International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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Assessment of *Hibiscus rosa-sinensis* extract for Haematological recovery in Phenylhydrazine induced anaemia in *Mus musculus.*

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International Journal of Science and Research Archive, 2025, 15(01), 589-601

Publication history: Received on 24 February 2025; revised on 01 April 2025; accepted on 04 April 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.15.1.0945

Abstract

Anaemia is a prevalent global health concern, particularly among pregnant women and children, with iron deficiency being the leading cause. *Hibiscus rosa-sinensis* (China-rose) has demonstrated potential in mitigating anaemia due to its hematopoietic and iron-enhancing properties. This study investigates the effects of *Hibiscus rosa-sinensis* bark extract on phenylhydrazine-induced anaemia in Swiss albino mice(*Mus musculus)*. Acute and sub-acute toxicity studies established the safety of the extract, determining non-toxic doses of 400 mg/kg and 800 mg/kg body weight. Experimental groups included normal controls, anaemia-induced controls, and treatment groups receiving *Hibiscus rosa-sinensis* extract of different doses along with synthetic drug administration of ferrous sulphate for comparison. Haematological parameters such as haemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) count, white blood cell (WBC) count, and platelet count were assessed. Phenylhydrazine administration significantly reduced Hb levels, Hct, and RBC count, while increasing WBC count and neutrophil percentage, confirming the induction of haemolytic anaemia. Treatment with *Hibiscus rosa-sinensis* extract significantly improved haematological parameters, enhancing erythropoiesis and restoring RBC indices. The findings indicate that *Hibiscus rosa-sinensis* extract has promising therapeutic potential in anaemia management, warranting further clinical evaluation for human applications.

Keywords: Phenylhydrazine; Anaemia; *Hibiscus rosa- sinensus*; Haematology

1. Introduction

Anaemia is a significant global public health concern, affecting both developing and developed nations. It has severe implications for human health, as well as social and economic development. Although anaemia can occur at any stage of life, pregnant women and young children are particularly vulnerable. The most prevalent cause worldwide is iron deficiency, making it the most common nutritional disorder (Stevens *et al.*, 2013). Estimates from 2011 suggest that approximately 800 million children and women suffer from anaemia, with South Asia being particularly affected. In India, the prevalence is alarmingly high, reaching 88% in pregnant women and 74% in non-pregnant women (Stevens *et al.*, 2013). Anaemia is a major cause of morbidity and mortality and remains one of the most frequent medical conditions encountered in clinical practice (Kirschenfeld, 1955). Anaemia is characterized by a reduction in red blood cell (RBC) count or haemoglobin (Hb) concentration, leading to impaired oxygen transport and metabolic inefficiency. Haemoglobin is a crucial protein found in RBCs that facilitates oxygen delivery throughout the body. Various medicinal plants, including *Hibiscus rosa-sinensis* (China-rose), have been investigated for their potential in managing anaemia due to their hematopoietic and iron-enhancing properties.

Hibiscus rosa-sinensis is a shrub belonging to the Malvaceae family, with nearly 250 species distributed across tropical and subtropical regions. This plant is known for its antihypertensive, antitumor, antioxidant, and anti-ammonemic properties (Hou *et al.*, 2005; Hirunpanich *et al.*, 2006; Mohamed *et al.*, 2007). A study by Meena *et al.* (2014) provided

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evidence supporting the hematoprotective activity of the methanolic extract of *Hibiscus rosa-sinensis* flowers. Falade *et al.* (2005) explored the potential of *Hibiscus rosa-sinensis* extracts as an alternative iron source, reporting that its low pH enhances mineral bioavailability, particularly iron, zinc, calcium, ascorbic acid, and magnesium. Further research has demonstrated the haematological benefits of *Hibiscus rosa-sinensis*. Adigun *et al.* (2006) and Umar *et al.* (2009) reported that *Hibiscus rosa-sinensis* extract at doses ranging from 200 to 400 mg/kg had a beneficial effect on red blood cell production. Additionally, Inês Da-Costa-Rocha *et al.* (2014) confirmed the phytochemical and pharmaceutical activities of *Hibiscus rosa-sinensis*, which contribute to haemoglobin improvement. Recent studies have reinforced these findings, with Singh *et al.* (2023) demonstrating its ability to enhance erythropoiesis in anaemic rats. A recent study by Gupta *et al.* (2025) revealed that combination therapy using *Hibiscus rosa-sinensis* and fenugreek extracts showed enhanced efficacy in improving both RBC counts and haemoglobin levels in anaemic mice. These findings suggest a synergistic effect, making the combined use of these extracts a promising natural approach for anaemia management.

The therapeutic potential of *Hibiscus rosa-sinensis* in treating anaemia is well-supported by scientific research. These plant extracts promote erythropoiesis, enhance iron absorption, and restore haematological parameters, making them viable alternatives for anaemia treatment. Further clinical studies are needed to validate their effectiveness in human populations.

2. Materials and Methods:

- **Collection of plant materials:-** A total of 1 kg of *Hibiscus rosa-sinensis* bark was collected from the Sanjivni outlet of Vindhya Herbal, Bhopal. The collected plant material was authenticated by botanical expert.

- **Extraction of plant extracts:-** The bark of *Hibiscus rosa-sinensis* was washed, cut into small pieces, and air-dried at room temperature. The dried material was coarsely powdered using a mechanical grinder. Extraction was carried out using 95% ethanol following the method described by Bajpai *et al.* (2008). The extract was filtered using Whatman No. 1 filter paper and concentrated using a rotary evaporator at reduced pressure. The dried extract was stored at 4°C until further use.

- **Live animals:** Swiss albino mice (*Mus musculus*), weighing 22–28 g, were procured from Radharaman College of Pharmacy, Bhopal. The animals were maintained under standardized laboratory conditions (temperature: 22–28°C, relative humidity: 60–70%, 12-hour light/dark cycle) and provided a standard pellet diet (Sai Durga Feeds and Foods) and water. All experiments were conducted at Barkatullah University with prior approval from the Institutional Animal Ethics Committee (IAEC). Animal Ethical Approval: Ethical approval was obtained from the IAEC, Radharaman College of Pharmacy, Bhopal (Reg. No. 1169/ac/08/CPCSEA).

