***Original Research Article***

# Comparative Investigation of the Effects of Different Aqueous Preparations of *Hibiscus sabdariffa* (Zobo Drinks) on Haematological Parameters in Normal Wistar Albino Rats.


## ABSTRACT

**Aim:** Investigating and comparing the effect of administration of different preparations of the commonly-consumed *Hibiscus sabdariffa Linn* (Zobo) drinks on haematological parameters.
**Study design:** Comparative study using animal models (Wistar Albino Rats) with daily administration of the same concentration of different zobo drink samples.
**Place and Duration of Study:** University of Port Harcourt, Choba, Rivers State, Nigeria and its environs between November 2014 and February 2015.
**Methodology:** Thirty (30) Wistar albino rats were grouped into six (6) groups of five rats each. Group A served as the control and B was administered an unblended zobo drink. Groups C – E were administered locally-produced zobo samples and group F was a National Agency for Food and Drug Administration and Control (NAFDAC)-branded zobo drink. A concentration of 200 mg/kg body weight of the samples was administered orally to groups B – F for 21 days. Packed cell volume (PCV), haemoglobin count, white blood cell (WBC) count, red blood cell (RBC) count, platelets, neutrophils, lymphocytes, mean cell haemoglobin (MCH), mean corpuscular volume (MCV) and mean cell haemoglobin concentration (MCHC) were analyzed and compared.
**Results:** The White blood cell (WBC) count and percentage lymphocytes were significantly lower ($P < 0.05$), while Haemoglobin, Packed cell volume, Red blood cell (RBC) count and Platelet count were significantly higher *($P < 0.05$)* when compared with the control. Percentage neutrophils showed no significant difference *($P > 0.05$)* compared with the control. **Conclusion:** The zobo drinks possess haematocrit properties that result in higher levels of blood volume and may be used for the management of anaemia. They also possess the ability to reduce WBC count.



*Keywords:* ***[Hibiscus sabdariffa; Zobo; blood; haematology; anaemia; leukocytosis]***


## 1. INTRODUCTION

*Hibiscus sabdariffa* Linn, a tropical plant, belongs to the super order Malvaceae. It is believed to have originated from East Africa [1]. *H. sabdariffa* plants are cultivated and consumed as vegetable and tea, whereas other *Hibiscus* varieties are cultivated because of their rich fibre content. *H. sabdriffa* is commonly known as Roselle and sorrel in English and Zobo and Isapa in Nigerian [2]. Various types of highly valued food and medicinal products are produced from parts of the *Hibiscus sabdariffa* including the seeds, leaves, fruits and roots. Among them, the fleshy red calyces are the most popular [3]. The flowers are large, short-peduncled, red to yellow with dark center. The accrescent large and fleshy sepals become enlarged and succulent, making excellent jelly. *Zobo* drink being made with part of a plant is believed to be highly nutritive and has many medical potentials including reduction in blood pressure, anti-diabetic, reduction in weight, antihyperlipidemic, hepatoprotective, anti-cancer, as well as an antioxidant and others [4, 5, 6, 7, 8].

In Nigeria, the dried calyces of this plant are processed into a refreshing non-alcoholic local beverage commonly called *zobo, zoborodo* or Isapa (pronounced Isakpa) [9].

Haematology is the branch of medicine concerned with the study of blood, the blood-forming organs and blood diseases. Haematology includes the study of blood, diagnosis, treatment and prevention of blood-related diseases which affect the production of blood and its components. These components include (blood cells, haemoglobin and blood proteins). Haematological Indices also called Heme Profile or Complete Blood Count (CBC), is often carried out to relate the extent of organ damage (Liver and Pancreas) especially those associated with diabetes mellitus. These include the measurement of white blood cells (WBCs), red blood cells or erythrocytes (RBCs), Haemoglobin (Hb), Haematocrit or packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular

haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), erythrocyte sedimentation rate (ESR), Lymphocytes, Monocytes, Basophils, Eosinophil and Platelets [10].

Following increased religious and health awareness against consumption of alcoholic beverages in Nigeria and the consequent decrease in the consumption of alcoholic beverages in certain areas, Zobo drink has great potential as a local alternative to imported red wines in particular and alcoholic beverages in general [7]. Moreover, production of this and similar local beverages has become the main source of income in many homes in the rural communities and more recently in the urban areas where these have grown to cottage business proportions as a result of following governmental interventions through the poverty alleviation schemes, thereby alleviating poverty among the people [8].

Zobo is mostly consumed by low and middle class people due to its relative low cost, because the *Hibiscus sabdariffa* calyces and the ingredients are cheap and easy to find. It has recently gained wider acceptance, being consumed by several millions of people from different socio-economic classes and background in the West Africa sub-region and particularly in Nigeria.

