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Effect of Ethanolic Extract of *Hibiscus sabdariffa* Leaves on the White Blood Cells, IgG, Interleukins and Antioxidants of Male Wistar Rats

***Gospel Onyinyeamara Okereke[1], Cornelius Maduabuchi Nwozor[1], Chibuike Onyeka Maduakor[1], Okwuchukwu Prince Egbuatu[1], Chibuzor Calistus Okpanum[1], Nnaemeka Chiemelie Nwachukwu[1], Makuochukwu Immaculata Ojimba**

[1] Department of Human Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria

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****Corresponding Author***

Gospel Onyinyeamara Okereke

Address:

Department of Human Physiology, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria

E-mail: *okerekeg097@gmail.com*

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ABSTRACT

Background; *Hibiscus sabdariffa*, broadly referred to by the common name Roselle, is a species indigenous to West Africa and selected parts of Asia. Its historical record of human utilization extends to Java, where a French botanist documented its edibility as early as 1576. At present, the plant is under large-scale cultivation across multiple countries for fiber, food, and medicinal purposes. Within Nigeria, it is colloquially designated "Zobo," a term of Hausa linguistic derivation.

Methods; This study constituted of three experimental groups which are the following:
Control Group: No active pharmacological treatment; vehicle alone (distilled water or physiological saline) was administered. This group provided the comparative reference baseline.
Low Dose Group: Received 100 mg/kg body weight of Hibiscus sabdariffa extract daily, to assess minimum effective biological activity.
High Dose Group: Received 300 mg/kg body weight of extract daily, to evaluate maximal pharmacological response and any potential adverse effects.
All treatments were administered once daily via oral gavage for 28 consecutive days.

Results; The results showed that the immunoglobin level increased at low dose of extract for decreased at high dose of extract, the interleukin 2 and 6 level increased compared to the control groups when extract was administered. The SOD level decreased compared to the control group while the GSH level decreased with low dose of extract and decreased with high dose extract and the WBC decreased with low dose and increased with high dose of extract.

Conclusion; This study provides evidence that the ethanolic leaf extract of *Hibiscus sabdariffa* significantly alters immune and antioxidant indices in male Wistar rats in a dose-dependent manner. Low-dose administration was associated with enhanced immunoglobulin synthesis, improved glutathione status, and potentiated immune signaling, all indicative of immunostimulatory and antioxidant benefit. High-dose administration, in contrast, resulted in reduced

List of Abbreviations:

Superoxide Dismutase (SOD),
Immunoglobulin G (IgG)
White Blood Cell (WBC)
Reduced Glutathione (GSH)
Interleukin (IL)

INTRODUCTION:

Hibiscus sabdariffa, broadly referred to by the common name Roselle, is a species indigenous to West Africa and selected parts of Asia. Its historical record of human utilization extends to Java, where a French botanist documented its edibility as early as 1576. At present, the plant is under large-scale cultivation across multiple countries for fiber, food, and medicinal purposes. Within Nigeria, it is colloquially designated "Zobo," a term of Hausa linguistic derivation.

The introduction of *Hibiscus sabdariffa* into Nigeria is generally attributed to merchants who traversed ancient trans-Saharan trade corridors linking North Africa to western sub-Saharan regions, transporting the plant's seeds as part of broader commercial exchanges. The plant proved highly adaptive to the Nigerian climate, establishing itself particularly in semi-arid northern zones. Key cultivating states like Kano, Kaduna, Katsina, and Sokoto remain important production centers owing to their climatically suitable conditions.(Rao, 1996)

Several factors account for Zobo's widespread acceptance in Nigeria. From a nutritional standpoint, the plant offers substantial quantities of vitamin C, anthocyanins, and organic acids, qualifying it as both a dietary supplement and a traditional remedy. Culturally, it has been assimilated into the national cuisine as a

popular thirst-quenching drink and a constituent of indigenous medicinal formulations. Economically, it sustains the livelihoods of smallholder farmers and micro-scale enterprises whose operations depend on calyx-based products such as beverages, syrups, and jams. Low input requirements and adaptability to local climatic conditions render it an accessible crop across commercial and subsistence farming scales.

