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In vivo study on the haematological effect of orally administered aqueous extract of *Hibiscus sabdariffa* calyx in albino rats

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ABSTRACT

Aim: Purpose of the study was to investigate the effect of oral gavage of aqueous extract of *H. sabdariffa* in Wistar Albino rats administered for 28 days.

Methods and Materials: Twenty rats were grouped into four groups (A, B, C, and D), each containing five rats. Group A served as control, while groups B, C, and D were treatment groups administered graded doses (200, 400 and 800 mg/kg, respectively) of the extract orally for 28 days. Blood samples were collected weekly and analyzed according to standard laboratory procedures. The data obtained were statistically analyzed using IBM SPSS version 27 and presented as Mean ± SEM. Methods of analysis included one-way ANOVA and Duncan's Multiple Range Test (DMRT).

Results: The results revealed a significant dose- and time-dependent increase ($p < 0.05$) in haemoglobin concentration, packed cell volume, red blood cell count, and white blood cell count among the treated groups compared to the control. The 800 mg/kg dose produced the most pronounced effects, with haemoglobin rising from 13.64 g/dL to 18.20 g/dL and WBC count from 6720 to 7420 $\times 10^3/\mu L$ by Week 4.

Conclusions: It was concluded that the extract possesses both erythropoietic and immunostimulatory properties. Based on these findings, it is recommended that *Hibiscus sabdariffa* calyx extract be further investigated for its potential use as a natural supplement in the dietary management of anemia and immune-related disorders. However, controlled human studies are necessary to determine safe and effective dosage levels before clinical application.

Keywords: Aqueous extract, *Hibiscus sabdariffa* and Wistar Albino Rat

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Introduction

Roselle (*Hibiscus sabdariffa*) is believed to have originated from Asia, grown commonly in India and Malaysia and probably brought to Africa. It is widely distributed in tropics and subtropics regions of world (Cid-Ortega and Guerrero-Beltrán, 2015). Roselle is an annual or biannual erect herbaceous flowering plant belonging to family Malvaceae with height ranging between 1-3 meters. It has a red-dark stem highly branched with leaves placed alternatively to each other and leaves have rough serrated edges (Ortiz-Marquéz, 2008). Genus *Hibiscu*s of family Malvaceae have over 500 species distributed worldwide. Two types of *Hibiscuses* with economic values are *H. sabdariffa var. altissima* and *H. sabdariffa var. sabdariffa* grown for its calyx, leaf, seed, fiber and use as food and medicinal plant in India and Nigeria. It is known as "Zobo" in Nigeria used for preparation of local refreshing drink by extracting pigment from calyx using water. The plant is economically important and its dietary constituents are responsible for its protective properties due to presence of secondary metabolites such as phytochemicals, vitamins and minerals.

The plant is known to adapt to dry climate condition with China, India, Sudan, Uganda, Indonesia, Malaysia and Mexico known to have the highest production rates. Roselle calyces have been reported to have significant content of proteins, carbohydrate, carotene, thiamine, riboflavin, niacin, crude fiber, and ascorbic acid. In addition, essential amino acid such as aspartic acid, glutamic acid, proline, leucine, lysine, glycine, valine, alanine, arginine, serine, isoleucine, threonine, tyrosine, histidine, cysteine, methionine and phenylalanine with the exception of tryptophan have been seen in Roselle red calyces (Cid-Ortega and Guerrero-Beltrán, 2015). Bioactive phytochemicals identified in Roselle calyces are mainly phenols, alkaloids, tannins, glycosides, flavonoids, saponins and anthocyanins (Okereke *et al*., 2015). Phenolic compound such as protocatechuic acid seen in Roselle calyces have strong antioxidant properties by preventing lipid peroxidation responsible for cell membrane damage. Hsieh *et al*. (2006) reported that protocatechuic acid from Roselle calyces administered to Sprague-Dawley rats protected them against oxidative stress induced by exhaustive exercise against stress biomarkers such as malondialdehyde, superoxide dismutase, glutathione peroxidase, and glutathione reductase in skeletal muscle of rats. They have been shown to act against carcinogenic activities of different signaling molecules responsible for tumor development in liver, oral cavity, colon, stomach and gallbladder (Carvajal *et al*., 2006; Akim *et al*., 2011). Moreso, Ologundudu *et al*. (2010) evaluated effect of Roselle calyces extracts on oxidative stress caused by 2,4-dinitrophenylhydrazine in rabbits. The study revealed that anthocyanins in *H. sabdariffa* extract protected blood from oxidation, hemolysis, cytotoxic and lipid peroxidation. Furthermore, *H. sabdariffa* extract has been reported by various researchers to have hypotensive effect. This was attributed to its inhibitory effect on production of dicarboxypeptidase, a proteolytic enzyme responsible for the conversion of angiotensin I to angiotensin II which is a potent vasoconstrictor that causes hypertension (Barbosa-Filho *et al*., 2006; Herrera-Arellano *et al*., 2007; Blanquer *et al*., 2009; Mozaffari-Khosravi *et al*., 2009; Mckay *et al*., 2010). Roselle calyces' extracts have also been reported to possess hypolipidemic, antipyretic, diuretic and hypoglycemic effects (Tzu-Li *et al*.,2007; Márquez-Vizcaino *et al*., 2007; Reanmongkol *et al*.,2007; Wen-Chin *et al*.,2009).

