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Comparative Investigation of *Hibiscus rosa-sinensis* and *Trigonella foenum-graecum* Extracts for Restoring Haematological Parameters in Phenylhydrazine-Induced Anaemia in *Mus musculus*

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Abstract: Anaemia is a prevalent haematological disorder characterized by reduced haemoglobin levels, impaired erythropoiesis, and associated immune dysregulation. This study evaluates the therapeutic potential of *Hibiscus rosa-sinensis* (China-rose) and *Trigonella foenum-graecum* (Fenugreek) extracts in anaemia-induced mice by assessing their haematological effects. Anaemia was induced in mice, followed by oral administration of *H.rosa-sinensis* and *T.foenum-graecum* extracts at different doses (400 mg/kg and 800 mg/kg), individually and in combination, for 60 days. Haematological parameters, including haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, and differential leukocyte counts, were analyzed. The results showed a significant, dose-dependent improvement in Hb and HCT levels, with the highest efficacy observed at 800 mg/kg doses. Combination therapy exhibited a synergistic effect, closely approaching the standard ferrous sulfate treatment. WBC normalization and a reduction in neutrophil, eosinophil, and basophil counts suggest anti-inflammatory and immunomodulatory effects. Additionally, platelet recovery and the restoration of monocyte and lymphocyte levels further highlight the hematopoietic potential of these extracts. The observed effects are likely attributed to the bioactive compounds, including flavonoids, polyphenols, and saponins, which enhance erythropoiesis, reduce oxidative stress, and modulate immune responses. These findings support the potential use of *H.rosa-sinensis* and *T.foenum-graecum* as natural alternatives for anaemia management, either individually or in combination, offering a promising plant-based therapeutic approach.

Keywords: *Anaemia, Haematology, T.Foenum-Graecum, H.Rosa-Sinensis, Standard Drug.*

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I. INTRODUCTION

Anaemia is a global health concern characterized by reduced haemoglobin levels, leading to impaired oxygen transport and physiological dysfunction. It affects millions worldwide, particularly in developing countries, where nutritional deficiencies and chronic diseases are prevalent (Kassebaum *et al*., 2014). Conventional treatments include iron supplementation and erythropoiesis-stimulating agents; however, these interventions often present limitations such as gastrointestinal side effects and high costs (Camaschella, 2015). Consequently, there is growing interest in exploring plant-based alternatives for managing anaemia.

Trigonella foenum-graecum (Fenugreek) and *Hibiscus rosa-sinensis* (China-rose) have been traditionally used in herbal medicine for their purported hematopoietic and antioxidant properties. *T.foenum-graecum* is rich in iron, flavonoids, and saponins, which contribute to erythropoiesis and red blood cell (RBC) stabilization (Nair *et al*., 2018). Similarly, *H.rosa-sinensis* contains anthocyanins and polyphenols that exhibit antioxidant and anti-inflammatory effects, potentially benefiting haematological parameters (Bhawya *et al*., 2019). Despite their traditional use, scientific validation of their efficacy in anaemia management remains limited.

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This study evaluates the effects of *T.foenum-graecum* and *H.rosa-sinensis* extracts on anaemia-induced mice over a 60-day period. Haematological parameters such as haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, and differential leukocyte counts were analyzed to assess their therapeutic potential.

II. MATERIALS AND METHODS

➢ *Collection of Plant Materials*

1 kg each of *Trigonella foenum-graecum* seed and *Hibiscus rosa-sinensis* bark were collected from the Sanjivni outlet of Vindhya Herbal, Bhopal. The collected plant material was authenticated by a botanical expert.

➢ *Extraction of Plant Extracts*

The bark of *Hibiscus rosa-sinensis* and seeds of *Trigonella foenum-graecum* were washed, 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.

➢ *Animal Model and Ethical Approval*

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 ad libitum. All experiments were conducted at Barkatullah University with prior approval from the Institutional Animal Ethics Committee (IAEC). Ethical approval was obtained from the IAEC, Radharaman College of Pharmacy, Bhopal (Reg. No. 1169/ac/08/CPCSEA).

