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AQUEOUS EXTRACTS OF PROCESSED *HIBISCUS SABDARIFFA* SEEDS ATTENUATE HAEMOLYTIC ANAEMIA IN WISTAR ALBINO RATS

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ABSTRACT

Anaemia is a haematological disorder characterized by reduced red blood cells (RBCs) or haemoglobin (HB) or lowered ability of blood to carry oxygen leading to increased risk of morbidity and mortality. The anti-anaemic potential of aqueous extracts of Hibiscus sabdariffa seeds (HSS) was evaluated in this study. One-hundred and eighty (180) adult albino rats were divided into twelve groups with three replicates of 5 rats each (n =15) and designated as Group 1 (normal control), Group 2 (positive control, treated with standard drug), Group 3 (negative control, untreated) and Groups 4 – 6 (treated with 200, 400 and 600 mg.kg^{-1} of aqueous extracts of raw, HSS respectively), Groups 7 – 9 (treated with 200, 400 and 600 mg.kg^{-1} of aqueous extracts of boiled HSS respectively) and Groups 10 – 12 (treated with 200, 400 and 600 mg.kg^{-1} of aqueous extracts of fermented HSS respectively). The acute toxicity test revealed no toxic effect of HSS at 5000 mg.kg^{-1} and haemolytic anaemia was induced with 350 mg.kg^{-1} b.w of Zidovudine. After 28 days of extract administration, the animals were humanely sacrificed and blood samples collected for haematological analysis using Automated Haematology Analyzer. RBC count, HB concentration and packed cell volume (PCV) decreased significantly ($p<0.05$) after Zidovudine induction, but the aqueous extracts of raw, boiled and fermented HSS significantly ($p<0.05$) increased HB, RBC, PCV, white blood cells (WBC) and neutrophil levels when compared with negative control. The results suggest that aqueous extracts of HSS attenuated hematological indices by increasing RBC and haemoglobin production and could be utilized in management of anaemia.

Keywords: Anti-anaemia, Haematology, Haemoglobin, *Hibiscus sabdariffa* seeds, Red blood cells, Zidovudine

INTRODUCTION

Anaemia, with a global prevalence of 24.8 % (1.62 billion people), is a common blood disorder affecting several categories of individuals – with infants, elderly and child-bearing age women more vulnerable (WHO, 2011). The prevalence of anaemia is higher in developing nations and anaemia can develop in diseased persons, malnourished persons and patients with blood parasites (Ogbe *et al.,* 2010). Haemolytic anaemia is a consequence of the premature destruction of RBCs (Schick and Nagalla, 2017). Haemolytic anaemia kicks in when bone marrow activity cannot compensate for RBC loss. Anti-anaemic drugs are usually

very expensive, making it tough for poor people to access in Nigeria and most rural dwellers cannot get access to them. Thus, plants and herbal products like *Fagara zanthoxylum, Carica papaya, Cajanus cajan, Terminilia catappa, Piper guinensis* and *Telfairia occidentalis* become good alternatives in the treatment of sickle cell anaemia (Oboh, 2004; Singh *et al.,* 2013).

Hibiscus sabdariffa Linn (Family: Malvaceae), is a herb that is cultured for its leaf, fleshy calyx, seed or fiber (Okasha *et al.,* 2008) and it is understood to come from East Africa (Ilondu and Iloh, 2007). *H. sabdariffa* is largely cultivated in the tropics (e.g. Central America, Africa, India, Caribbean, Brazil, Hawaii etc.) as a home-garden crop. It serves as a notable export crop in Sudan after millet and Sesamum (Leung and Foster, 1996; Gautam, 2004). *H. sabdariffa* seeds (HSS) are generally considered to have medicinal value and contain a high level of nutrients (Tounkara *et al*., 2011). *H. sabdariffa* seeds have been used in the treatment of anaemia by the natives in Western Sudan (Ahmed *et al.,* 2013).

H. sabdariffa seeds are fermented into Mungza ntusa, a condiment in Northern Nigeria (Omobuwajo *et al.,* 2000). In northern Cameroon, *H. sabdariffa* seeds are used to prepare Mbuja, another fermented condiment. Mbuja is also called Bikalga, Dawadawa botso, Datou and Furundu in Burkina Faso, Niger, Mali and Sudan respectively (Parkouda *et al.,* 2008).