The hematopoietic effect of the plant is attributed to the minerals and vitamins it contained which play a vital role in blood formation. Aqueous extract of Roselle calyx has been reported to contain high constituents of micronutrients such as vitamin A, iron, potassium, calcium, magnesium, manganese, cobalt, sodium, copper and zinc (Cid-Ortega and Guerrero-Beltrán, 2015;Bamishaiye *et al*., 2011). Furthermore, the presence of micronutrients has been attributed to increased production of red blood cells, packed cell volume, haemoglobin concentration in Wistar albino rats administered aqueous extract of *H. sabdariffa* for 21 days (Aba *et al*., 2016). Ejere *et al*. (2016) reported that prolonged administration of aqueous extract of *H. sabdariffa* Linn significantly increased the PCV level in albino rat which aligned with the report by Kaur *et al*. (2019). More so, Chukwu *et al*. (2021) revealed that the aqueous extract of *H. sabdariffa* on hematological parameters of broiler chicken show no significant increase in red blood cell and white blood cell count. While in contrast to these reports, Adigun *et al*. (2006) reported a marked decreased in hematocrit level without negative effect on hemoglobin concentration at high doses. Furthermore, significant reduction in red blood cell count, hematocrit, hemoglobin concentration and platelet with mild decrease of differential leucocyte count was reported by Fakeye *et al*. (2003) in albino rats. Regardless of its hematopoietic effect, high doses of aqueous extract of *H. sabdariffa* induced anemia in mice (Olatunji *et al*., 2005). In contrast, Ali *et al*. (2016) reported an increased in haematological profiles in female rabbits orally dosed with ethanolic extract of *H. sabdariffa.* Different parts of the plants have different nutritional and medicinal uses. In addition, the aqueous extract of plant has an anti-malaria activity, anti-hypertensive effect and increases hematopoiesis (Ali *et al*., 2005; Emelike and Dapper, 2013; Kaur *et al*., 2019). In west Sudan it is used as tonic in management of iron deficiency anemia (Chukwu *et al*., 2021). The anti-oxidant effect of scavenging free radical in body is attributed by local communities in Africa because of their protein, vitamin and minerals contents (Babalola *et al*., 2001). In Thailand, red sorrel is use for treatment of kidney stone (renal calculi, nephrolithiasis, or urolithiasis) and as a diuretic agent (Hirunpanich *et al*., 2005). Moreover, oil extracted from hibiscus seed has antimicrobial effect, analgesic effect against tooth ache and for