In spite of the plant's established global and historical relevance, the particular ways in which Zobo contributes to Nigerian culinary heritage, therapeutic practice, and rural economies remain incompletely understood and deserve more focused inquiry. The present investigation was therefore undertaken to examine the prospective health-related properties of *Hibiscus sabdariffa*, with emphasis on its effects upon hematological indicators and its suitability as an adjunct or alternative therapeutic resource.(Rao, 1996)

Aim: The primary aim of this study is to ascertain the impact of the ethanolic leaf extract of *Hibiscus sabdariffa* (Zobo) on Immunoglobulin G (IgG), White Blood Cell (WBC) count, Interleukins (IL-2 and IL-6), Superoxide Dismutase (SOD) and Reduced Glutathione (GSH) in male albino Wistar rats.

Specific Objectives:

1. To assess how treatment with the ethanolic leaf extract of Hibiscus sabdariffa modifies serum IgG concentrations in male Wistar rats.
2. To determine the impact of the same extract on circulating IL-2 and IL-6 levels in male Wistar rats.
3. To quantify changes in total white blood cell counts in male Wistar rats following extract administration.
4. To measure the effect of Hibiscus sabdariffa ethanolic leaf extract on SOD activity and GSH concentrations in male Wistar rats.

Research Questions.

1. How does administration of the ethanolic leaf extract of Hibiscus sabdariffa influence IgG levels in male Wistar rats?
2. What changes in IL-2 and IL-6 levels are observed in male Wistar rats following treatment with the extract?
3. In what way does exposure to the extract alter total WBC counts in male Wistar rats?
4. What are the measurable effects of this extract on SOD activity and GSH levels in male Wistar rats?

Research Hypotheses

Null Hypothesis (H_0):

Administration of the ethanolic leaf extract of *Hibiscus sabdariffa* will produce no statistically significant changes in IgG, IL-2, IL-6, white blood cell count, SOD, or GSH levels in male Wistar rats.

Alternative Hypothesis (H_1):

Administration of the ethanolic leaf extract of *Hibiscus sabdariffa* will yield statistically significant changes in one or more of the following parameters in male Wistar rats: IgG, IL-2, IL-6, white blood cell count, SOD and GSH levels.

METHODS

Study Area;

A quantity of dried *Hibiscus sabdariffa* (Zobo) leaves sufficient for experimental requirements was sourced from the main market in Onitsha, Anambra State, Nigeria.

Experimental Animals

Male albino Wistar rats were selected as the animal model for this study.

Other Materials

The following were used in the study:

1. Wooden cage with iron mesh panels (animal housing)
2. Standard rodent feed (grower's mash)
3. Distilled water
4. 95% absolute ethanol (extraction solvent)
5. Sawdust (cage litter/bedding)
6. Protective clothing: laboratory coat and examination gloves
7. Oral dosing equipment: syringes and gavage cannula
8. Weighing scale, test tubes, and graduated measuring cylinders
9. Prepared Hibiscus sabdariffa leaf extract
10. Feed bowls/plates

(A) Extraction Method

Dried Zobo leaves from Onitsha Main Market, Anambra State, were washed under running tap water to remove particulate contamination. After washing, the leaves were laid flat for ambient air-drying over a two-week period before being processed into fine powder in an electric blender. Maceration in 1000 mL of 95% absolute ethanol (JHD Chemicals, Guangdong, China) was carried out over 48 hours. Coarse filtration was accomplished using clean porcelain cloth, followed by fine filtration via Whatman Qualitative Filter Paper (Grade 1, Sigma Aldrich, WHA1001042, USA). The filtrate was concentrated under reduced pressure using a rotary evaporator (Digital TT-52, Techmel & Techmel, USA) and the resulting residue was air-dried to a powder. The extract powder was preserved in sealed containers in a domestic refrigerator (Nexus model) until use. The

procedure was adapted from Attar and Abu-Zeid (2013) with minor protocol adjustments.