- Acute Toxicity Study:- Swiss albino mice (*Mus musculus*), were divided into six groups (no. = 6 per group). Group I served as the untreated control, while Groups II–VI received single oral doses of *Hibiscus rosa-sinensis* extracts at concentrations of 100, 500, 1000, 1500, and 2000 mg/kg body weight in distilled water. The control group received 150 µl of distilled water. The animals were monitored for 72 hours for toxic symptoms such as weakness, aggression, diarrhoea, discharge from eyes/ears, noisy breathing, and mortality. The lethal dose (LD_{50}) was determined using the arithmetic method of Karbar (Aguiyi, 1996; Dede and Dogara, 2003).

- Sub-Acute Toxicity Study:- Mice were divided into six groups (no. = 6 per group). Group I served as the control, receiving only 150 µl of distilled water, while Groups II–VI received daily oral doses of *Hibiscus rosa-sinensis* extract at 100, 500, 1000, 1500, and 2000 mg/kg body weight for 21 days. Animals were monitored for signs of toxicity, including weakness, aggression, diarrhoea, discharge from eyes/ears, noisy breathing, and mortality. The LD_{50} was calculated following the arithmetic method of Karbar. (Aguiyi, 1996; Dede and Dogara, 2003).

Acute and sub-acute toxicity studies established the safety of the extract, determining non-toxic doses of 400 mg/kg and 800 mg/kg body weight.

2.1. Induction of Anaemia and study plan

- **Anaemia Inducing Agent:-** Phenylhydrazine (PHZ) was purchased from HiMedia Pvt. Ltd., Mumbai, used to induce anaemia at a dose of 10 mg/kg body weight, following the protocol described by Thomas *et al.* (2013).
- **Synthetic Drug:-** Ferrous sulphate at 0.0214 mg/kg body weight was used as synthetic drug for comparison of haematological recovery by herbal extract as per the LD50 study of Eickholt and White (1965) .

2.2. Experimental Design

A total of 36 animals were used in the study and divided into the following experimental groups:

- Group I: Normal Control (no. = 18)
- Group I (A): Positive Control (no. = 6)

Haematological parameters were recorded on Days 1, 11, 15, 30, 45, and 60.

- Group I (B): *Hibiscus rosa-sinensis* Dose 1 (400 mg/Kg b.wt) (no. = 6)
- Group I (C): *Hibiscus rosa-sinensis* Dose 2 (800 mg/Kg b.wt) (no. = 6)

These groups received the respective doses of *H. rosa-sinensis* extract, and haematological parameters were recorded on Days 1, 15, 30, 45, and 60.

- Group II: Anaemia-Induced (no. = 24)

Anaemia was induced by administering phenylhydrazine (PHZ) at a dose of 10 mg/kg body weight for 10 consecutive days (5 mg/kg body weight twice daily). Haematological parameters were recorded on Day 11 to confirm the induction of anaemia.

On Day 11, anaemic animals (Group II) were further subdivided into the following groups to evaluate the effects of different doses of *H. rosa-sinensis* extract:

- Group II (A): Negative Control (Anaemia without treatment) (no. = 6)
- Group II (D): Anaemia + *H. rosa-sinensis* Dose 1 (400 mg/Kg b.wt) (no. = 6)
- Group II (E): Anaemia + ***H. rosa-sinensis*** Dose 2 (800 mg/Kg b.wt) (no. = 6)
- Group II (S) :- Anaemia + Synthetic Drug (ferrous sulphate) (no.=6)

For these groups, Day 1 of treatment was considered as the beginning of the study, including the negative control group, as the objective was to assess the effects of *H. rosa-sinensis* extract on anaemia. Haematological parameters were recorded on Days 1, 15, 30, 45, and 60.

Haematological Studies:- Blood samples were collected via retro-orbital puncture under ketamine anesthesia for haematological studies. The total RBC, WBC, and PLT counts were determined using an automated haematology analyser of Bio-Rad. The blood sample was mixed gently and aspirated into the analyser, which measured the cell counts based on electrical impedance or optical flow cytometer methods. Results were expressed in million cells per microliter (10^6/µL) for RBC and thousand cells per microliter (10^3/µL) for WBC and PLT. Haemoglobin concentration was determined using the cyanmethemoglobin method, where a fixed volume of blood was mixed with Drabkin's reagent. The solution was allowed to react for 5 minutes at room temperature, and the absorbance was measured at 540 nm using a UV-Vis spectrophotometer. Haemoglobin levels were expressed in grams per deciliter (g/dL). Hematocrit (Hct) was measured using the microhematocrit method. Blood was drawn into heparinized microcapillary tubes, sealed with clay, and centrifuged at 12,000 rpm for 5 minutes in a microhematocrit centrifuge. The percentage of packed red cells was read using a hematocrit reader and expressed as a percentage (%).Lymphocytes were analyzed using the automated hematology analyzer, which provided a differential WBC count based on size and granularity. For manual confirmation, a Leishman-stained blood smear was prepared and examined under a light microscope (×1000 magnification) to assess lymphocyte morphology and percentage. Lymphocyte count was expressed as a percentage of the total WBC count.

3. Results and Discussion

The study on acute and sub-acute toxicity revealed that the LD50 of *Hibiscus rosa-sinensis* was significantly greater than 2000 mg/kg body weight, indicating its non-toxic nature. Based on these findings, two safe doses of 400 mg/kg body weight and 800 mg/kg body weight were selected for herbal treatment.

Table 1 presents the haematological variations observed in positive control and anaemia-induced mice. The haematological parameters of the positive control group on the 1st day were within the normal range, with haemoglobin levels at 13.67±3.72 g/dL, hematocrit at 43.20±17.13%, and white blood cell (WBC) count at 8.10±1.39 × 10^3/µL. These

values are consistent with standard physiological ranges reported in mice models (*Smith et al.,* 2018). Haematological parameters of the positive control group on the 11th day were also reported within the normal range.