➢ *Acute Toxicity Study of H. Rosa-Sinensis and T. Foenum-Graecum Extracts Individually*

Swiss albino mice were divided into six groups (n = 6 per group). Group I served as the untreated control, while Groups II–VI received single oral doses of respective plant extract 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, diarrhea, discharge from eyes/ears, noisy breathing, and mortality. The lethal dose (LD_{50}) was determined using the arithmetic method of Karbar (Aguiyi, 1996; Dede & Dogara, 2003).

➢ *Sub-Acute Toxicity Study of H. Rosa-Sinensis and T. Foenum-Graecum Extracts Individually*

Mice were divided into six groups (n = 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 respective plant 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, diarrhea, discharge from eyes/ears, noisy breathing, and mortality. The LD_{50} was calculated following the arithmetic method of Karbar.

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

➢ *Induction of Anaemia and Study Plan*

- *Anaemia Indusing Agent*

Phenylhydrazine (PHZ) was purchased from HiMediaPvt. Ltd., Mumbai, was 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)

- *Experimental Design*

A total of 78 animals were used in the study and divided into the following experimental groups:

- *Group I:* ***Normal Control (n = 30)***

Group I (A): Positive Control (n = 6)
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)
Group I (F): *Trigonella foenum-graecum* Dose 1 (400 mg/Kg b.wt) (n = 6)
Group I (G): *Trigonella foenum-graecum* Dose 2 (800 mg/Kg b.wt) (n = 6)

- *Group II:* ***Anaemia-Induced (n = 48)***

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 *Trigonella foenum-graecum* and *H.rosa sinensis* extract:

Group II (A): Negative Control (Anaemia without treatment) (n = 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)

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Group II (H): Anaemia + *Trigonella foenum-graecum* Dose 1 (400 mg/Kg b.wt) (n = 6)
Group II (I): Anaemia + *Trigonella foenum-graecum* Dose 2 (800 mg/Kg b.wt) (n = 6)
Group II (J): Anaemia + *Trigonella foenum-graecum* + *Hibiscus rosa sinensis* Dose of 400 mg/kg body weight of each (n = 6)
Group II (K): Anaemia + *Trigonella foenum-graecum* + *Hibiscus rosa-sinensis* Dose of 800 mg/kg body weight of each (n = 6)
Group II (S): Anaemia + Ferrous sulphate 0.0214 mg/kg b. wt. (n = 6)

For these experimental, Day 1 of treatment was considered the beginning of the study, including the negative control group, to assess the combined effects of *Trigonella foenum-graecum* + *Hibiscus rosa-sinensis* extract compared to standard drug ferrous sulphate on anaemia. Haematological parameters were recorded on Days 1, 15, 30, 45, and 60.

- *Haematological Analysis*

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.

III. RESULTS AND DISCUSSION

The study on acute and sub-acute toxicity revealed that the LD_{50} of *Trigonella foenum-graecum* and *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.

The haematological parameters presented in the **Table 1** and **Graph 1** indicates significant alterations in response to anaemia induction, reflecting disruptions in erythropoiesis and immune responses.

Table 1 Haematological Parameters of Control and Anaemia-Induced Mice

Parameters	Control		Anaemia induced
	0th Day	11th Day	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	24.60±07.71
WBC (x1000)	8.10±1.39	8.06±1.35	15.30±4.61
Platelets (x100000)	3.21±0.82	3.22±0.83	0.95±0.50
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	1.90±1.57
Lymphocyte (%)	74.15±3.95	73.13±3.52	51.00±5.04

The haemoglobin (Hb) levels remained stable in the control group (13.67±3.72 to 13.70±3.73 g/dL), whereas anaemia induction caused a sharp decline to 6.90±0.85 g/dL. A similar trend was observed in hematocrit (HCT), which dropped from 43.12±17.09% in the control to 34.40±13.15% in anaemic mice. This decline suggests impaired red blood cell production or increased destruction, characteristic of anaemia (Gupta *et al*., 2020). Mean corpuscular volume per RBC (MCV) also showed a considerable reduction, dropping from approximately 46.31±18.51 fL in control mice to 24.60±07.71 fL in anaemic mice. This aligns with microcytic anaemia, which is often associated with iron deficiency or chronic disease (Patel *et al.,* 2021).