(B) Acute Toxicity Study

The lethal dose (LD50) of the ethanolic *Hibiscus sabdariffa* leaf extract was established at the Department of Physiology, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University, Nnewi Campus, following the method of Lorke (1983). Thirteen rats were enrolled in two successive phases; all doses were administered by the oral route.

Phase I

Nine animals were divided equally into three groups. Group 1 received 10 mg/kg; Group 2, 100 mg/kg; Group 3, 1000 mg/kg. All animals were kept under observation for 24 hours post-dosing. No clinical abnormalities or fatalities were detected in any group during Phase I.

Phase II

Four animals (one per group) received 1200, 1600, 2900, and 5000 mg/kg respectively. Observation was maintained for 24 hours. No mortality was observed in any Phase II animal.

Table 1: Acute Toxicity Study Analysis

Phase	Dose	Deaths	Observation
1	10 mg/kg 100 mg/kg 1000 mg/kg	0/3 0/3 0/3	All rats clinically normal All rats clinically normal All rats clinically normal
2	1200 mg/kg 1600 mg/kg 2900 mg/kg 5000 mg/kg	0/1 0/1 0/1 0/1	Animal remained healthy Animal remained healthy Animal remained healthy No mortality recorded

LD50 is computed as the geometric mean of the maximum dose with 0% mortality and the minimum dose with 100% mortality: LD50 = $\sqrt{(A \times B)}$. Since no fatalities occurred at any dose level, the LD50 of the ethanolic extract of *Hibiscus sabdariffa* is above 5000 mg/kg, indicating a wide margin of safety for animal and human use.

(C) Experimental Design

Three experimental groups were constituted as follows:

Control Group: No active pharmacological treatment; vehicle alone (distilled water or physiological saline) was administered. This group provided the comparative reference baseline.
Low Dose Group: Received 100 mg/kg body weight of Hibiscus sabdariffa extract daily, to assess minimum effective biological activity.
High Dose Group: Received 300 mg/kg body weight of extract daily, to evaluate maximal pharmacological response and any potential adverse effects.
All treatments were administered once daily via oral gavage for 28 consecutive days.

(D) Animal Source and Care

Wistar rats were obtained from a certified breeding establishment. On arrival at the facility, animals were housed in clean, ventilated cages in the animal house of the Department of Physiology, Nnamdi Azikiwe University. Housing conditions were maintained at a 12-hour photoperiod cycle, 22–25°C ambient temperature, and 40–60% relative humidity. A two-week acclimatization period was observed before experimentation commenced; throughout this period and the study itself, animals received standard rat chow and unrestricted water. All animal procedures complied with approved ethical protocols, and formal institutional ethical clearance was secured before the study began.

(E) Statistical Analysis

Experimental data were analyzed by inferential statistical methods to determine the significance of inter-group differences. Statistical computations were carried out using SPSS (Statistical Package for the Social Sciences) and Microsoft Excel. Continuous data are presented as group mean ± standard deviation (SD). Between-group comparisons were performed using one-way Analysis of Variance (ANOVA); where significant main effects were found, pairwise distinctions were identified using Tukey's or Bonferroni's post hoc tests. The threshold for statistical significance was set at $p < 0.05$, corresponding to a less than 5% probability of observed differences due to chance. Findings are presented in both tabular and graphical form to facilitate interpretation.

Duration and Location of Study

This research was carried out at the animal facility of the Human Physiology Department, Nnamdi Azikiwe University, College of Health Sciences, Nnewi, Anambra State. The total study span was five weeks comprising two weeks of acclimatization followed by a three-week active experimental phase. During acclimatization, all animals received standard feed and free access to water. Male albino Wistar rats with an initial body mass of 90–120 g were housed in adequately spaced, ventilated cages maintained at appropriate temperatures throughout the study.