The high global pervasiveness of anaemia, coupled with low economic wellbeing and poor nutritional status of many Nigerians, have called for serious consideration and search for cheaper and more effective management models. This may well embrace the usage of locally available plants which possess certain medicinal potentials to alleviate some types of anaemia. This study was aimed at evaluating the anti-anaemic potentials of extracts of *H. sabdariffa* seeds on Zidovudine-induced haemolytic anaemia in rats.

MATERIALS AND METHODS

Plant Sample Collection and Preparation: *H. sabdariffa* seeds were collected from a local farm in Mangu Local Government Area of Plateau State, Nigeria. The sample was identified (Chukwu *et al.,* 2019; WFO, 2021) and authenticated at the Herbarium of Plant Science and Biotechnology, University of Port Harcourt (Voucher Specimen Number: UPH/H/011). They were sorted, cleaned and stored in sealed container pending use.

Processing of *Hibiscus sabdariffa* Seeds

Raw: The raw seeds of *H. sabdariffa* were gotten rid of debris, properly cleaned and dried. They were ground into powder and stored in a sealed plastic container in a refrigerator.

Boiling: A modified method of Mariod *et al.* (2013) was adopted. Raw *H. sabdariffa* seeds (600 g) were boiled in 500 ml of distilled water for 40 minutes till they become softened when squeezed between the fingers. The boiled seeds were drained, dried, milled into powder and stored in a sealed container for further analyses.

Fermentation: A modified method of Parkouda *et al.* (2008) was employed. After boiling and draining off water from boiled seeds, the seeds were left in a sealed container and allowed to ferment for four days. They were dried, ground into powder with an electric blender and stored in sealed container in a refrigerator.

Preparation of Plant Extract: 100 g each of the ground samples of *H. sabdariffa* seeds were separately macerated in 400 ml of distilled water. They were left to stand for 24 hours, with intermittent shaking for proper homogenization and extraction. The resultant solutions were then filtered using a clean muslin cloth and the resulting filtrate was further filtered using Whitman No. 1 filter paper. The final extract was refrigerated at 4 ^{0}C pending use.

Acute Toxicity Test (LD_{50}): The median lethal doses (LD_{50}) tests of *H. sabdariffa* seed extract was carried out in two phases using the method of Lorke (1983).

Experimental Animals: One-hundred and eighty adult rats weighing 180 – 240 g acquired from the Animal House (Department of Physiology, University of Port Harcourt) were used for the study. The weights were balanced in each group in order to eliminate errors. The adult animals were acclimatized to the laboratory environment for seven days and marked for easy identification and monitoring after their baseline weights had been taken. All animals had access to normal rat feed (Vital Feeds® with 18.0 % crude protein and 2800 kcal/kg metabolizable energy) and water *ad libitum.* Weekly weights were recorded accordingly. All processes and procedures in handling the rats were in compliance with the guidelines of the National Research Council (NRC, 2010). Ethical approval (UPH/CEREM/REC/MM61/009) was granted by the Research Ethics Committee of The University of Port Harcourt.

Induction of Anaemia: Haemolytic anaemia was induced by administration of 350 $mg.kg^{-1}$ of Zidovudine-containing Highly Active Antiretroviral Therapy (HAART) regimen for 1 week via oral intubation – after their baseline haematological parameters were determined. Anaemia was established by comparing the values of PCV after one week of induction with the baseline values determined before the induction. There were significant reductions (≤22 %) in the PCV values, which are characteristic of anaemic conditions.

Experimental Design: In a Completely Randomized Design (CRD), the animals were weighed and distributed evenly into twelve treatment groups, replicated thrice with each replicate containing five rats. Group 1 served as normal control and treated with normal saline. Group 2 served as positive control treated with standard anaemia drug (Erythropoietin). Group 3 served as negative control (NGC) induced with anaemia but left untreated. Groups 4 – 12 were treated with 200, 400 and 600 $mg.kg^{-1}$ of aqueous extracts of raw, boiled and fermented *H. sabdariffa* seeds respectively. At the end of the experiment, the animals were sacrificed under mild anaesthesia with chloroform. Blood samples were obtained into EDTA anticoagulant tubes for haematological analysis.

Determination of Bodyweight: The bodyweights of the experimental animals were measured weekly using an electronic weighing balance and used in calculation of weight gain.