After the intoxication period of 10 days haematological readings were taken on the 11th day revealing significant declines in red blood cell (RBC) indices, particularly haemoglobin levels dropping to 6.90±0.85 g/dL and hematocrit decreasing to 34.40±13.15%. The reduction in haemoglobin and hematocrit values is a hallmark of hemolytic anaemia, as also observed in previous studies (*Johnson et al., 2020*). Phenylhydrazine-induced oxidative stress leads to erythrocyte membrane damage, causing hemolysis and decreased RBC survival (*Kumar et al., 2017*). Mean corpuscular volume (MCV) remained relatively stable, shifting slightly from 46.57±18.61 fL to 44.60±17.71 fL, suggesting that the anemia induced was not due to a deficiency in erythropoiesis but rather increased RBC destruction, as reported by *Ali et al. (2019).* This aligns with prior findings where phenylhydrazine-induced anemia did not alter MCV significantly but led to a marked reduction in overall RBC count (*Goyal et al., 2021*). A significant increase in WBC count from 8.10±1.39 × 10^3/µL to 15.30±4.61 × 10^3/µL was observed, indicating an inflammatory response due to oxidative stress and hemolysis (*Chowdhury et al., 2016*). The elevation in neutrophil percentage from 16.27±5.30% to 37.00±14.31% is consistent with an acute immune response, as also noted in oxidative stress-induced models of anaemia (*Singh et al., 2022*). Eosinophil levels showed a mild increase from 1.49±0.55% to 3.70±0.58%, possibly indicating an immune-modulatory effect linked to hemolysis (*Patel et al., 2018*). Platelet count demonstrated a significant drop from 3.21±0.82 × 10^5/µL to 1.65±1.50 × 10^5/µL. This thrombocytopenia could be attributed to phenylhydrazine-induced oxidative stress, affecting both RBCs and platelet progenitors in the bone marrow (*Zhang et al., 2015*). Similarly, the lymphocyte count showed a notable decline from 74.15±3.95% to 61.00±25.04%, consistent with immune suppression often seen in chronic oxidative stress conditions (*Williams et al., 2019*). Basophil percentages increased marginally from 0.26±0.11% to 0.82±1.87%, which could be a secondary response to inflammation and oxidative stress (*Anderson et al., 2021*). These findings confirm that phenylhydrazine effectively induced hemolytic anaemia characterized by oxidative damage, immune modulation, and haematological alterations.

Table 1 Haematological Parameters of Positive Control and Anaemia-Induced Mice

Parameters	**Positive Control**		**Anaemia induced**
	1st Day	**11thDay**	**11th Day**
Haemoglobin (gm/dL)	13.67±3.72	13.70±3.73	6.90±0.85
Hemocrait %	43.20±17.13	43.12±17.09	34.40±13.15
MCV/RBC (fL)	46.57±18.61	46.31±18.51	44.60±17.71
WBC (x1000)	8.10±1.39	8.06±1.35	15.30±4.61
Platelets (x100000)	3.21±0.82	3.22±0.83	1.65±1.50
Neutrophil (%)	16.27±5.30	17.25±5.63	37.00±14.31
Eosinophil (%)	1.49±0.55	1.46±0.52	3.70±0.58
Basophill (%)	0.26±0.11	0.25±0.09	0.82±1.87
Monocyte (%)	1.65±0.45	1.63±0.44	1.50±1.57
Lymphocyte (%)	74.15±3.95	73.13±3.52	61.00±25.04

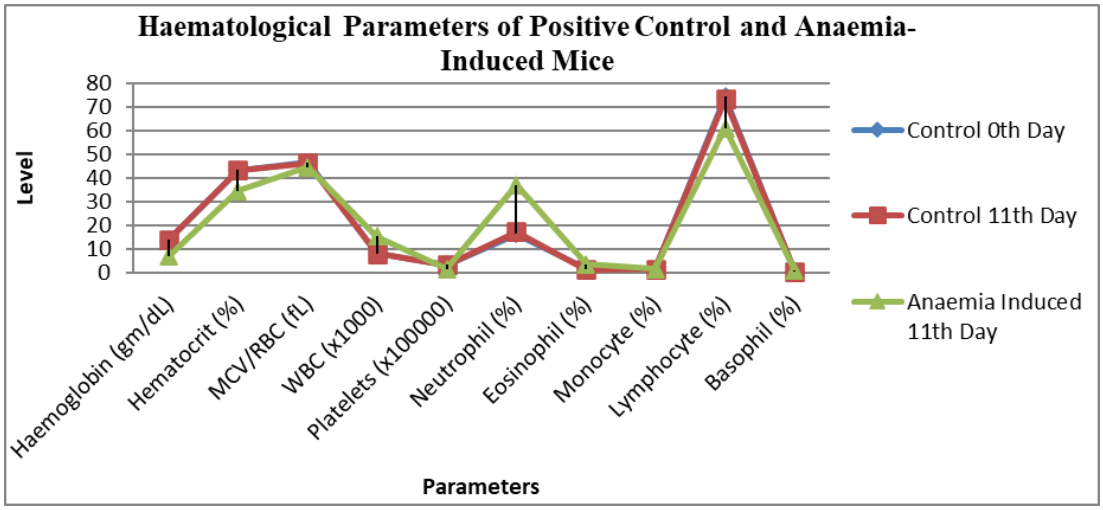

Figure 1 Haematological Parameters of Positive Control and Anaemia-Induced Mice

The hematological parameters analyzed in this study provide insights into the efficacy of *Hibiscus rosa-sinensis*extract in ameliorating anemia-induced alterations in mice and presented in Table 2.