Ethical Consideration

Ethical approval was obtained from the Research Ethics Committee of the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.

RESULTS

Evaluation of Body Weights of the Experimental Animals

Table 2: Descriptive Table for the initial weights of Animals

					95% Confidence Interval for Mean			
Reading	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
A	4	158.7500	25.94064	12.97032	117.4727	200.0273	135.00	190.00
B	4	183.7500	17.96988	8.98494	155.1559	212.3441	160.00	200.00
C	4	182.5000	12.58306	6.29153	162.4775	202.5225	170.00	200.00
Total	12	175.0000	21.42641	6.18527	161.3863	188.6137	135.00	200.00

Table 3: Descriptive Table for the Final Weights of Animals

					95% Confidence Interval for Mean			
Reading	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
A	4	156.5000	16.34013	8.17007	130.4992	182.5008	144.00	180.00
B	4	172.7500	8.80814	4.40407	158.7343	186.7657	165.00	185.00
C	4	170.5000	17.21434	8.60717	143.1082	197.8918	158.00	195.00
Total	12	166.5833	15.20442	4.38914	156.9229	176.2438	144.00	195.00

EVALUATION OF HEAMATOLOGICAL PARAMETERS

ANOVA COMPARISON FOR IMMUNOGLOBULIN

Table 4.1: Descriptive Table for Immunoglobulin

					95% Confidence Interval for Mean			
Reading	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
A	4	162.0000	6.96324	3.48162	150.9199	173.0801	156.60	171.80
B	4	200.8750	38.84494	19.42247	139.0640	262.6860	166.30	235.60
C	4	142.4500	15.25003	7.62501	118.1838	166.7162	132.00	164.70
Total	12	168.4417	33.63692	9.71014	147.0698	189.8135	132.00	235.60

Table 4.2: ANOVA

Reading

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7075.932	2	3537.966	5.930	.023
Within Groups	5369.937	9	596.660		
Total	12445.869	11			

Post Hoc Tests

Table 4.3: Multiple Comparisons

Dependent Variable: Reading
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-38.87500	17.27223	.153	-89.5400	11.7900
	C	19.55000	17.27223	.861	-31.1150	70.2150
B	A	38.87500	17.27223	.153	-11.7900	89.5400
	C	58.42500*	17.27223	.024	7.7600	109.0900
C	A	-19.55000	17.27223	.861	-70.2150	31.1150
	B	-58.42500*	17.27223	.024	-109.0900	-7.7600

*. The mean difference is significant at the 0.05 level.

ANOVA COMPARISON FOR INTERLEUKINS 2

Table 5.1: **Descriptive Table for Interleukins 2**

Reading	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	4	6.3250	.60208	.30104	5.3670	7.2830	5.80	7.10
B	4	8.4250	.99121	.49561	6.8478	10.0022	7.30	9.70
C	4	7.5000	.63770	.31885	6.4853	8.5147	6.90	8.10
Total	12	7.4167	1.13284	.32702	6.6969	8.1364	5.80	9.70

Table 5.2: ANOVA for Interleukins 2

Reading	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.862	2	4.431	7.588	.012
Within Groups	5.255	9	.584		
Total	14.117	11			

Post Hoc Tests

Table 5.3: Multiple Comparisons for Interleukins 2

Dependent Variable: Reading
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-2.10000*	.54032	.011	-3.6849	-.5151
	C	-1.17500	.54032	.173	-2.7599	.4099
B	A	2.10000*	.54032	.011	.5151	3.6849
	C	.92500	.54032	.363	-.6599	2.5099
C	A	1.17500	.54032	.173	-.4099	2.7599
	B	-.92500	.54032	.363	-2.5099	.6599

*. The mean difference is significant at the 0.05 level.