Determination of Haematological Parameters: The haematological parameters including haemoglobin (Hb), red blood cell (RBC) count, packed cell volume (PCV), white blood cell (WBC) count, platelets, neutrophils, lymphocytes, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were assayed using MY-B002B Auto Hematology Analyzer (Maya Medical, China). The Auto Hematology Analyzer was powered on and the blood samples in the EDTA bottles were presented to the sample probe in the Auto Hematology Analyzer one after another for aspiration. The samples were analyzed immediately the blood samples are aspirated. The result for each sample was displayed on the display unit and then printed.

Statistical Analysis: Analysis of Variance (ANOVA) was used to test for differences between the treatment groups. The data were considered significant at *p*-values less than 0.05 ($p<0.05$) using Tukey's Post Hoc test. All analyses were done using Statistical Product and Service Solutions (SPSS) version 20.0 (IBM Statistics, United Kingdom). All values are reported as means ± standard error of mean (SEM).

RESULTS

Acute Toxicity (LD_{50}) Test: The acute toxicity test (Table 1) showed that the aqueous extracts of raw, boiled and fermented HSS caused no mortality at maximum concentration of 5000 $mg.kg^{-1}$ and thus safe for the study.

Changes in Bodyweight: The result of the variations in average bodyweight of the animals (Table 2) showed 7.97 % loss in the bodyweight of the negative control (NGC) animals.

Table 1: Acute toxicity test of aqueous extracts of *Hibiscus sabdariffa* seed

Experiment	Dose (mg.kg $^{-1}$/ b.w)	Mortality rate
Phase 1		
Group 1	10	0/3
Group 2	100	0/3
Group 3	500	0/3
Phase 2		
Group 1	1000	0/3
Group 2	2900	0/3
Group 3	5000	0/3

Table 2: Effect of administration of aqueous extract of raw, boiled and fermented *Hibiscus sabdariffa* seeds on mean bodyweight of animals after 28 days

S/N	Group	Initial weight (g)	Final weight (g)	Weight difference (%)
1	**NC**	184.75 ± 2.22	200.40 ± 16.33	8.47
2	**PC**	206.67 ± 21.38	280.23 ± 67.82	35.59
3	**NGC**	213.20 ± 39.25	196.20 ± 8.94	-7.97
4	**HSR 1**	236.40 ± 19.85	242.35 ± 28.63	2.52
5	**HSR 2**	218.33 ± 5.50	240.40 ± 5.30	10.11
6	**HSR 3**	202.20 ± 13.02	244.90 ± 29.66	21.12
7	**HSB 1**	198.20 ± 10.45	216.70 ± 16.50	9.33
8	**HSB 2**	212.63 ± 7.50	241.90 ± 9.50	13.77
9	**HSB 3**	218.10 ± 15.25	265.20 ± 18.45	21.60
10	**HSF1**	196.64 ± 44.50	184.25 ± 43.36	-6.13
11	**HSF2**	228.20 ± 24.10	272.50 ± 33.47	19.41
12	**HSF3**	198.75 ± 19.55	215.30 ± 19.18	8.33

Values in the Table are means ± Standard Error (SE) and n = 15. (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS)

Reduction of weight was also observed in rats administered 200 mg.kg^{-1} of fermented HSS (HSF1) (6.13 %). Highest weight gain was observed in the positive control group (PC) (35.59 %). There was no remarkable difference in the weight change of normal control (NC) (8.87 %), HSB1 (9.33 %) and HSF3 (8.33 %) and also between HSR3 (21.12 %), HSB3 (21.60 %) and HSF2 (19.41 %).

Effect of Administration of Aqueous Extracts of Raw and Processed HSS on Haematological Indices of Zidovudine-Induced Anaemic Albino Rats: The results of the effect of raw, boiled and fermented HSS on haematological indices of Zidovudine-induced anaemia in albino Wistar rats are presented in Figures 1 – 10.

The haemoglobin (HB) (Figure 1), red blood cell (RBC) count (Figure 2), packed cell volume (PCV) (Figure 3), white blood cell (WBC) count (Figure 4), platelet (PLT) count (Figure 5) and neutrophils (NEU) (Figure 6) in NGC group were significantly lower ($p<0.05$) than the corresponding parameters in all the other groups.