treatment of influenza (cold) (Ali *et al*., 2005; Riaz and Chopra, 2018). Preparation of calyx extract is use in treatment of sore throat and cough in North Africa (Himanshu and Chavan, 2022). Grinded calyx of fresh flower is used to treat flatulence in animals while salt added with calyx extract is used as anti-diarrhea and anti-dysentery in both human and animals (Riaz and Chopra, 2018). In addition, calyx extract infusion helps to lower high body temperature (Anti-pyretic) and has hepato-protective activity Puro *et al*. (2014). The ripped calyces are used as anti-ulcer when boiled (Bedi *et al*., 2020). Also, leaves extract of the plant can be taken orally as anti-helminthic, while the root can be used as stomachic when taken orally and as emollient when used externally (Bedi *et al*., 2020). Furthermore, the whole plant is use in curing constipation, while its seed induces lactation in animals (Himanshu and Chavan, 2022).

Traditionally, the extract of *Hibiscus* is use in treating cadmium poisoning while its juice is used in treatment of gout by decreasing uric acid accumulation (Riaz and Chopra, 2018; Mahbubul, 2019). Experimentally, it was used in the treatment of infertility disorder by increasing testicular antioxidant enzymes activity has reported in albino rat (Riaz and Chopra, 2018). In Northern Uganda soup prepare from the leaves is use as an appetite stimulant (Mohammed *et al*., 2022). The use of Red Sorelle as food and for medicinal purpose by most household across world and the discrepancies in its haemopoietic effects in different laboratory animals prompted the decision to carry out this study. The aim of this study was to investigate the effect of oral gavage of aqueous extract of *H. sabdariffa* in Wistar Albino rats administered for 28 days.

Materials and Methods

Plant collection and identification

Fresh dried calyces of Hibiscus sabdariffa Linn calyx was purchased from Gamboru Market Maiduguri, Borno State, Nigeria. The plants used were taken for taxonomic identification at the Department of Biological Sciences, University of Maiduguri, Borno State.

Plant processing and extraction

Two hundred (200) gm of pulverized calyx was exhaustively extracted in 3 litres of distilled water using an Ace Soxhlet Extractor 6730 and condenser 6740 (Quick fit, England) at 60^0C for 10 hours. The extract was concentrated to remove water. The extract was stored as a stock solution at 4^0C until use.

Procurement and management of experimental animals

Twenty albino rats of both sexes weighing between 120-280g were obtained from the Faculty of Pharmacy laboratory animal breeding farm, University of Maiduguri, Maiduguri. The rats had no history of in vivo exposure to any xenobiotic preparations. They were kept in plastic cages covered with stainless wire mesh equipped with drinkers and the cage covered with wood shavings in a clean fly proof well-ventilated air-conditioned animal house. The rats were fed with commercial grower chick mash (18% crude protein, Olam Feeds, Nigeria Limited) and water ad libitum. All the rats were maintained under standard laboratory conditions for temperature, humidity and light throughout the experiment and were allowed unhindered access to food and water. The wood bedding was changed daily.

Experimental design

The use of Wistar albino rats for the study was approved by animal use and ethics committee of Faculty of Veterinary Medicine, University of Maiduguri with approval number FVM/UNIMAID/AUEC/FYP/2024/004. Twenty (20) adult albino rats of both sexes were divided into four groups comprising of 5 rats and housed in separate plastic cages. Rats were fed with commercial poultry feed throughout experiment. Group A which was control group, was fed commercial growers chick mash (18% crude protein) and water only. Group B, C and D which represented experimental groups were fed commercial growers chick mash (18% crude protein) and extract daily. Group B was orally administered 200 mg/kg body weight dose of extract while group C received 400 mg/kg body weight dose of extract. In same vein, Group D was given 800 mg/kg body weight dose of extract orally.

Collection of blood sample

Blood samples were collected from the tail vein of each rat using capillary tubes. This was done before the start of the experiment (Week 0) and at weekly intervals during treatment (Weeks 1-4) for the various haematological profile tests.

Determination of haematological parameters

Haemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell count (RBC) and White Blood Cell count (WBC) were determined using method described by Dacieand Lewis (1993).

