared with normal control. Treatment with phenylhydrazine and Sobo (200 mg/kg, p.o.) significantly reversed ( $p < 0.05$ ) the effect of phenylhydrazine on PCV when compared with anemic group.

**Table 1. Effect of sobo on hemoglobin concentration in rats treated with phenylhydrazine**

Group	Pre-induction (g/dl)	Post-treatment (g/dl)
Control		15.83 $\pm$ 0.39
Anemic	15.79 $\pm$ 0.42	10.47 $\pm$ 0.12 ####
Anemic + Sobo	10.45 $\pm$ 0.15	15.13 $\pm$ 0.22 ***

Values are expressed as mean  $\pm$  SEM,  $n = 10$ , #### $p < 0.05$  vs. control group; \*\*\* $p < 0.05$  vs. Anemic group

**Table 2. Effect of sobo on packed cell volume in rats treated with phenylhydrazine**

Group	Pre-induction (%)	Post-treatment (%)
Control		49.33 $\pm$ 1.45
Anemic	48.78 $\pm$ 2.01	33.00 $\pm$ 0.58 ####
Anemic + Sobo	33.02 $\pm$ 0.39	47.50 $\pm$ 1.00 ***

Values are expressed as mean  $\pm$  SEM,  $n = 10$ , #### $p < 0.05$  vs. control group; \*\*\* $p < 0.05$  vs. control Anemic group

### 3.3 Effect of Sobo on Phenylhydrazine-induced Mean Corpuscular Hemoglobin Alteration in Rats

The effect of Sobo on phenylhydrazine-induced alteration in mean corpuscular hemoglobin concentrations (MCHC) is shown in Table 3. Treatment with phenylhydrazine did not produce any significant change in MCHC levels when compared with normal control. Similarly, treatment with Sobo (200 mg/kg, p.o.) did not produce any significant change relative to control and anemic groups.

### 3.4 Effect of Sobo on Phenylhydrazine-induced Alteration in TWBC Counts in Rats

The effect of Sobo on phenylhydrazine-induced alteration in mean TWBC counts is shown in Table 4. As shown in the result, administration of phenylhydrazine caused a significant increase ( $p < 0.05$ ) in TWBC counts in the Anemic and Anemic + Sobo treated groups when compared with normal control. However, treatment with

Sobo (200 mg/kg, p.o.) failed to reverse the effect of phenylhydrazine on TWBC when compared with anemic group.

### 3.5 Effect of Sobo on Neutrophil Counts in Rats Treated with Phenylhydrazine

Table 5 shows the effect of aqueous leaf extract of Sobo on phenylhydrazine-induced alteration in neutrophil counts. Treatment with phenylhydrazine produced a significant increase in neutrophil counts when compared to control. Conversely, treatment with Sobo (200 mg/kg, p.o.) significantly reduced ( $p < 0.05$ ) phenylhydrazine-induced increase in neutrophil counts when compared with control and anemic groups respectively.

### 3.6 Effect of Sobo on Eosinophil Counts in Rats Treated with Phenylhydrazine

The effect of Sobo on phenylhydrazine-induced alteration in eosinophil counts is shown in Table 6. Phenylhydrazine induced a significant

**Table 3. Effect of sobo on mean corpuscular hemoglobin concentrations in rats treated with phenylhydrazine**

Group	Pre-induction (%)	Post-treatment (%)
Control		32.27 ± 0.20
Anemic	33.07 ± 0.12	31.67 ± 0.27
Anemic + Sobo	31.57 ± 0.20	31.83 ± 0.25

Values are expressed as mean ± SEM, n = 10; no significant difference amongst the groups

**Table 4. Effect of Sobo on Phenylhydrazine-induced alteration in TWBC counts in rats**

Group	Pre-induction ((X10 <sup>9</sup> /L)	Post-treatment (X10 <sup>9</sup> /L)
Control		10.00 ± 3.22
Anemic	10.52 ± 0.21	14.28 ± 1.55 <sup>###</sup>
Anemic + Sobo	14.27 ± 1.75	14.58 ± 3.37 <sup>***</sup>

Values are expressed as mean ± SEM, n = 10; <sup>###</sup> $p < 0.05$ , <sup>\*\*\*</sup> $p < 0.05$  vs. control group

**Table 5. Effect of Sobo on Neutrophil counts in rats treated with phenylhydrazine**

Group	Pre-induction (%)	Post-treatment (%)
Control		23.67 ± 6.98
Anemic	24.35 ± 2.66	89.67 ± 1.76 <sup>###</sup>
Anemic + Sobo	89.77 ± 1.67	31.75 ± 7.34 <sup>***</sup>