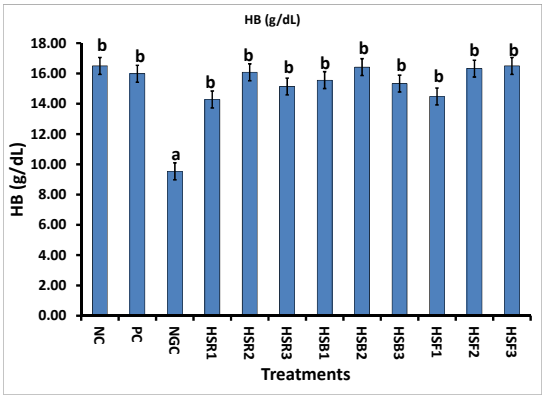

Figure 1: Effect of administration of aqueous extract of raw, boiled and fermented *Hibiscus sabdariffa* seeds on haemoglobin level of Zidovudine-induced anaemic albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At $p<0.05$, bars with different superscripts are significantly different*

These were shown by statistically significant elevation ($p<0.05$) of these parameters in the PC Group and different concentrations of raw,

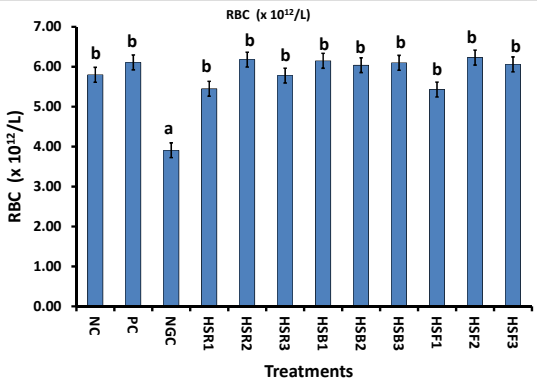

Figure 2: Effect of administration of aqueous extract of raw, boiled and fermented HSS on red blood cell count of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

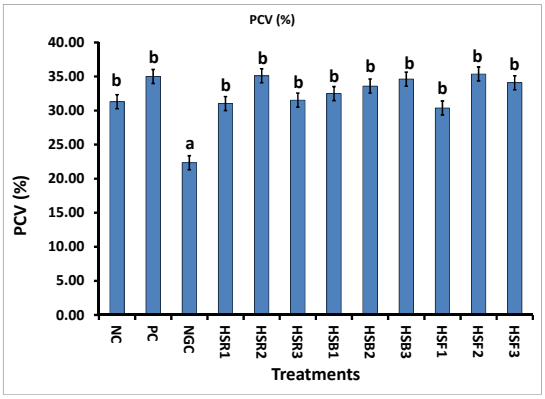

Figure 3: Effect of administration of aqueous extract of raw, boiled and fermented HSS on packed cell volume of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

boiled and fermented HSS. The levels of these parameters (HB, RBC, PCV, WBC and NEU) were not significantly different (p>0.05) when compared with PC Group treated with standard drug (rHU EPO), except for WBC in HSR3 (18.70 ± 4.20 x 10^9 L^{-1}) which was significantly higher

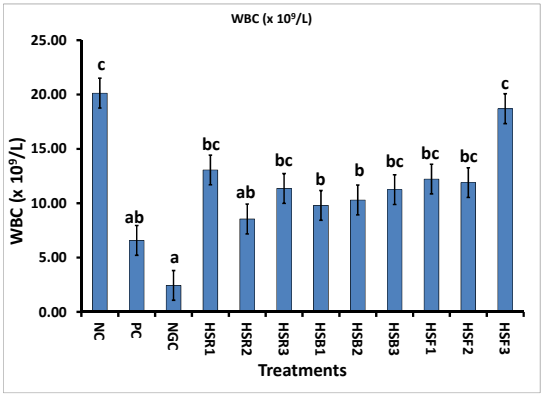

Figure 4: Effect of administration of aqueous extract of raw, boiled and fermented HSS on white blood cell of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