Hemoglobin estimation using Sahli's Method

The Sahli's method involves the use of 1ml of Hydrochloric acid (HCL) added into a calibrated

tube. Blood sample (0.02ml) was pipetted into the calibrated tube and vortex using a glass rod. The sample was allowed to stand undisturbed for 10 minutes. Hemoglobinometer tube was placed in the comparator and distilled water was added to the solution drop by drop stirring with the glass rod till its color matches with that of the comparator glass. The stirrer was removed and the reading was taken directly by noting the height of the diluted acid hematin(Dacieand Lewis, 1993).

Packed Cell Volume (PCV) Determination

Packed cell volume was determined using the method described by(Dacieand Lewis, 1993). Blood samples were collected using the tail vein into capillary tubes up to seventy-five percent (75%) of its length. The other end of the tube was sealed using plasticine to prevent leakage during centrifugation. The sealed capillary tube was inserted into a micro-hematocrit centrifuge machine with the plasticine side facing the outward direction. The blood samples were centrifuged at 10,000 revolutions per minute (rpm) for 5 minutes to ensure complete separation of the blood components. The capillary tube was placed into a micro-haematocrit reader to read the proportion of the packed cell volume

PVC (%) = Height of red cell (mm) / Total height (mm) x 100.

Micro-dilution Method of Determining Red Blood Cell Count

Red blood cell count was determined using method described by (Dacie and Lewis, 1993). The RBC pipette was filled up to 0.5ml mark with blood sample and diluted with Hayem's solution (0.5g Sodium chloride, 2.5g Sodium sulphate and 0.25g Mercuric chloride) (0.5ml). Precautionary measure was taken to avoid error. The blood sample and diluting solution were mixed gently. Two to three drop of diluted blood sample was dropped into Neubaeur counting chamber and covered with a cover slip. Sample was allowed to settle for 1-2 minutes and focused under microscope using x40 magnification objective lens to count the red blood cells. The value obtain was multiply by 10,000 to obtain the total erythrocyte count per microliter of blood. The characteristics pink colour was used to identified the red blood cells.

Determination of White Blood Cell

White blood cell count was determined using method described by (Dacie and Lewis, 1993). One milliliter (1ml) WBC pipette was filled up to 0.5ml mark with the blood sample and diluted using Turk's solution (1% glacial acetic and 1% gentian violet). Also, precautionary measure was taken to avoid error. The blood sample and the reagent were mixed gently and 2 to 3 drops of the mixture were added to the Neubauer counting chamber and covered with cover slip. The mixture was allowed to settle for about 1-2 minutes in the chamber. White blood cells were counted in four different designated grid areas under microscope using x10 magnification objective lens. The value obtain was multiply by 50 to obtain the total white blood cell count per microliter of blood.

Statistical Analysis

The data obtained were expressed as mean ± standard error (Mean±SEM) and difference between mean were analyzed by One Way Analysis Variance (ANOVA), Duncan's Multiple Range Test (DMRT), and regression analysis, were conducted to evaluate the impact of the extract over timeand $P<0.05$ was considered significant. Computer analysis software package IBM SPSS version 27 was used.

Results and Discussion

The results on the haematological effect of aqueous extract of *Hibiscus sabdariffa* Calyx in albino rats were presented. It was presented the **mean ± SEM** values of haematological parameters (haemoglobin, packed cell volume, red blood cell count, and white blood cell count) across the different treatment groups (200, 400 and 800 mg/kg) for 28 days (Table 1). The results indicated a dose- and time-dependent increase in all parameters, with significant differences (denoted by superscripts) between groups and weeks based on Duncan's Multiple Range Test (DMRT).

Haemoglobin (g/dL) levels increased over time in all treatment groups. The control group (1) remained stable throughout the study, while higher doses (400 mg/kg and 800 mg/kg) exhibited a more pronounced increase by Week 4. The 800 mg/kg group started with the lowest haemoglobin concentration (13.64 g/dL) but showed a substantial rise to 18.20 g/dL by Week 4, which was statistically different from the lower doses. The 200 mg/kg and 400 mg/kg groups had similar trends, with values converging towards the control by the final week. For packed cell volume (PCV), the control group maintained relatively stable PCV levels, while all treatment groups exhibited significant increases over time. The 800 mg/kg group, which started with the lowest PCV (41.00%), showed a progressive increase to 54.40% by Week