ANOVA COMPARISON FOR INTERLEUKINS 6

Table 6.1: Descriptive Table for Interleukins 6

Reading	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
A	4	57.2250	5.20408	2.60204	48.9441	65.5059	51.10	62.80
B	4	66.2750	6.82856	3.41428	55.4092	77.1408	60.50	76.10
C	4	63.2750	3.61697	1.80849	57.5196	69.0304	60.20	68.50
Total	12	62.2583	6.25510	1.80569	58.2840	66.2326	51.10	76.10

Table 6.2: ANOVA for Interleukins 6

Reading	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	170.007	2	85.003	2.938	.104
Within Groups	260.382	9	28.931		
Total	430.389	11			

Post Hoc Tests

Table 6.3: Multiple Comparisons for Interleukins 6

Dependent Variable: Reading
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
A	B	-9.05000	3.80338	.124	-20.2065	2.1065
	C	-6.05000	3.80338	.438	-17.2065	5.1065
B	A	9.05000	3.80338	.124	-2.1065	20.2065
	C	3.00000	3.80338	1.000	-8.1565	14.1565
C	A	6.05000	3.80338	.438	-5.1065	17.2065
	B	-3.00000	3.80338	1.000	-14.1565	8.1565

ANOVA COMPARISON FOR SUPEROXIDE DISMUTASE (SOD)

Table 7.1: Descriptive Table for SOD

Reading	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	95% Confidence Interval for Mean Upper Bound	Minimum	Maximum
A	4	22.2500	1.67631	.83815	19.5826	24.9174	20.50	24.50
B	4	21.6750	.99791	.49896	20.0871	23.2629	20.60	22.90
C	4	19.8500	1.92787	.96393	16.7823	22.9177	18.00	22.40
Total	12	21.2583	1.78705	.51588	20.1229	22.3938	18.00	24.50

Table 7.2: **ANOVA for SOD**

Reading	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.562	2	6.281	2.505	.137
Within Groups	22.567	9	2.507		
Total	35.129	11			

Post Hoc Tests

Table 7.3: Multiple Comparisons for SOD

Dependent Variable: Reading
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	.57500	1.11971	1.000	-2.7095	3.8595
	C	2.40000	1.11971	.182	-.8845	5.6845
B	A	-.57500	1.11971	1.000	-3.8595	2.7095
	C	1.82500	1.11971	.413	-1.4595	5.1095
C	A	-2.40000	1.11971	.182	-5.6845	.8845
	B	-1.82500	1.11971	.413	-5.1095	1.4595

ANOVA COMPARISON FOR GLUTATHIONE (GSH)

Table 8.1: Descriptive Table for GSH

Reading	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	4	37.0000	2.55734	1.27867	32.9307	41.0693	34.90	40.60
B	4	38.5250	1.60702	.80351	35.9679	41.0821	36.40	40.00
C	4	33.6000	1.91659	.95830	30.5503	36.6497	31.90	35.50
Total	12	36.3750	2.84832	.82224	34.5653	38.1847	31.90	40.60

Table 8.2: **ANOVA for GSH**

Reading	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.855	2	25.427	5.962	.022
Within Groups	38.388	9	4.265		
Total	89.242	11			

Post Hoc Tests

Table 8.3: Multiple Comparisons for GSH

Dependent Variable: Reading
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-1.52500	1.46036	.971	-5.8087	2.7587
	C	3.40000	1.46036	.135	-.8837	7.6837
B	A	1.52500	1.46036	.971	-2.7587	5.8087
	C	4.92500*	1.46036	.025	.6413	9.2087
C	A	-3.40000	1.46036	.135	-7.6837	.8837
	B	-4.92500*	1.46036	.025	-9.2087	-.6413

*. The mean difference is significant at the 0.05 level.