Values are expressed as mean ± SEM, n = 10, <sup>###</sup> $p < 0.05$  vs. control, <sup>\*\*\*</sup> $p < 0.05$  vs. anemic group

**Table 6. Effect of Sobo on Eosinophil counts in rats treated with phenylhydrazine**

Group	Pre-induction (%)	Post-treatment (%)
Control		0.67 ± 0.33
Anemic	0.65 ± 1.13	0.33 ± 0.33 <sup>###</sup>
Anemic + Sobo	0.32 ± 0.69	1.25 ± 0.29 <sup>***</sup>

Values are expressed as mean ± SEM, n = 10; <sup>###</sup> $p < 0.05$  vs. control, <sup>\*\*\*</sup> $p < 0.05$  vs. anemic group

decrease ( $p < 0.05$ ) in eosinophil counts when compared with normal control. But treatment with Sobo (200 mg/kg, o.p.) significantly increased ( $p < 0.05$ ) eosinophil counts in rats when compared with anemic and control groups respectively

### 3.7 Effect of Sobo on Phenylhydrazine-induced Alteration in Lymphocyte Counts in Rats Treated

Table 7 shows the effect of Sobo on phenylhydrazine-induced alteration in lymphocyte counts. Treatment with phenylhydrazine produced a significant decrease in lymphocyte counts when compared with normal control. However, treatment with Sobo (200 mg/kg, p.o.) significantly increased ( $p < 0.05$ ) lymphocyte count when compared with the anemic group.

## 4. DISCUSSION

The finding from this study revealed that administration of aqueous leaf extract of *H. sabdariffa* popularly known as Sobo reverses the hematological alterations induced by repeated administration of phenylhydrazine, as evident in increased hemoglobin concentration, packed cell volume and decreased neutrophil, eosinophil and lymphocytes counts respectively. However, no significant changes were observed in mean corpuscular hemoglobin and white blood cell counts respectively. Indeed, recent studies have shown that Hibiscus tea naturally lowers blood pressure similarly to some standard hypertensive drugs. This is not brand new information, as Hibiscus has been used to treat high blood pressure in both African and Asian traditional medicine [23,24]. Similarly, [25] in a study presented to the American Heart Association Scientific Session observed that drinking three cups of hibiscus tea a day lowered blood pressure by as much as 13.2% in pre- and mildly hypertensive adults. According to [14] *H. sabdariffa* is highly rich in vital minerals and nutrients such as iron, copper, calcium, magnesium, manganese required for healthy

growth in humans. Okereke, had also suggested that plant derived medicines are relatively safer than synthetic alternative, offering profound therapeutic benefits and more affordable treatment; *H. sabdariffa* readily provide such benefits [14]. Also documented, is that some plant chemicals present in the extract such as alkaloids, tannins, saponins, glycosides, phenols and flavonoids are the medicinal benefits of *H. sabdariffa* that cause reduction in hypertension and cholesterol [14]. [26] reported that hibiscus is a natural diuretic that opens the arteries and may act as natural angiotensin-converting enzyme inhibitor that slows the release of hormones which constrict blood vessels.

Two weeks administration of the aqueous leaf extract of *H. sabdariffa* showed a marked improvement in the hemoglobin and packed cell volume values in anemic rats when compared with control groups respectively. These results agree with the findings of [27-29]. However, differences exist in the part of the *H. sabdariffa* used and the method of induction of anemia; this study employed phenylhydrazine-induced anemia and the leaves of *H. sabdariffa* was used. Others, employed hemorrhagic anemia and used the calyx and seeds of *H. sabdariffa* respectively.

Hemoglobin (Hb) is the iron-containing oxygen transport metalloprotein in the RBCs of all vertebrates [30] with the exception of the fish family *channichthyidae* [31] as well as the tissues of some invertebrates. Hb plays an important role in the delivery of oxygenated blood to various organs of the body [32]. There it releases the oxygen to permit aerobic respiration to provide energy for metabolism. A healthy individual has 12 to 20 grams of Hb in every 100 ml of blood [32]. From the results obtained the Hb concentration in the anemic+ Sobo treated group showed increased level as compared to anemic group. This implies that *H. sabdariffa* consumption improved the Hb concentration in the animal. Similar trend was observed with the packed cell volume. Hematocrit is the volume

**Table 7. Effect of Sobo on Phenylhydrazine-induced alteration in lymphocyte counts in rats**

Group	Pre-induction (%)	Post-treatment (%)
Control		72.67 ± 3.84
Anemic	72.27 ± 8.09	10.00 ± 2.08 ###
Anemic + Sobo	10.10 ± 1.38	68.00 ± 7.64 ***