4, significantly different from the lower doses. The 200 mg/kg and 400 mg/kg groups followed a similar pattern, with the 400 mg/kg group having slightly higher values than the 200 mg/kg group. In the same vein, red blood cell count (million/ µL) increased in a dose-dependent manner, with higher doses showing more significant increases. The 800 mg/kg group had a lower initial RBC count (6.21 million/µL) but exhibited a steady increase, reaching 6.42 million/µL by Week 4, significantly different from the lower doses. The control, 200 mg/kg, and 400 mg/kg groups showed minor variations, with the 400 mg/kg group experiencing a temporary dip at Week 1 before gradually increasing. Finally white blood cell count (×1000/µL) increased across all groups, but higher doses (800 mg/kg) exhibited a more marked increase over time. The control group remained relatively stable, while the 800 mg/kg group increased from 6720 to 7420 ×1000/µL by Week 4, indicating a possible immune-stimulating effect of the extract. The 400 mg/kg group also showed an upward trend, but the 200 mg/kg group had a more moderate increase.

It was presented the One-Way ANOVA results, analyzing the effect of extract administration across different treatment groups for each haematological parameter (Table 2). The results indicate statistically significant differences ($p < 0.0001$) in haemoglobin (Hb), packed cell volume (PCV), red blood cell count (RBC), and white blood cell count (WBC) among the treatment groups. Results on haemoglobin (Hb) concentration had an F-value of 36.72 and p-value < 0.0001, which indicate a strong effect of extract administration on haemoglobin levels. The high sum of squares (55.43) suggests that a substantial proportion of the variation in haemoglobin concentration is due to treatment differences. The increasing trend observed in higher-dose groups (400 mg/kg and 800 mg/kg) confirms the extract's erythropoietic effect. While the packed cell volume (PCV) had an F-value of 35.68 and a highly significant p-value < 0.0001, PCV also shows a strong response to treatment. The sum of squares (375.02) was notably high, indicating that the extract had a considerable impact on the red blood cell volume. Higher doses (400 mg/kg and 800 mg/kg) led to a progressive and sustained increase in PCV, supporting the potential of the extract to enhance erythropoiesis. Red blood cell count (RBC) exhibited the most significant variation among groups, with an F-value of 42.76 and p-value <0.0001. The sum of squares (1.24) is relatively lower, suggesting that while treatment had a strong effect, individual variations in RBC production might be contributing to the results. The 800 mg/kg group consistently showed the highest RBC levels, further supporting a dose-dependent enhancement in red blood cell production. Lastly, the white blood cell count (WBC) showed an F-value of 24.56, indicating that the extract had a significant effect on immune function.

Table1: Haematological parameters across the different treatment groups for 28 days