ANOVA COMPARISON FOR WHITE BLOOD CELLS (WBC)

Table 9.1: Descriptive Table for WBC

Reading	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	4	37.0000	2.55734	1.27867	32.9307	41.0693	34.90	40.60
B	4	38.5250	1.60702	.80351	35.9679	41.0821	36.40	40.00
C	4	33.6000	1.91659	.95830	30.5503	36.6497	31.90	35.50
Total	12	36.3750	2.84832	.82224	34.5653	38.1847	31.90	40.60

Table 9.1: ANOVA for WBC

Reading	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.855	2	25.427	5.962	.022
Within Groups	38.388	9	4.265		
Total	89.242	11			

Post Hoc Tests

Table 9.3: Multiple Comparisons for WBC

Dependent Variable: Reading
Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-1.52500	1.46036	.971	-5.8087	2.7587
	C	3.40000	1.46036	.135	-.8837	7.6837
B	A	1.52500	1.46036	.971	-2.7587	5.8087
	C	4.92500*	1.46036	.025	.6413	9.2087
C	A	-3.40000	1.46036	.135	-7.6837	.8837
	B	-4.92500*	1.46036	.025	-9.2087	-.6413

*. The mean difference is significant at the 0.05 level.

EVALUATION OF BODY WEIGHTS OF EXPERIMENTAL ANIMALS

Table 10. Initial and Final Body Weights of Experimental Rats

Parameters	Control Group	Low Dose	High Dose
Initial Weight (g)	158.75 ± 25.94	183.75 ± 17.96	182.5 ± 12.58
Final Weight (g)	156.50 ± 15.12*	172.75 ± 8.80*	170.50 ± 8.60*

Values presented as mean ± SD
* Significantly different from control values ($p < 0.05$)

Pre- and post-treatment body weight measurements were obtained for all Wistar rats. Analysis of initial versus final weight data revealed a significant net decrease in body mass across both treatment groups. The control group showed mixed weight trajectories (both gains and losses) during the experimental period, whereas both the low-dose and high-dose groups exhibited consistent significant weight reductions by study end.

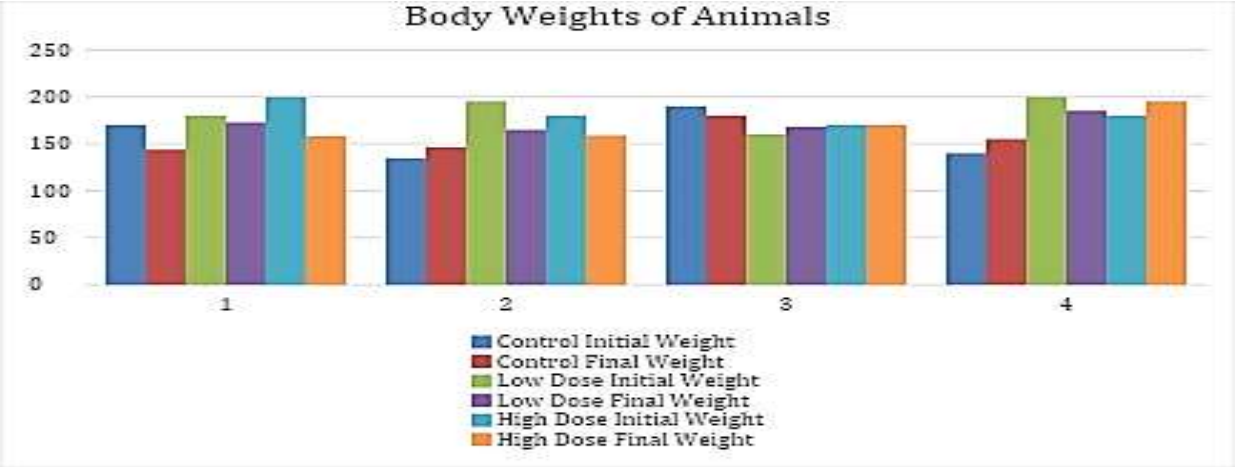

Figure 1: Bar chart of initial and final body weights across Control, Low Dose, and High Dose groups (n=4 per group), with color-coded pre- and post-treatment values