Within the University of Port Harcourt and Choba community, there is high rate of consumption of Zobo drink by both students and staff. This is reflective of the perception by many, that, zobo drink is highly nutritious, medicinal and of course, cheaper (following the prevailing economic downturn in Nigeria) than other non-alcoholic beverages sold around and within the environment.
Therefore, the present study aimed to elucidate the impacts of different preparations of Zobo drink on haematological parameters using Wister albino rats.

## 2. MATERIAL AND METHODS

### 2.1 Chemicals and Reagents

All chemicals and reagents used in this study were of high analytical grade.

### 2.2 Plant Material

Dried calyces of the plant were bought from Choba market, Port Harcourt. They were authenticated in the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt as *Hibiscus sabdariffa.*

### 2.3 Samples and Preparations

The unblended zobo drink sample (ZSTD) was prepared using the method described by Ogundapo *et al,* 2014 [11]. The dry calyces of *Hibiscus sabdariffa* (HS) were carefully sorted to remove dirt and other unwanted materials. Sixty grams (60g) of the dry HS calyces were washed with cold water and added to two litres of boiling distilled water. It was allowed to boil for 15 minutes and then cooled. After cooling, the mixture was sieved with muslin cloth and filtered with Whatman No. 1 filter paper. The clear filtrate was covered with aluminium foil and stored in the refrigerator at $4^0$C until use.
The ZOBO COLA was also used for comparison. This is a commercially sold branded 40cl Zobo drink produced by Zobo Cola Company Ltd, Nigeria, certified by National Agency for Food and Drug Administration and Control (NAFDAC) and sold in shops within the community. The ingredients used to prepare the Zobo Cola include: purified water, HS extracts, aspartame, sugar, Cola flavour, ginger and citric acid. Other drink samples were obtained from shops around the community. ZAP1 (Zobo drink sold within Abuja Campus Park): This was prepared using the following ingredients: Dried HS calyces, tap water, ginger, zobo pepper and flavourings. The flavouring used was Joccy® Pineapple flavour with NAFDAC No. A1-2269, manufactured by Kaadan Nigeria Ltd, Kano. Half rubber (about 3 litres capacity) of cleaned *Hibiscus sabdariffa* calyces were added to about 20L of boiling water. Chopped ginger and zobo pepper were then added to the boiling solution. The mixture was allowed to boil for about 25 minutes and then allowed to cool overnight. In the morning, it was sieved, the flavour added and then mixed properly. The ready zobo drink was then packed in recycled 50cl plastic containers and cooled with blocks of ice.
ZAP2 (another Zobo drink sold around Abuja Park by another local producer) and ZCHO (Zobo drink sold in one of the shops in Choba campus) were also used.
The local producers use almost the same method and ingredients as stated above. The differences are either with the proportions of the ingredients used and the particular flavouring (particularly pineapple, orange and cola flavours).

## 2.4 Experimental Animals

Thirty (30) Wistar Albino rats weighing between 110 - 195g were used for the study. The animals were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, Choba, Rivers state. All the animals were housed in the animal house, University of Port Harcourt, Choba Campus – using plastic cages covered with wire gauze and given standard food pellets (Top Feeds' grower's mash) and water *ad libitum*. They were acclimatized for 2 weeks under normal conditions of light (12/24 hour) and temperature (26±4 $^{0}$C). They were marked for easy identification and monitoring, after their baseline weights were taken. All procedures and techniques in handling the animals were according to standard methods and complied with the guidelines of the National Institutes of Health of the United States [12].

## 2.5 Experimental Design and Administration of Samples

The acclimatized albino rats were sorted according to their weights into six groups of five rats each. Group A was fed the normal rat feed with water and served as the control. Groups B – F served as experimental groups and were administered 200 mg/kg body weight of the respective samples via oral intubation for a period of 21 days. Group B was administered the unblended zobo drink (ZSTD). Group F was administered the Zobo Cola (ZCOLA), while groups C, D and E were administered with other drink samples (ZAP1, ZAP2 and ZCHO respectively) obtained from shops around the community. All animals were allowed access to water and food for the 21 days.

## 2.5 Sacrificing of Animals and Collection of Blood Samples

All the animals from the groups were sacrificed at the end of the administration period. The animals were incapacitated with chloroform in a desiccator. Under this condition, the rats were dissected using dissecting tools and the blood was collected and put into lithium heparin and EDTA anticoagulant tubes (to prevent blood clotting) for haematological analyses.