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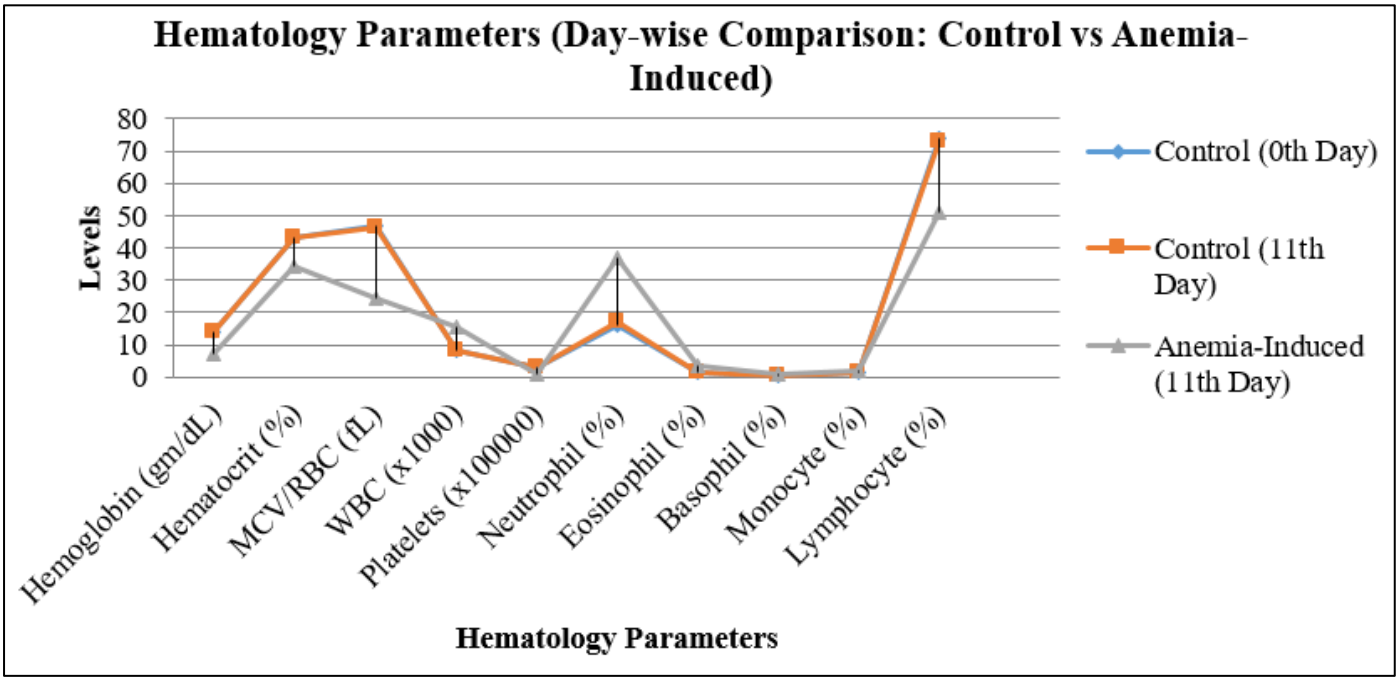