EVALUATION OF HEMATOLOGICAL PARAMETERS ANOVA COMPARISON FOR IMMUNOGLOBULIN (mg/dL)

Table 11. Serum Immunoglobulin G (IgG) Levels across Study Groups

ANOVA	Control Group	Treatment Group	p-value
	162.00 ± 6.96	Low Dose: 200.87 ± 38.8*	0.023
		High Dose: 142.45 ± 15.25*	

Values presented as mean ± SD; * significantly different from control ($p < 0.05$)

Serum IgG concentrations differed significantly between the control and treatment groups ($p = 0.023$). A dose-dependent biphasic pattern was observed: the low-dose group demonstrated elevated IgG, while the high-dose group exhibited a reduction relative to control values, consistent with immunostimulatory effects at low extract concentrations and immunosuppression at high concentrations.

ANOVA COMPARISON FOR INTERLEUKINS 2 (pg/mL)

Table 12. Interleukin-2 (IL-2) Levels Across Study Groups

ANOVA	Control Group	Treatment Group	p-value
	6.32 ± 0.60	Low Dose: 8.42 ± 0.99*	0.012
		High Dose: 7.50 ± 0.63	

Values presented as mean ± SD; * significantly different from control ($p < 0.05$)

IL-2 concentrations reached statistical significance in the low-dose group ($p = 0.012$); the high-dose group change did not achieve significance. These results indicate a concentration-dependent variation in the extract's capacity to modulate IL-2-mediated immune signaling.

ANOVA COMPARISON FOR INTERLEUKINS 6 (pg/mL)

Table 13. Interleukin-6 (IL-6) Levels Across Study Groups

ANOVA	Control Group	Treatment Group	p-value
	57.22 ± 5.20	Low Dose: 66.27 ± 6.82	0.104
		High Dose: 63.27 ± 3.61	

Values presented as mean ± SD; * significantly different from control ($p < 0.05$)

IL-6 concentrations did not show a statistically significant difference between control and treatment groups at either dose level (p = 0.104), suggesting that the extract did not substantially alter IL-6 secretion under the conditions of this experiment.

ANOVA COMPARISON FOR SUPEROXIDE DISMUTASE (SOD) (U/g Hb)

Table 14. Superoxide Dismutase (SOD) Activity Across Study Groups

ANOVA	Control Group	Treatment Group	p-value
	22.25 ± 1.67	Low Dose: 21.67 ± 0.99	0.137
		High Dose: 19.85 ± 1.92	

Values presented as mean ± SD; * significantly different from control (p < 0.05)

No statistically significant differences in SOD enzymatic activity were observed between control and extract-treated groups (p = 0.137), indicating that the extract did not produce a measurable alteration in SOD levels at the doses and duration employed.

ANOVA COMPARISON FOR GLUTATHIONE (GSH) (mg/dL)

Table 15. Reduced Glutathione (GSH) Concentrations Across Study Groups

ANOVA	Control Group	Treatment Group	p-value
	37.00 ± 2.55	Low Dose: 38.52 ± 0.60**	0.022
		High Dose: 33.60 ± 1.91*	

Values presented as mean ± SD; * significantly different from control (p < 0.05)

GSH levels showed a statistically significant difference across groups (p = 0.022). The low-dose group exhibited a marginal gain in GSH, whereas the high-dose group demonstrated a significant depletion, supporting a dose-dependent biphasic antioxidant response similar to that observed for IgG.

ANOVA COMPARISON FOR WHITE BLOOD CELLS (WBC) (cells/L)

Table 16. White Blood Cell (WBC) Counts Across Study Groups

ANOVA	Control Group	Treatment Group	p-value
	4.48 ± 0.22	Low Dose: 4.29 ± 0.30**	0.000
		High Dose: 6.75 ± 0.45**	

Values presented as mean ± SD; * p < 0.05; ** p < 0.01

Total WBC counts differed highly significantly across experimental groups (p = 0.000). The low-dose group showed a slight decrease while the high-dose group exhibited a marked leukocytosis, suggestive of either immune activation or a pro-inflammatory systemic response at the higher extract concentration.