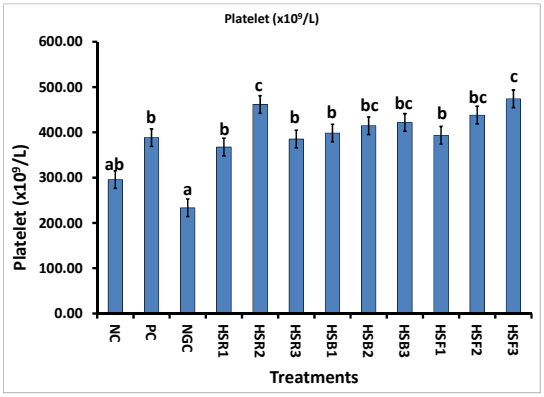

Figure 5: Effect of administration of aqueous extract of raw, boiled and fermented HSS on platelet count of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

(p<0.05) than that of the PC Group (6.58 ± 1.02 x 10^9 L^{-1}). The platelet count was observed to be least in the NGC group (233.40 ± 38.01 x 10^9/L), while the count for the treatment groups were between 367.60 ± 41.90 x 10^9 L^{-1} in HSR1

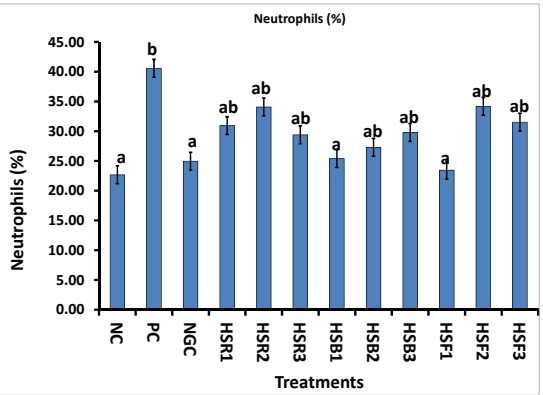

Figure 6: Effect of administration of aqueous extract of raw, boiled and fermented HSS on neutrophils count of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

and 474.00 ± 37.76 x 10^9 L^{-1} in HSF3. Only HSF3 differed significantly (p<0.05) from the normal control (295.60 ± 50.17 x 10^9L^{-1}) and NGC. No significant difference (p>0.05) was observed for lymphocytes (%) (Figure 7) in all the extract-treated groups (57.05 ± 7.86 – 69.16 ± 3.19) when compared with the NC (68.73 ± 1.59) and NGC (64.96 ± 3.20).

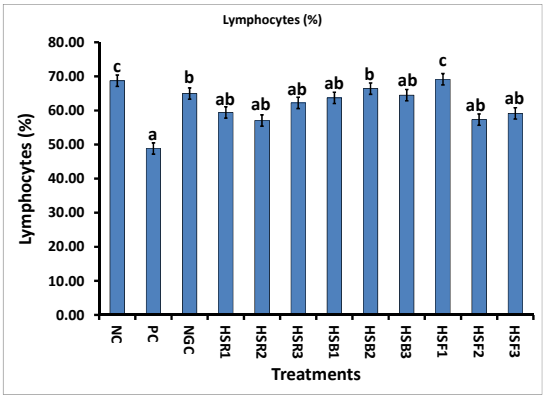

Figure 7: Effect of administration of aqueous extract of raw, boiled and fermented HSS on lymphocytes count of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

However, HSF1 (69.16 ± 3.19), HSB2 (66.40 ± 2.40) and NC (68.73 ± 1.59) were significantly higher (p<0.05) than the PC Group (48.85 ± 5.30). There was an insignificant difference (p>0.05) in MCV (Figure 8) and MCH (Figure 9) within all the groups. However, the level of MCH in NC (28.40 ± 0.74) was significantly lower than NGC, HSR1, HSR2 and HSR3 (23.80 ± 0.51, 25.70 ± 0.55, 25.83 ± 0.69 and 26.13 ± 0.24 respectively).