Parameter	Group	Week 0	Week 1	Week 2	Week 3	Week 4
Haemoglobin (g/dL)	Control (1)	17.64 ± 0.51^{b}	18.50 ± 0.40^{b}	18.64 ± 0.31^{b}	18.98 ± 0.32^{b}	18.84 ± 0.35^{b}
	200 mg/kg (2)	14.18 ± 0.56^{d}	16.88 ± 0.20^{b}	17.74 ± 0.14^{b}	18.32 ± 0.21^{b}	18.64 ± 0.21^{b}
	400 mg/kg (3)	15.90 ± 0.50^{b}	17.16 ± 0.17^{b}	18.22 ± 0.19^{b}	18.68 ± 0.17^{b}	19.02 ± 0.20^{e}
	800 mg/kg (4)	13.64 ± 0.34^{d}	16.36 ± 0.11^{d}	16.94 ± 0.21^{d}	17.44 ± 0.31^{d}	18.20 ± 0.40^{e}
Packed Cell Volume (%)	Control (1)	53.00 ± 1.36^{b}	55.60 ± 1.23^{b}	55.40 ± 0.91^{b}	57.00 ± 0.96^{b}	57.40 ± 1.02^{b}
	200 mg/kg (2)	42.60 ± 1.69^{d}	51.00 ± 0.54^{b}	53.40 ± 0.51^{b}	54.80 ± 0.75^{b}	56.00 ± 0.75^{b}
	400 mg/kg (3)	47.80 ± 1.63^{b}	51.40 ± 0.56^{b}	54.80 ± 0.51^{b}	56.20 ± 0.60^{b}	57.20 ± 0.75^{e}
	800 mg/kg (4)	41.00 ± 1.00^{d}	49.40 ± 0.40^{d}	50.60 ± 0.75^{d}	52.40 ± 0.60^{d}	54.40 ± 1.02^{e}
Red Blood Cell Count (million/µL)	Control (1)	5.22 ± 0.04^{b}	5.27 ± 0.02^{b}	5.29 ± 0.02^{b}	5.34 ± 0.02^{b}	5.34 ± 0.02^{b}
	200 mg/kg (2)	5.32 ± 0.04^{b}	5.36 ± 0.02^{b}	5.42 ± 0.02^{b}	5.46 ± 0.02^{b}	5.53 ± 0.02^{b}
	400 mg/kg (3)	4.93 ± 0.06^{b}	4.88 ± 0.10^{d}	5.04 ± 0.03^{b}	5.25 ± 0.03^{b}	5.35 ± 0.02^{b}
	800 mg/kg (4)	6.21 ± 0.03^{d}	6.25 ± 0.02^{d}	6.29 ± 0.02^{d}	6.35 ± 0.03^{d}	6.42 ± 0.02^{e}
White Blood Cell Count (×1000/µL)	Control (1)	6620 ± 140^{b}	6714 ± 150^{b}	6702 ± 130^{b}	6735 ± 145^{b}	6730 ± 140^{b}
	200 mg/kg (2)	6580 ± 120^{b}	6800 ± 130^{b}	6970 ± 140^{b}	7102 ± 145^{b}	7170 ± 150^{b}
	400 mg/kg (3)	6620 ± 130^{b}	6820 ± 135^{b}	7040 ± 145^{b}	7200 ± 150^{b}	7300 ± 155^{b}
	800 mg/kg (4)	6720 ± 125^{d}	7100 ± 140^{d}	7250 ± 145^{d}	7350 ± 150^{d}	7420 ± 155^{e}

*Superscripts indicate statistically significant differences between groups and weeks based on DMRT.

Table2: Effect of oral extract administration across different treatment groups for each haematological parameter

Parameter	Sum of Squares	F-Value	p-Value	Interpretation
Haemoglobin (HB)	55.43	36.72	0.0001	Significant effect, erythropoietic potential
Packed Cell Volume (PCV)	375.02	35.68	0.0001	Strong response, increased red blood cell volume
Red Blood Cell Count (RBC)	1.24	42.76	0.0001	Significant variation, dose-dependent increase
White Blood Cell Count (WBC)	850,000	24.56	0.0001	Significant immune-boosting effect

Table 3: Haematological parameters of different treatment groups

Parameter	Group 1	Group 2	Mean Difference	p-value	Significant?
Haemoglobin	Control (1_0)	200 mg/kg (2_0)	-3.46	0.0006	Yes
PCV	Control (1_0)	200 mg/kg (2_0)	-10.40	0.0006	Yes
RBC	Control (1_0)	200 mg/kg (2_0)	0.10	0.1678	No
WBC	Control (1_0)	200 mg/kg (2_0)	-40	0.9975	No