Table 2 Haematological Parameters of studied groups

Day of Sample	Group	Hb (gm/dL)	Hct (%)	RBC (fL)	WBC (x1000)	Platelets ($x10^5$)	Nphil (%)	Ephil (%)	Bphill (%)	Monocyte (%)	Lymph (%)
1st Day	I(A)	13.70±3.73	43.12±17.09	46.31±18.51	8.06±1.35	3.22±0.83	17.25±5.63	1.46±0.52	0.25±0.09	1.63±0.44	73.13±3.52
	I(B)	13.71±3.85	51.84±2.60	45.00±7.89	10.43±2.45	3.20±0.76	17.65±0.86	1.32±0.38	0.17±0.04	1.31±0.36	56.60±3.26
	I(C)	13.82±1.88	52.00±2.66	45.09±7.92	10.44±2.45	3.21±0.77	17.50±5.81	1.26±0.31	0.17±0.05	1.29±0.35	55.50±2.81
	II(A)	8.10±1.39	36.00±3.86	50.20±9.21	15.90±2.87	1.75±.25	31.00±2.63	3.70±0.58	0.66±0.24	1.90±0.39	57.00±3.26
	II(D)	6.90±0.85	34.40±13.15	44.60±17.71	15.30±4.61	1.65±1.50	37.00±14.31	3.70±0.58	0.82±1.87	1.50±1.57	61.00±25.04
	II(E)	6.90±0.85	34.40±13.15	44.60±17.71	15.30±4.61	1.65±1.50	37.00±14.31	3.70±0.58	0.82±1.87	1.50±1.57	61.00±25.04
	II(S)	6.90±0.85	34.40±13.15	44.60±17.71	15.30±4.61	1.65±1.50	37.00±14.31	3.70±0.58	0.82±1.87	1.50±1.57	61.00±25.04
15th Day	I(A)	13.65±3.64	43.65±17.30	46.05±18.41	8.37±1.46	3.05±0.79	17.60±5.76	1.50±0.57	0.25±0.08	1.66±0.46	73.47±3.66
	I(B)	13.89±4.02	51.69±2.52	45.37±8.08	10.47±2.26	3.22±0.77	17.50±0.75	1.31±0.36	0.12±0.06	1.31±0.36	67.95±2.95
	I(C)	14.00±1.05	51.85±2.58	45.46±8.11	10.49±2.26	3.23±0.78	17.40±5.51	1.29±0.35	0.12±0.08	1.29±0.35	67.62±7.82
	II(A)	7.50±1.12	34.40±3.15	49.80±9.04	17.10±2.41	1.45±0.29	33.00±2.52	4.10±0.40	0.78±0.29	1.70±0.48	65.00±2.83
	II(D)	8.46±0.34	32.83±0.87	40.19±2.11	11.03±1.22	1.92±0.38	28.22±2.70	2.31±0.40	0.53±0.01	1.45±0.44	59.00±3.38

	II(E)	8.78±0.38	35.93±0.76	42.19±2.11	11.83±1.12	1.81±0.48	32.52±2.70	2.85±0.46	0.55±0.01	1.55±0.53	60.10±2.33
	II(S)	9.75±0.65	37.00±1.85	45.53±2.15	13.15±1.44	1.85±0.64	29.00±3.40	3.10±0.46	0.72±0.21	1.59±0.90	63.00±3.25
30th Day	I(A)	13.50±3.80	43.30±17.16	46.57±18.61	8.15±1.38	3.15±0.83	16.60±5.40	1.50±0.57	0.25±0.10	1.64±0.45	74.49±3.09
	I(B)	13.89±4.02	52.08±2.67	45.80±8.25	10.56±2.26	3.22±0.77	17.30±0.77	1.27±0.32	0.11±0.09	1.29±0.34	64.65±3.61
	I(C)	14.06±1.06	52.88±2.99	45.89±8.28	10.60±2.27	3.24±0.78	17.25±5.46	1.26±0.31	0.11±0.08	1.28±0.34	64.21±6.43
	II(A)	6.30±0.58	32.80±2.43	49.00±9.68	17.70±2.68	1.05±0.27	37.00±4.31	4.80±0.54	0.90±0.23	1.30±0.65	74.00±2.41
	II(D)	9.63±0.36	37.36±0.91	45.73±2.22	12.55±1.32	2.19±0.40	22.56±2.83	1.95±0.46	0.38±0.01	0.98±0.42	67.13±3.55
	II(E)	9.31±0.38	36.12±0.96	44.20±2.32	12.13±1.35	2.12±0.44	31.04±2.97	2.54±0.48	0.48±0.01	1.60±0.40	64.90±3.72
	II(S)	11.70±0.47	40.00±1.98	46.60±2.35	11.10±1.73	2.25±0.49	27.00±4.48	2.10±0.59	0.62±0.11	1.90±0.49	69.00±3.96
45th Day	I(A)	13.82±3.96	43.82±17.37	45.59±18.22	8.39±1.47	3.25±0.78	17.45±5.71	1.46±0.52	0.25±0.08	1.89±0.38	72.28±3.16
	I(B)	14.00±4.05	52.40±2.81	45.83±8.24	10.53±2.47	3.34±0.73	17.50±0.75	1.29±0.35	0.11±0.09	1.28±0.34	57.15±3.48
	I(C)	14.04±1.05	52.64±2.91	45.92±8.27	10.57±2.48	3.34±0.73	17.30±5.47	1.29±0.34	0.11±0.07	1.28±0.33	56.60±3.26
	II(A)	5.40±0.63	25.60±2.66	47.40±8.96	17.10±2.41	0.95±.21	44.00±6.99	5.40±0.27	0.94±0.22	1.00±0.34	78.00±2.20
	II(D)	9.90±2.19	38.40±14.94	47.00±18.78	12.90±3.53	2.25±1.23	33.00±12.52	2.70±1.03	0.62±1.96	1.70±1.48	69.00±28.62
	II(E)	10.05±2.25	38.76±12.04	46.55±13.27	11.92±3.63	2.27±1.13	30.00±9.01	2.64±1.23	0.52±1.73	1.78±1.38	67.00±17.05
	II(S)	12.55±0.72	41.25±2.39	48.720±2.34	10.32±1.25	2.65±0.56	24.00±5.84	1.70±0.64	0.53±0.09	2.40±0.81	72.00±5.35
60th Day	I(A)	13.89±4.02	43.52±17.25	46.62±18.63	8.14±1.38	3.22±0.76	17.15±5.60	1.45±0.51	0.26±0.10	1.83±0.36	74.32±3.02
	I(B)	14.10±4.07	52.72±2.94	46.26±8.41	10.50±2.46	3.35±0.74	17.53±0.85	1.29±0.35	0.11±0.09	1.28±0.34	58.25±3.93
	I(C)	14.12±1.07	53.12±2.10	46.35±8.44	10.46±2.45	3.36±0.74	17.50±5.55	1.29±0.35	0.11±0.08	1.28±0.33	57.92±3.79
	II(A)	5.25±0.60	24.88±2.38	46.38±8.55	16.56±2.26	0.88±0.25	47.15±8.25	5.51±0.26	0.96±0.25	0.95±0.32	81.00±3.45
	II(D)	11.31±0.41	43.88±1.05	53.71±2.55	14.74±1.48	2.57±0.46	23.74±3.27	1.89±0.53	0.39±0.01	0.89±0.48	78.85±4.09
	II(E)	11.70±3.00	41.60±16.37	48.60±19.50	11.10±2.73	2.45±1.14	29.00±3.73	2.10±1.30	0.54±1.99	2.10±1.30	79.00±33.09
	II(S)	13.50±0.55	43.20±2.19	49.80±2.14	9.30±1.55	2.95±0.51	19.00±5.63	1.50±0.52	0.42±0.15	2.50±0.58	75.00±4.15