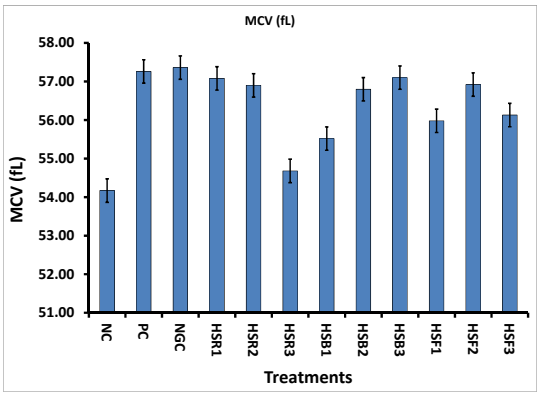

Figure 8: Effect of administration of aqueous extract of raw, boiled and fermented HSS on mean cell volume of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

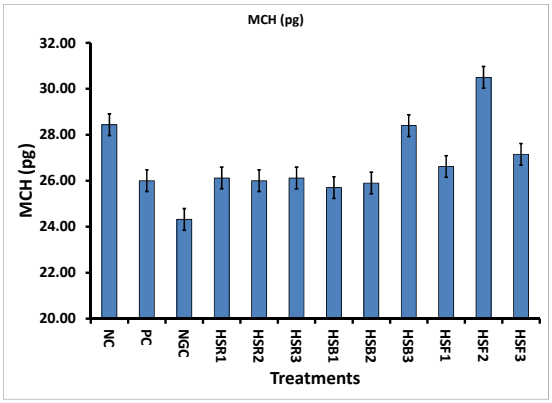

Figure 9: Effect of administration of aqueous extract of raw, boiled and fermented HSS on mean cell haemoglobin of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

MCHC (g.L^{-1}) (Figure 10) in NGC (42.52 ± 0.89) was lowest in all the groups but showed statistically significant difference ($p<0.05$) in NC (52.88 ± 0.46), HSR3 (47.94 ± 0.67), HSF1 (47.76 ± 1.22) and HSF3 (48.55 ± 1.17).

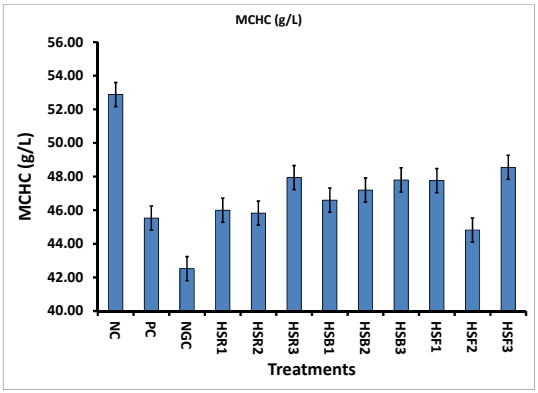

Figure 10: Effect of administration of aqueous extract of raw, boiled and fermented HSS on mean cell haemoglobin concentration of Zidovudine-induced anemia in albino rats. *Key: (NC = Normal Control, PC = Positive Control, NGC = Negative Control, HSR1, HSR2 and HSR3 = 200, 400 and 600 mg/kg of aqueous extract of raw HSS; HSB1, HSB2 and HSB3 = 200, 400 and 600 mg/kg of aqueous extract of boiled HSS. HSF1, HSF2 and HSF3 = 200, 400 and 600 mg/kg of aqueous extract of fermented HSS). Bars are means ± Standard error of mean (SEM) and n = 15. At p<0.05, bars with different superscripts are significantly different*

DISCUSSION

Toxicity of Extract: The acute toxicity test results of the aqueous extracts of *H. sabdariffa* on albino rats showed no death or adverse reactions in the rats as earlier reported by Amos *et al*. (2003) and Ali *et al*. (2005). This suggests its safety for use in experimental animals.

Effects on Body Weight: The changes in weight of the animals used in the anti-anaemic study showed that there was a 7.97 % reduction in the weight of the NGC animals; however, there was no relationship between increase in concentration of the extracts and weight gain. This suggested that the untreated anaemia in NGC probably affected the weight of the animals in that group. Earlier report had shown that polyphenolic components seeds of *H. sabdariffa* have the ability to manage the weight of rats fed high fat diets (Herrera-Arellano *et al.,* 2004). This study was in agreement with Herrera-Arellano *et al.* (2004) as seen in the groups administered 200 mg/kg aqueous extracts of HSR and HSF.