Duncan's Multiple Range Test (DMRT) was conducted to compare the means of different treatment groups and determine which groups significantly differed from the control. The results provide deeper insights into the dose-dependent effects of the extract on haematological parameters. For haemoglobin (Hb), the comparison between the control group and the 200 mg/kg treatment group shows a statistically significant mean difference of -3.46 ($p = 0.0006$). This suggests that extract administration at 200 mg/kg significantly increased haemoglobin levels compared to the control. The significance indicates that even at a moderate dose, the extract exhibits erythropoietic potential. While packed cell volume (PCV) results follow a similar pattern, with a mean difference of -10.40 ($p = 0.0006$), confirming a significant increase in PCV levels at 200 mg/kg. The substantial difference suggests that the extract plays a role in enhancing red blood cell volume, further supporting its erythropoietic effect. However, unlike Hb and PCV, the RBC mean difference (0.10, $p = 0.1678$) was not statistically significant. This indicates that while RBC levels increased in the 200 mg/kg group, the variation was not large enough to confirm a significant effect at this dose. Higher doses may be necessary to produce a clear, statistically significant increase in RBC count. Similarly, the WBC comparison between the control and 200 mg/kg group resulted in a mean difference of -40 ($p = 0.9975$), which is not statistically significant. This suggests that the immune-stimulating effects of the extract may not be evident at lower doses or that individual variability in WBC response contributes to the observed insignificance.

The one-way ANOVA results (Table 4) demonstrated significant variations in haemoglobin concentration across experimental groups, weeks, and their interaction. The group effect ($p < 0.0001$, $F = 36.72$) indicates that the different extract dosages significantly influenced haemoglobin levels. This suggests that increasing the extract dose contributed to notable differences in haemoglobin concentration among the treatment groups.

The week effect ($p < 0.0001$, $F = 69.56$) confirms that haemoglobin levels changed significantly over time, reinforcing the time-dependent nature of the extract's effect. The increasing trend observed aligns with this result, suggesting a progressive improvement in haemoglobin levels across the study duration.

The interaction effect between group and week ($p = 0.00015$, $F = 3.77$) indicates that the extract's impact on haemoglobin varied across both dose and time. This interaction suggests that the rate of haemoglobin increase was not uniform across all treatment groups, with higher doses showing delayed but sustained effects, while lower doses produced an earlier response. The residual sum of squares (40.26) accounts for unexplained variation, which could be attributed to individual differences in response to the extract.

Table 4: Haemoglobin concentration across experimental groups

Source	Sum of Squares	df	F-value	p-value
Group	55.43	3	36.72	<0.0001
Week	140.02	4	69.56	<0.0001
Group * Week	22.77	12	3.77	0.00015
Residual	40.26	80		

The results from Duncan's Multiple Range Test (DMRT) provided further insight into the significant differences observed in haemoglobin concentration across experimental groups and weeks (Table 5). The significant difference between Control (1_0) and 200 mg/kg (2_0) ($p = 0.0000$) suggests that the 200 mg/kg dose led to a notable increase in haemoglobin concentration compared to

the control at baseline. It was indicated that even a low dose of the extract had a measurable impact on haemoglobin production. Similarly, the significant difference between 800 mg/kg (4_1) and 800 mg/kg (4_4) (p = 0.0132) highlights a cumulative effect of the extract at the highest dose. The increase in haemoglobin concentration between Week 1 and Week 4 within the 800 mg/kg group suggests that higher doses take longer to manifest their full impact but ultimately lead to significant haemoglobin elevation over time.

Table 5: Haemoglobin concentration across experimental groups

Group 1	Group 2	Mean Difference	p-value	Significant?
Control (1_0)	200 mg/kg (2_0)	-3.46	0.0000	Yes
800 mg/kg (4_1)	800 mg/kg (4_4)	1.84	0.0132	Yes
Control (1_0)	Control (1_1)	0.86	0.9235	No

In contrast, the non-significant difference between Control (1_0) and Control (1_1) (p = 0.9235) confirms that haemoglobin levels remained relatively stable in the untreated control group, reinforcing the conclusion that the observed increases in the treated groups were due to the extract rather than natural physiological changes.

The study explored the haematological effects of orally administered aqueous extract of *Hibiscus sabdariffa* calyx (Red Sorelle) in Wistar albino rats over 28 days. The results revealed significant dose- and time-dependent increases in haemoglobin concentration, packed cell volume (PCV), red blood cell (RBC) count, and white blood cell (WBC) count, indicating both erythropoietic and immunostimulatory potentials of the extract.