## 2.6 Determination of Haematological Parameters

Haemoglobin concentration was estimated using Drabkin's Method or Haemoglobinocyanide (HICN) Technique. Packed Cell Volume (PCV) was determined by the Micro-haematocrit reader according to [13]. Total white blood cell count was estimated by visual count method using Turke's solution to lyse the red blood cell, leaving the white blood cells to be counted. The red cell count was estimated by visual method, viewed under the microscope. MCV was calculated as the ratio of the packed red cell volume to the volume of the red blood cell multiplied by the factor of ten, MCH as the ratio of the haemoglobin concentration to the red blood cell count and MCHC as the ratio of the haemoglobin concentration to the packed red cell volume – all as reported [13].

## 2.7 Statistical Analysis

All data obtained in this study were subjected to statistical analyses using One-way Analysis of Variance (ANOVA). Tukey's Multiple Range Test was used to test for differences between the administration groups. All analyses were done using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Statistics, UK). All the values were reported as means ± standard error of mean (SEM) and the results were considered significant at p-values of less than 0.05 ($P < 0.05$) i.e. at 95% confidence level.

# 3. RESULTS AND DISCUSSION

The results for the means and standard error of mean (SEM) of Haematological parameters [Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell count (RBC), White Blood Cell count (WBC), Platelet count (PLT), Neutrophils (Neu), Lymphocytes (Lym), Mean Corpuscular volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC)] of the Wistar albino rats are shown in "Table 1".

From the results of the haematological parameters, Haemoglobin (Hb) level was significantly higher *($P < 0.05$)* in groups B, D, E and F (13.65±0.51, 13.20±0.74, 12.35±0.56 and 12.33±0.34) when compared with the control (10.43±0.14), while the increase in group C (11.50±0.44) was not statistically different *($P > 0.05$)* when compared with the control (Group A). The packed cell volume

(PCV) was significantly higher *(P < 0.05)* in groups B, D and E (41.0±1.14, 39.5±1.20, 37.00±1.70 and 37.00±0.89) when compared with the control (31.00±1.18), while the increase in group C (34.5±0.59) was not statistically different *(P > 0.05)* when compared with the control (Group A). The red blood cell (RBC) count was significantly higher *(P < 0.05)* in groups B, D, E and F (5.50±0.16, 5.43±0.16, 5.32±0.22 and 4.86±0.04), while the increase in group C (4.70±0.13) was not statistically different *(P > 0.05)* when compared with the control (4.10±0.12). The white blood cell (WBC) count in groups B, C and D (5.30±0.22, 3.10±0.14 and 4.50±0.23) was significantly lower *(P < 0.05)* than the control (7.10±0.67) and there was no significant difference *(P > 0.05)* in groups E and F (6.60±0.21 and 6.00±0.32) when compared with the control (Group A). The platelet count in groups C, D, E and F (334±15.68, 300±15.68, 308±4.90 and 300±0.00) was significantly higher *(P < 0.05)* than the control (250±22.40), while there was no significant difference *(P < 0.05)* in group B (226±11.22) when compared with the control (Group A). The percentage lymphocytes was significantly lower *(P < 0.05)* in group B (59.60±1.63) when compared with the control (67.40±1.12), while there was no significant difference in groups C, D, E and F (67.80±1.85, 65.60±1.93, 68.00±1.22 and 66.40±1.57) when compared with the control (Group A). The percentage Neutrophil, MCH, MCHC and MCV were not significantly affected *(P > 0.05)* in this study.

**Table 1. Effect of 21 days administration of different zobo drinks on haematological parameters of wistar albino rats**

| GROUP | A (CNTRL) | B (ZSTD) | C (ZAP1) | D (ZAP2) | E (ZCHO) | F (ZCOLA) |
|---|---|---|---|---|---|---|
| **HB (g/dL)** | 10.43 ± 0.14[a] | 13.65 ± 0.51[a] | 11.50 ± 0.44[a] | 13.20 ± 0.74[b] | 12.35 ± 0.56[b] | 12.33 ± 0.34[b] |
| **PCV (%)** | 31.0 ± 1.18 [a] | 41.0 ± 1.14 [b] | 34.5 ± 0.59 [a] | 39.5 ± 1.20 [b] | 37.0 ± 1.70[b] | 37.0 ± 0.89[b] |
| **RBC ($x10^{12}$/L)** | 4.10 ± 0.12 [a] | 5.50 ± 0.16 [b] | 4.70 ± 0.13 [a] | 5.43 ± 0.16 [b] | 5.32 ± 0.22[b] | 4.86 ± 0.04[b] |
| **WBC ($x10^{9}$/L)** | 7.10 ± 0.67 [a] | 5.30 ± 0.22[b] | 3.10 ± 0.14 [b] | 4.50 ± 0.23 [b] | 6.60 ± 0.21[a] | 6.00 ± 0.32[a] |
| **PLT ($x10^{9}$/L)** | 250 ± 22.4 [a] | 226 ±11.22[a] | 334 ±15.68[b] | 300 ±15.81[b] | 308 ± 4.90 [b] | 300 ± 0.00 [b] |
| **Neu (%)** | 33.6 ± 1.57 [a] | 40.4 ± 1.63 [a] | 32.2 ± 1.85 [a] | 34.4 ± 1.94 [a] | 32.0 ± 1.22[a] | 33.6 ± 1.57[a] |
| **Lym (%)** | 67.4 ± 1.12 [a] | 59.6 ± 1.63 [b] | 67.8 ± 1.85 [a] | 65.6 ± 1.93 [a] | 68.0 ± 1.22[a] | 66.4 ± 1.57[a] |