Effect of Administration of Aqueous Extracts of Raw and Processed HSS on Haematological Indices of Zidovudine-Induced Anaemic Albino Rats: The mechanism of ZDV-induced anaemia is due to the inhibition of proliferation of blood cell progenitor cells in a time-and dose-dependent fashion (Groopman, 1990; Miles, 1992). The reduction in the haematocrit parameters is most likely as a result of the induction of anaemia using a Zidovudine-containing HAART regimen. The elevation of these parameters was in concordance with the work of Akase *et al.* (2004), whose study showed trends toward the improvement of the indices of anaemia i.e. RBC count, HB level, PCV and serum iron. Ahmed *et al.* (2013) reported a similar result for *H. sabdariffa* seeds extract on haematological parameters of anaemic rats. The outcome of this study was in agreement with the report of Agbor and Odetola (2001), who also observed a significant ($p<0.05$), progressive and dose-dependent increases in RBC count, HB, PCV and reticulocyte of anaemic rats administered 400, 800 and 1600 mg.kg $^{-1}$ of *Paraqutina ingrescens* aqueous extract daily for four weeks. Such dose-dependent haemoglobin correction was also observed with the aqueous extracts of raw, boiled and fermented *H. sabdariffa* at 200, 400 and 600 mg.kg $^{-1}$. HB level decreased significantly after induction with Zidovudine. However, this decrease was corrected by erythropoietin and the different doses of the HSS extracts – possibly by stimulating erythropoiesis. These findings possibly favour the traditional claim of using *H. sabdariffa* seed extract in the treatment of anaemia (Ahmed *et al.,* 2013). Also, these findings were in agreement with previous study conducted on the same plant; however, these were carried out on the aqueous extract of the calyces (Adigun *et al.,* 2006). In the above mentioned study, 200 and 400 mg.kg $^{-1}$ of the aqueous extract of the calyx administered to normal Wistar albino rats for 2 weeks. The extracts caused significant elevations in

haemoglobin (P = 0.004) and PCV (P = 0.03) values. However, these beneficial effects of the extract were not sustained at higher doses (1000 mg.kg $^{-1}$ body weight). Chukwu *et al.* (2018) also reported that *H. sabdariffa* calyx-based beverage possess haematocrit potentials of increasing blood volume and management of anaemia as evidenced by higher levels of PCV, Hb and RBCs. This anaemic potential stems down to the presence of phytochemicals like flavonoids, anthocyanins and other phytochemicals as reported by Chukwu *et al*. (2019), which influence this property. Furthermore, *H. sabdariffa* seed aqueous extract was found to cause small increases in the MCV, MCH and MCHC levels of the haemorrhagic anaemic rats. However, these increases were not significant. This was in agreement with Ahmed *et al.* (2013), which reported no significant increase in the MCV, MCH and MCHC levels of haemorrhagic rats treated with aqueous extract of *H. sabdariffa* seeds, while Ejere *et al.* (2013) reported a significant increase in the MCV and MCH values, but not significant increase in MCHC levels in a dose and duration-dependent manner after administration of aqueous extract of *H. sabdariffa* calyces on *Rattus novergicus*. The values of MCH in all the administration groups in this present study were within the normal range of 16 – 53 pg as outlined by Mitruka and Rawnsley (1977). The significant lower WBC count in NGC was better improved (in a dose-dependent manner) by the extracts of *H. sabdariffa* seed than the standard drug. The non-significant differences in the lymphocyte levels between the extract-treated groups when compared with the NC and NGC treatment groups was in agreement with the report of Olatunji *et al.* (2005), which studied the haematological effects of aqueous extract of *H. sabdariffa* petals in rats. However, the lymphocyte level was better improved by the aqueous extracts of *H. sabdariffa* seed than the standard drug. On the other hand, the standard drug (rHu EPO) was more effective for neutrophil synthesis than the aqueous extracts of *H. sabdariffa* seed. The effects on MCV, MCH and MCHC of the anaemic rats were the same for both the aqueous extracts of *H. sabdariffa* seed and the standard drug. Generally, 600 mg.kg $^{-1}$ of HSF better improved the HB, PLT, WBC and MCHC of the anaemic rats.

Conclusion: The aqueous extract of raw, boiled and fermented *H. sabdariffa* seeds attenuated the haemolytic anaemia in the animals by significantly increasing the levels of haemoglobin, RBC, PCV, WBC and neutrophils, and compared favourably with the standard anti-anaemic drug. This study strongly posits that the cheap and locally-available *H. sabdariffa* seeds could be exploited in the management of anaemia in our society. Further studies on the mechanism of the anti-anaemic property of *H. sabdariffa* seeds is recommended for better understanding and utilization.

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