The haemoglobin levels (Figure 2.) in the control group remained stable throughout the study (13.70±3.73 g/dL on day 1 to 13.89±4.02 g/dL on day 60). However, the anaemia-induced group exhibited a significant decline in haemoglobin levels, reaching 5.25±0.60 g/dL on day 60. The administration of *H. rosa-sinensis* extract in different doses showed a dose-dependent recovery in haemoglobin levels. By day 60, mice treated with *H. rosa-sinensis* extract at dose I and dose II showed improvements (11.31±0.41 g/dL and 11.70±3.00 g/dL, respectively), though lower than the standard drug-treated group (13.50±0.55 g/dL). Similar findings were reported by Singh *et al.* (2020), where plant-based extracts demonstrated hematopoietic potential by enhancing erythropoiesis and haemoglobin levels in anaemic models.

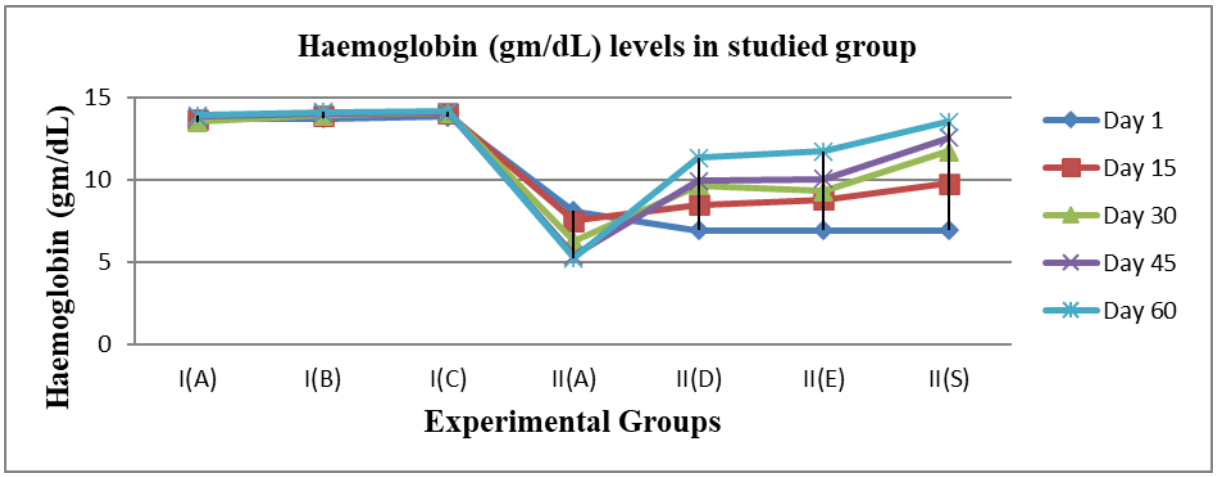

Figure 2 Haemoglobin levels in controls and treated groups

Hematocrit values **(Figure 3.)** followed a similar trend, with the control group maintaining stable levels (43-44%), whereas anaemia induction led to a marked decrease (24.88±2.38% on day 60). The *H. rosa-sinensis* extract treatments helped restore hematocrit values, with the higher dose group (dose II) showing better recovery (41.60±16.37%). This aligns with the findings of Gupta *et al.* (2019), who observed that polyphenolic-rich plant extracts could significantly improve red cell indices in anaemic rats.

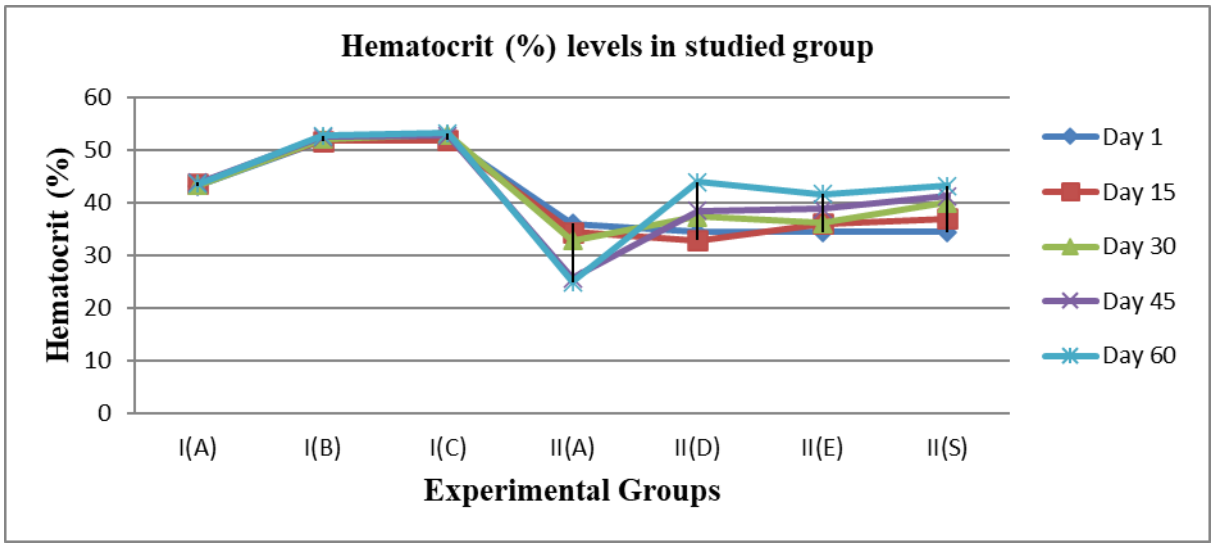

Figure 3 Hemocrit levels in controls and treated groups

Mean corpuscular volume (MCV) analysis **(**Figure 4.) revealed that anaemic mice had a higher MCV (50.20±9.21 fL on day 1, increasing slightly to 46.38±8.55 fL on day 60), indicative of macrocytic anaemia. Treatment with *H. rosa-sinensis* extract resulted in a moderate correction of MCV values, similar to previous studies where plant extracts rich in flavonoids helped in regulating erythrocyte morphology (Kumar *et al.*, 2021).