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|---|---|---|---|---|---|---|
| **MCV (fL)** | 7.54 ± 0.08 [a] | 7.43 ± 0.03 [a] | 7.23 ± 0.30 [a] | 7.33 ± 0.09 [a] | 6.97 ± 0.31[a] | 7.58 ± 0.15[a] |
| **MCHC (g/L)** | 0.34 ± 0.01 [a] | 0.33 ± 0.00 [a] | 0.33 ± 0.01 [a] | 0.33 ± 0.01 [a] | 0.33 ± 0.00[a] | 0.33 ± 0.00[a] |
| **MCH (pg)** | 2.55 ± 0.06[a] | 2.47 ± 0.04 [a] | 2.42 ± 0.12 [a] | 2.42 ± 0.02 [a] | 2.33 ± 0.10[a] | 2.52 ± 0.05[a] |

Values in the table are means ± Standard error of mean (SEM) and n = 5. At ($P < 0.05$), mean values with different superscripts in a row are significantly different from the control. Hb = Haemoglobin, PCV = Packed Cell Volume, RBC = Red blood cell count, WBC = White blood cell count, PLT = Platelet count, Neu = Neutrophils, Lym = Lymphocytes, MCV = Mean Cell Volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration.

Physiological and nutritional status of animals can be ascertained using blood parameters. Changes in haematological parameters have been used to elucidate the impact of nutritional factors and/or additives supplied in diets of living organisms [14]. The significant higher levels of PCV, Hb and RBC in groups B, D, E and F (also in group C, but not significantly) corroborates the results of [15], which showed significant elevation of Hb, PCV and RBC levels in the study of effect of aqueous extract of *Hibiscus sabdariffa* calyces on haematological characteristics of rattus novergicus. This suggests that the extract may possess haematocrit properties that ultimately result in increased blood volume. This clearly indicated that there was an increase in the rate of production of RBCs (erythropoiesis) as well as a decrease in the destruction of matured RBCs during the study period [15]. This means that the extract has the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production [16, 17]. This is in line with the earlier report of [18] which showed that the PCV, Hb content and RBC count are associated with the total population of red blood cells. The haematological results is also in line with the reports of [19] and [20], who observed significant elevation *($P < 0.05$)* in PCV, Hb, WBC and RBC values of rats following treatment with aqueous H. sabdariffa calyx extracts – suggesting the use of *Hibiscus sabdariffa* in the management of anemia. Severity and patients' response to treatment of anaemia is usually monitored using Haemoglobin concentration and packed cell volume (PCV) [13]. Therefore, the results suggest that the drink samples – ZSTD, ZAP2, ZCHO and ZCOLA were all beneficial in terms of increasing total blood volume and management of anaemia, because of the significant higher levels of these indices in animals fed with these samples when compared with control. According to [21], some studies have shown that some substances like food and leaves have been associated with increase or decrease in Hb and PCV. Elevated levels of Hb, RBC and PCV of albino rats in this study are in consonance with this. However, this present study is not in consonance with the elevation of the WBC values which was significantly lower in this study. Also, this study is at variance with the report of [22], which showed that the administration of the aqueous extract of *H. sabdariffa* calyx showed no significant effect on haematocrit, haemoglobin, red blood cell count and platelet count when compared with the control. A high number of WBC results in an abnormality called leukocytosis. Therefore, the significant lower levels of WBC in groups B, C and D suggest that the drinks may be beneficial in the management of leukocytosis (abnormal high level of white blood cell count). However, further research is suggested to ascertain the possible active constituents that are responsible for the beneficial effects highlighted in this study.

## 4. CONCLUSION

This study aimed at investigating and comparing the effects of 21 days administration of different preparations of *Hibiscus Sabdariffa* (Zobo) drink consumed around the University of Port Harcourt community on haematological parameters using normal albino rats. From the study, zobo drinks have haematocrit potentials of increasing blood volume and management of anaemia as evidenced by

higher levels of PCV, Hb and RBCs in the groups administered with the different zobo drinks. Furthermore, all the zobo drinks regardless of the different preparations are believed to have positive impact on haematological parameters as well as possessing properties which may be significantly important in the management of leukocytosis.

## 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 where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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