Values are expressed as mean ± SEM, n = 10, ###p < 0.05 vs. control, \*\*\*p < 0.05 vs. anemic group

percentage of RBCs in blood. It is normally 40.7% - 50.3% for men and 36.1% - 44.3% for women [32]. Hematocrit levels that are too high or too low may indicate a blood disorder, dehydration, or other medical conditions [33]. An abnormally low hematocrit may suggest anemia, which is a reflection of the result ( $33.00 \text{ vol}\% \pm 0.58$ ) obtained in the anemic group. The anemic+Sobo treated group showed improved hematocrit ( $47.50 \text{ vol}\% \pm 1.00$ ) indicating the impact of the aqueous extract on anemic conditioned rats. However, there was no significant difference in the MCHC among the groups. The MCHC is an indicator of changes in size and color intensity of the red cells. It implies that consumption of *H. sabdariffa* may not affect the size or color intensity of RBCs. However, further elucidation is required to clarify this assertion.

The number of leukocytes in the blood is often an indicator of disease [34]. The WBC count is an important subset of the complete blood count [35]. The normal white cell count usually lies between  $4 \times 10^9/\text{L}$  and  $11 \times 10^9/\text{L}$  and usually expressed as 4,000 – 11,000 WBCs/ $\mu\text{L}$  [36]. Previous studies have established that WBC makes up approximately 1% of the total blood volume in a healthy adult [37] making them substantially less numerous than the RBCs at 40% to 50%. Different reports have suggested that an increase in the number of leukocytes over the upper limits is usually linked to response to infection or inflammation, whereas a decrease below the lower limit weakens the immune system [38,37] suggesting that consumption of the hibiscus extract in an already prevalent medical condition associated with anemic condition may increase leukocyte counts and boost immune system in response to infection.

Lymphocytes are white blood cells [34]; also one of the body's main types of immune cells made in the bone marrow and found in blood and lymph tissues [39-41]. The normal lymphocyte range has been shown to fall between 1,000 and 4,800 lymphocytes in 1 microliter ( $\mu\text{L}$ ) of blood [34]. Lymphocyte counts above the normal range can be harmless and temporary situation due to the body's normal response to an infection or inflammatory condition [42]. Lymphocytosis is frequently associated with autoimmune diseases such as inflammatory bowel disease [42]. Lymphocyte counts below the normal range can also be temporary, however, they can occur after a cold or another infection, or be caused by intense physical exercise, severe stress, or malnutrition [43,44]. From the result obtained

( $72.67\% \pm 3.84$ ;  $10.00\% \pm 2.08$ ; and  $68.00\% \pm 7.64$ ) for control, anemic and anemic + Sobo-treated groups respectively; the lymphocyte counts in the anemic group was significantly lower as compared to control; this indicates that there was an infection in the anemic animals. However, in the anemic + Sobo-treated group, the lymphocyte count was significantly increased when compared to the anemic group. This increase may be attributable to the effect of hibiscus consumption, which enhanced the immune system to fight against infections. Similarly, an insignificant decrease in eosinophils count was observed in the anemic group as compared to control group. There was however an increased level of eosinophil when compared with control and anemic groups respectively. Eosinophils are known to destroy invading germs like viruses, bacteria, or parasites [45]. They have a role in the inflammatory response to allergies such as asthma and in various other respiratory and gastrointestinal diseases [46,42, 47]. The hibiscus extract enhanced the production of eosinophils to fight against infection and improved total well-being of the animals. Also, neutrophils are a type of WBC or granulocyte that protects the body from infections, among other functions similarly to eosinophil [48]. They make up approximately 40% to 60% of the WBCs in the body [43,42]. Studies have reported that neutrophil count may increase during infections, due to increased production in the bone marrow as with leukemia [49,50] or due to physical or emotional stress [44, 51]. They are usually the first cells to arrive on the scene when there is bacterial infection [52]. In this study, administration of aqueous extract of *H. sabdariffa* was found to reduce the neutrophils level, showing an increased improvement in anemic condition related to decreased immunity.

## 5. CONCLUSION

The findings from this study revealed that the aqueous extract of *H. sabdariffa* demonstrates anti-amnesic effect in rats treated with phenylhydrazine, suggesting its ethnopharmacological utility in conditions associated with anemic conditions.

## ETHICAL APPROVAL

All authors hereby declare that strict adherence and compliance to guidance for the care and use of laboratory animals as stipulated by the National Research Council (2011) was maintained.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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