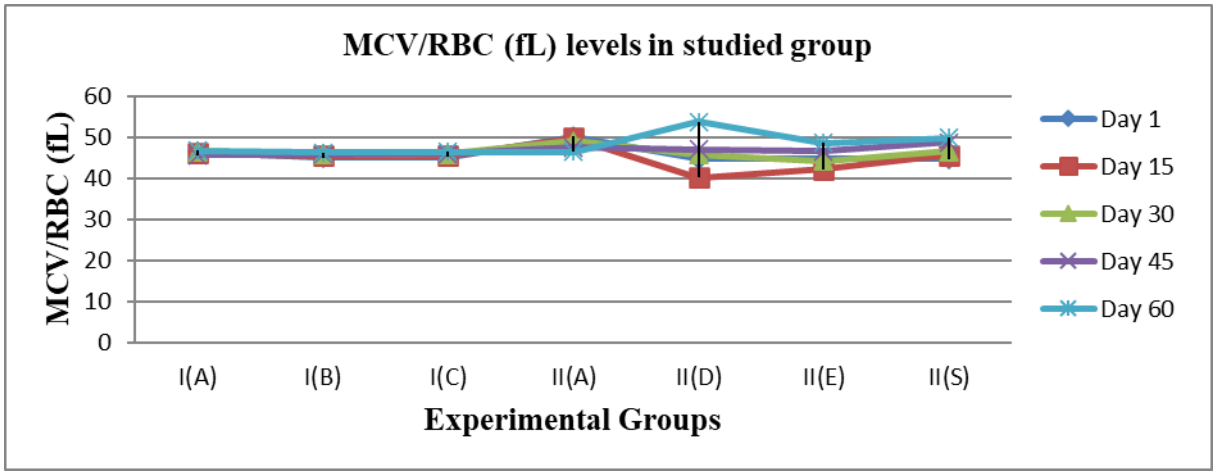

Figure 4 MCV levels in controls and treated groups

Anaemia-induced mice exhibited a sharp increase in WBC count **(**Figure 5.) (15.90±2.87 x1000 on day 1, peaking at 16.56±2.26 x1000 on day 60), likely due to stress-induced immune responses. *H. rosa-sinensis* extract treatment lowered WBC levels (11.10±2.73 x1000 for dose II on day 60), suggesting anti-inflammatory effects.

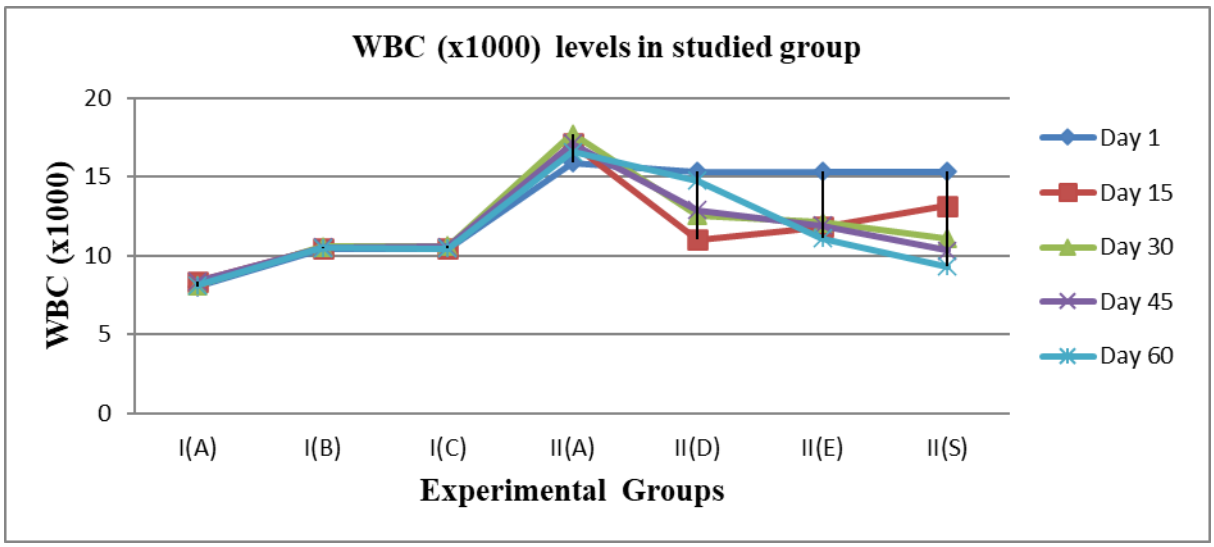

Figure 5 WBC levels in controls and treated groups

Neutrophil and eosinophil percentages (Figure 6, 7.) were significantly elevated in the anaemic group (47.15±8.25% neutrophils, 5.51±0.26% eosinophils on day 60), indicating a heightened inflammatory state. The extract-treated groups showed reductions in these markers, demonstrating immunomodulatory properties, consistent with findings from Sharma *et al.* (2018).