DISCUSSION

The present investigation was carried out to characterize the effects of the ethanolic leaf extract of *Hibiscus sabdariffa* on immune and antioxidant parameters specifically IgG, WBC, IL-2, IL-6, SOD, and GSH in male Wistar rats. Results indicated that the extract elicited concentration-dependent effects across measured hematological and immunological endpoints.

IgG levels increased at the low dose but fell at the high dose, suggesting that dilute concentrations of the extract upregulate antibody production while supraphysiological phytochemical loads suppress it. This pattern is consistent with observations made by Okereke (2015), who noted a dose-threshold relationship in the immune-modulating properties of medicinal herbs. The polyphenol-mediated immunomodulatory activity of *H. sabdariffa* in a dose-dependent fashion has also been established by Da-Costa-Rocha et al. (2014). The suppressive effect at high dose may represent immune exhaustion or oxidative overload and contrasts with the sustained immunostimulatory response reported by Gurrola-Díaz et al. (2010) in metabolic syndrome subjects, pointing to possible population- or condition-specific variability in response.

Both treatment groups exhibited elevations in IL-2 and IL-6 compared to the control, implying that *H. sabdariffa* extract activates interleukin-mediated immune cascades. This aligns with findings reported by Augustine et al. (1998) relating to hibiscus polyphenol-driven cytokine production. The persistence of elevated IL-6 is, however, a feature of chronic inflammation and may indicate that high-dose administration tips the physiological balance toward a pro-inflammatory rather than a protective immune state.

SOD activity was reduced in both low- and high-dose groups, a result partially at odds with Crawford et al.'s (1998) report of enhanced antioxidant enzyme function. A plausible explanation is that the extract augments cellular oxidative burden, accelerating enzymatic consumption beyond the rate of replenishment; the greater SOD reduction at the high dose supports dose-proportionate oxidative stress accumulation.

GSH showed a marginal increase at low dose but a significant decline at high dose, reinforcing the hypothesis that moderate *H. sabdariffa* intake bolsters antioxidant defenses while excessive intake depletes them a pattern consistent with Da-Costa-Rocha et al. (2014).

WBC counts were slightly reduced at the low dose but rose markedly at the high dose. The observed leukocytosis corroborates Haji and Haji (1999), who documented stimulation of immune cell proliferation by hibiscus extract; however, excessive leukocytosis may reflect systemic inflammatory stress, reinforcing the concept that high-dose use poses a potential health risk.

In aggregate, these observations confirm the immunomodulatory and antioxidant nature of *Hibiscus sabdariffa*, beneficial at conservative doses but potentially detrimental in excess, providing scientific grounding for dosage-conscious applications in traditional and integrative medicine.

CONCLUSION

This study provides evidence that the ethanolic leaf extract of *Hibiscus sabdariffa* significantly alters immune and antioxidant indices in male Wistar rats in a dose-dependent manner. Low-dose administration was associated with enhanced immunoglobulin synthesis, improved glutathione status, and potentiated immune signaling, all indicative of immunostimulatory and antioxidant benefit. High-dose administration, in contrast, resulted in reduced IgG, depleted glutathione, decreased SOD activity, and elevated WBC counts and interleukin levels, collectively suggesting immune suppression and oxidative imbalance. These findings are consistent with a dose-dependent biphasic pharmacological profile, underscoring the importance of dosage precision in therapeutic applications of *Hibiscus sabdariffa*.

Competing Interests

There is no conflict of interest.

Authors' Contributions

GO; wrote the study design, CM; analysed the data generated from the study, MO; wrote the introduction of the study, OP and CC; wrote the literature review, NC and MI discussed the results.

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