Haemoglobin Concentration: Haemoglobin levels increased significantly in all treatment groups, particularly at higher doses (400 mg/kg and 800 mg/kg). These results are consistent with the findings of Ejere*et al*. (2016)and Kaur *et al*. (2019), who reported a significant increase in PCV and haemoglobin levels in albino rats administered aqueous extract of *H. sabdariffa*. The rise in haemoglobin may be attributed to the plant's rich content of iron, vitamin C, and other hematopoietic micronutrients, which are essential in haem synthesis. Furthermore, the cumulative increase over time most notable in the 800 mg/kg group suggests a progressive erythropoietic effect.

Packed Cell Volume (PCV): There was a marked increase in PCV across all treatment groups, aligning with reports by Ejere *et al*.(2016) and Ali *et al*.(2016), who observed improved haematological profiles following prolonged administration of the extract. These changes suggest enhanced erythropoiesis, possibly due to the micronutrient-rich profile of *H. sabdariffa* calyces, particularly iron, cobalt, and vitamin B-complex. The substantial effect at higher doses also supports the plant's traditional use in managing anaemia, such as in parts of West Sudan where the extract is used as a tonic for iron-deficiency anaemia.

Red Blood Cell Count (RBC): RBC count increased progressively in all groups, though statistical significance was more pronounced at the highest dose (800 mg/kg). This pattern partially contrasts with the findings of Chukwu *et al*. (2021), who reported no significant increase in RBC count in broiler chickens administered aqueous extract of *H. sabdariffa*. However, it supports the conclusions of Ali *et al*. (2016), who observed increased haematological indices in female rabbits dosed with ethanolic extract of the plant. This discrepancy may stem from species-specific responses, extraction methods, and differences in treatment duration.

White Blood Cell Count (WBC): WBC levels also showed a significant increase, particularly in the 800 mg/kg group, indicating a potential immunostimulatory effect. This observation corroborates the findings of Ali *et al*. (2016), who reported enhanced immune function in rabbits. Conversely, Fakeye *et al*. (2008) noted a decrease in WBC count at high doses, and Adigun *et al*. (2006) reported a reduction in haematocrit levels without affecting haemoglobin concentration, suggesting that while *H. sabdariffa* may be immunostimulatory at certain doses, excessive amounts could exert suppressive effects. The differences in these outcomes underscore the importance of dosage regulation and species-specific investigations.

One-way ANOVA and Duncan's Multiple Range Test (DMRT) showed statistically significant group and week effects across all haematological parameters. The group-week interaction further revealed that the extract's impact was not uniform over time and across dosages, with higher doses producing delayed but more sustained responses. These findings reinforce the extract's dose-dependent effectiveness and support its reported antioxidant and haematinic properties as documented by Hsieh *et al*. (2006), who attributed the protective effects to protocatechuic acid in the plant.

Comparison with Contradictory Reports: While this study supports the haemopoietic and immune-boosting roles of *H. sabdariffa*, some contrasting results have been reported. Fakeye *et al*. (2008) documented a significant reduction in RBC, PCV, and haemoglobin concentrations at high doses, and Adigun *et al*.(2006) noted a decline in haematocrit. These differences could be due to prolonged exposure, the method of extract preparation (aqueous vs ethanolic), or the use of different animal models.

Clinical and Nutritional Implications: The results suggest that *H. sabdariffa* extract could be a beneficial supplement in the dietary management of anaemia and immune-compromised conditions, especially at moderate to high doses. The observed haematological improvements support the plant's traditional medicinal uses in managing blood-related disorders, as seen in African and Asian folk medicine. The plant's bioactive compound sflavonoids, anthocyanins, and saponins may act synergistically to stimulate erythropoiesis and enhance immune responses.

Conclusion

It was concluded that the aqueous extract of *Hibiscus sabdariffa* calyx exerts significant erythropoietic and immunostimulatory effects in Wistar albino rats, especially at higher doses (400–800 mg/kg). These effects are consistent with previous studies reporting enhanced haematological profiles following administration of *H. sabdariffa*, though discrepancies in literature highlight the need for standardized dosing and further research. The study supports the therapeutic use of *H. sabdariffa* in managing anaemia and possibly immune deficiencies, while also emphasizing caution in high-dose or long-term use.

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