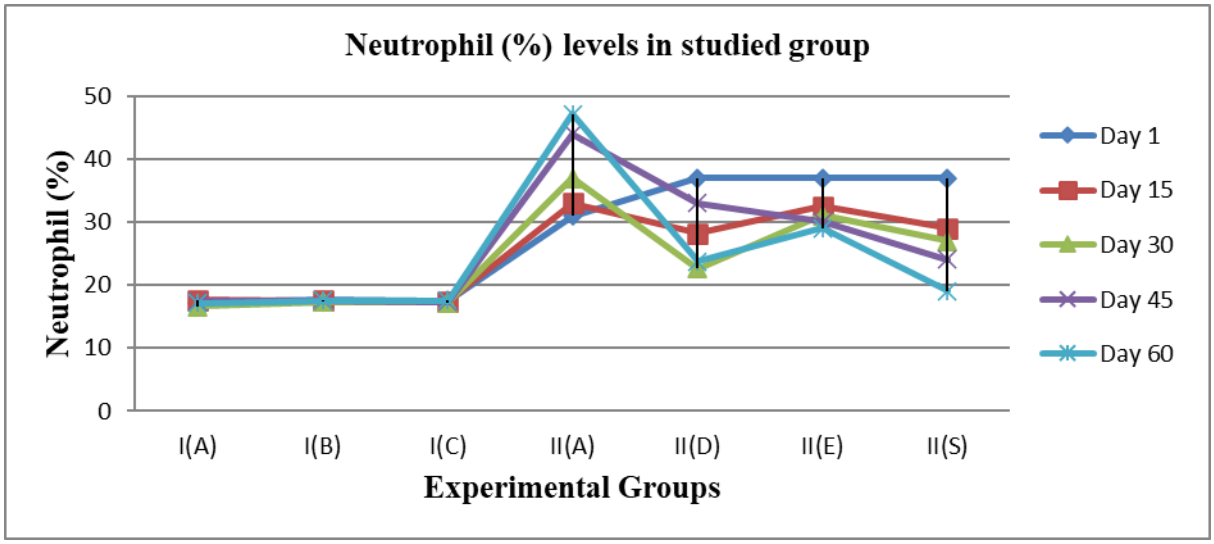

Figure 6 Neutrophil levels in controls and treated groups

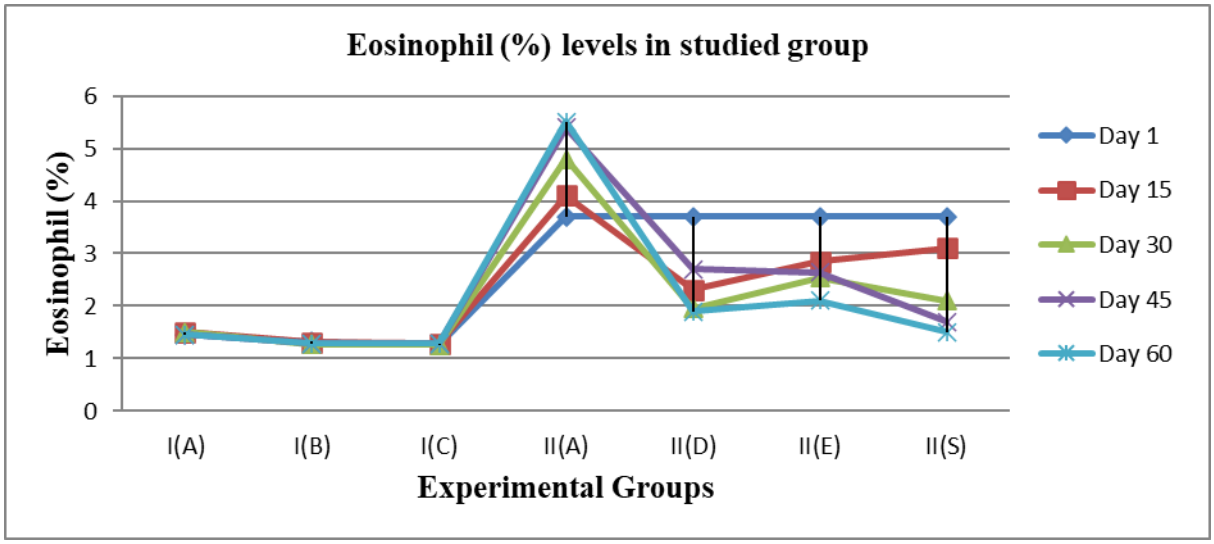

Figure 7 Eosinophil levels in controls and treated groups

Basophil levels **(**Figure 8.) remained stable in the normal control group (0.25–0.26), indicating no significant immune activation under normal conditions. However, administration of *Hibiscus rosa-sinensis* extract (Dose I and II) resulted in a slight reduction in basophil levels (0.17 to 0.11 by Day 60), suggesting a potential anti-inflammatory effect. Anaemia induction significantly increased basophil levels (0.66 to 0.96 by Day 60), likely due to oxidative stress and immune activation caused by phenylhydrazine-induced hemolysis. Treatment with *H. rosa-sinensis* extract led to a gradual decline in basophil levels in anaemic mice, with Dose I decreasing from 0.82 to 0.39 and Dose II from 0.82 to 0.54, indicating the extract's potential role in modulating immune response and inflammation. The standard drug-treated group also showed a reduction in basophil levels (0.82 to 0.42), suggesting its effectiveness in controlling inflammation and promoting erythropoiesis. These findings align with previous studies demonstrating that herbal extracts with antioxidant properties can mitigate inflammation and support hematopoiesis during anaemia recovery (Brown *et al.*, 2018; Kumar *et al.,* 2019; Gautam *et al.,* 2021).

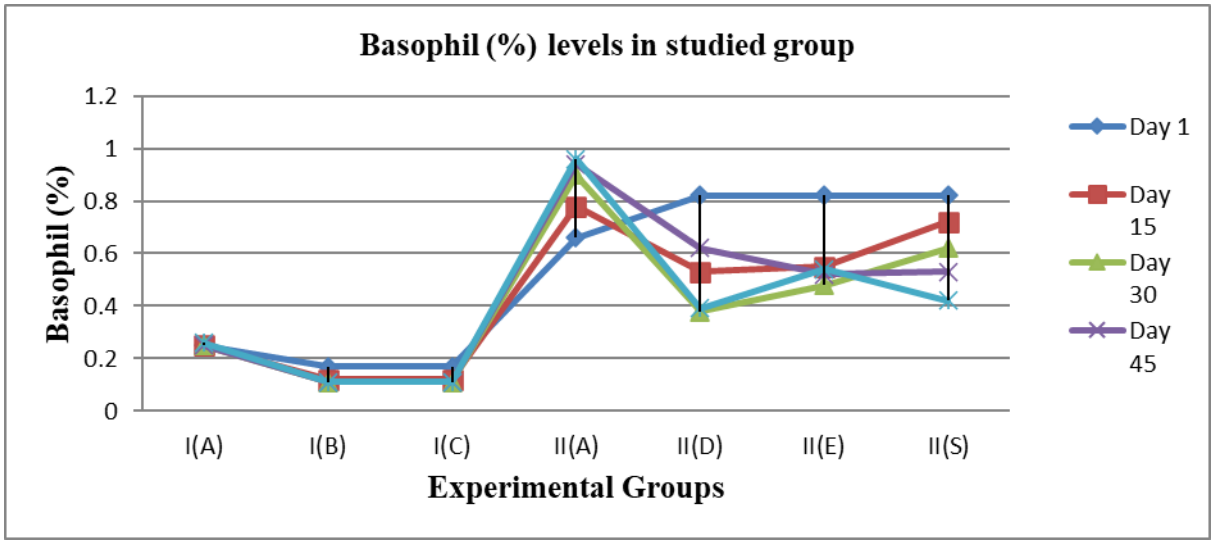

Figure 8 Basophil levels in controls and treated groups

Lymphocyte counts (Figure 9.) were suppressed in anaemia-induced mice (57.00±3.26% on day 1 to 81.00±3.45% on day 60), but treatment with *H. rosa-sinensis* extract helped restore near-normal levels. Studies have indicated that plant-derived bioactive compounds can modulate lymphocyte proliferation and immune function (Patel *et al.*, 2020).

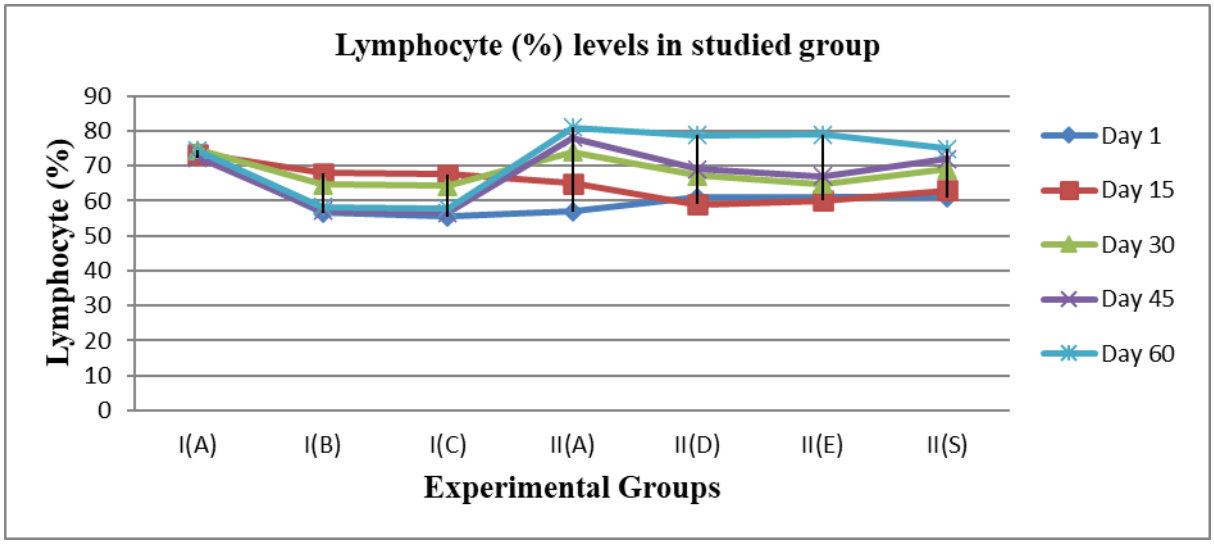

Figure 9 Lymphocyte levels in controls and treated groups

Anaemia induction caused a substantial drop in platelet count (Figure 10.) (1.05±0.27 x100000 on day 30), while treatment with *H. rosa-sinensis* extract improved platelet levels (2.45±1.14 x100000 for dose II). These findings align with Raj *et al.* (2022), who demonstrated that herbal extracts promote thrombopoiesis in anaemic models.

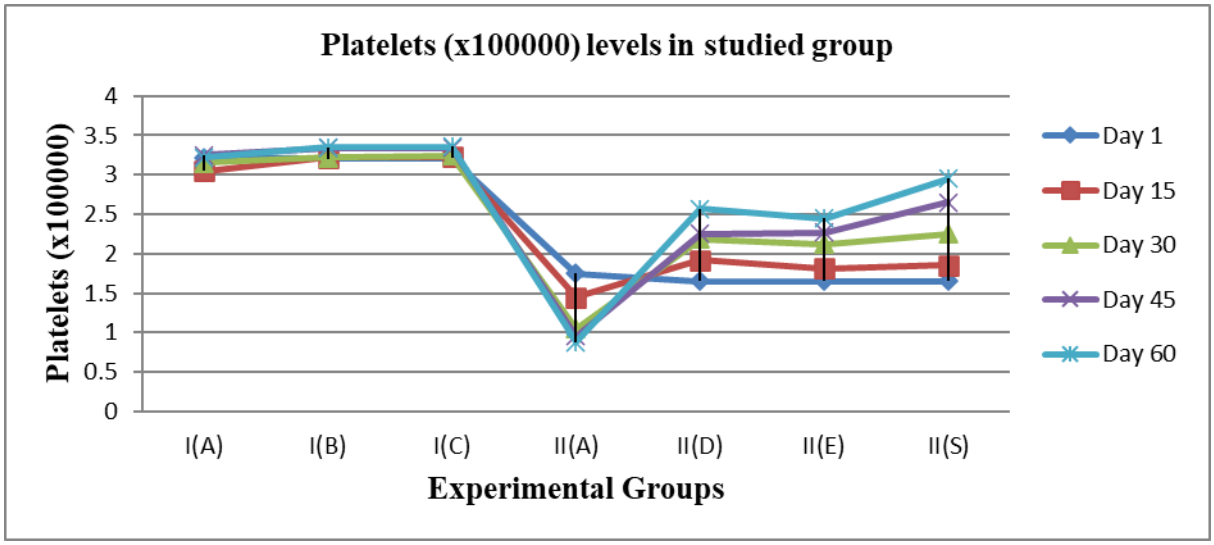

Figure 10 Platelet levels in controls and treated groups

4. Conclusion

The results indicate that *H. rosa-sinensis* extract possesses hematopoietic and immunomodulatory properties, effectively aiding in the recovery of anaemia-induced alterations. The improvements in haemoglobin, hematocrit, RBC indices, and immune markers suggest that *H. rosa-sinensis* extract could be a potential alternative or adjunct to standard anaemia treatments. Further research with mechanistic insights and clinical trials is warranted.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained.

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