Graph 1 Haematological Parameters of Control and Anaemia-Induced Mice

White blood cell (WBC) count increased significantly in anaemia-induced mice (15.30±4.61 x 1000) compared to controls (8.06±1.35 x 1000). Elevated WBC levels often indicate an immune response to oxidative stress or inflammation due to hemolysis (Singh *et al.,* 2019). Neutrophil percentage also rose from 17.25±5.63% to 37.00±14.31%, indicating a shift towards an inflammatory state. Conversely, lymphocytes decreased from 73.13±3.52% to 51.00±5.04%, potentially due to stress-induced immunomodulation (Sharma *et al.,* 2018). Platelet count showed a drastic decline in anaemic mice (0.95±0.50 x 100000), suggesting impaired thrombopoiesis or increased platelet destruction, common in severe anaemia (Kumar *et al.,* 2022). Eosinophils, basophils, and monocytes also exhibited fluctuations, with eosinophils increasing to 3.70±0.58% and basophils to 0.82±1.87%, possibly linked to inflammatory responses (Verma *et al.,* 2020).

Haematological parameters are crucial indicators of anaemia and the effectiveness of therapeutic interventions. This study evaluates the effects of *T.foenum-graecum* and *H.rosa-sinensis* extracts on anaemia-induced mice over a 60-day period by assessing haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), white blood cell (WBC) count, platelet count, and differential leukocyte counts 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)	13.70±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(C)	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(F)	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(G)	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
	II(A)	6.90±0.85	34.40±13.15	24.60±07.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04
	II(D)	6.90±0.85	34.40±13.15	44.60±17.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04
	II(E)	6.90±0.85	34.40±13.15	44.60±17.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04
	II(H)	6.90±0.85	34.40±13.15	24.60±07.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04

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	II(I)	6.90±0.85	34.40±13.15	24.60±07.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04
	II(J)	6.90±0.85	34.40±13.15	24.60±07.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04
	II(K)	6.90±0.85	34.40±13.15	24.60±07.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04
	II(S)	6.90±0.85	34.40±13.15	24.60±07.71	15.30±4.61	0.95±0.50	37.00±14.31	3.70±0.58	0.82±1.87	1.90±1.57	51.00±5.04
15th Day	I(A)	13.72±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
	I(F)	13.76±2.96	50.97±2.48	44.73±7.97	10.32±2.23	3.17±0.76	17.25±0.74	1.29±0.35	0.12±0.06	1.29±0.35	67.00±2.91
	I(G)	13.83±1.04	51.23±2.55	44.91±8.01	10.36±2.23	3.19±0.77	17.19±5.44	1.27±0.35	0.12±0.08	1.27±0.35	66.81±7.73
	II(A)	7.50±1.12	34.40±3.15	49.80±9.04	17.10±2.41	0.88±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	1.81±0.48	28.22±2.70	2.31±0.40	0.53±0.01	1.45±0.44	48.00±3.38
	II(E)	8.78±0.38	35.93±0.76	42.19±2.11	11.83±1.12	1.92±0.38	32.52±2.70	2.85±0.46	0.55±0.01	1.32±0.53	43.10±2.33
	II(H)	7.85±0.45	36.65±0.79	34.30±3.25	13.64±2.30	1.03±0.39	28.65±2.72	2.83±0.45	0.71±0.01	1.73±0.35	59.10±3.45
	II(I)	9.05±0.42	36.21±0.92	35.43±2.14	13.67±1.96	1.24±0.47	32.67±3.00	3.02±0.66	0.61±0.04	1.81±0.67	65.36±3.16
	II(J)	8.47±0.53	36.48±1.11	32.86±2.94	13.63±1.32	1.08±0.26	35.95±3.47	3.30±0.65	0.61±0.03	1.82±0.62	59.79±3.72
	II(K)	8.64 ± 0.54	37.21 ± 1.13	33.52 ± 3.00	13.90 ± 1.35	1.10±0.27	36.67 ± 3.54	3.37 ± 0.66	0.62 ± 0.03	1.86±0.63	60.99±3.79
	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)	13.75±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
	I(F)	13.76±2.96	51.35±2.63	45.16±8.13	10.41±2.23	3.17±0.76	17.06±0.76	1.25±0.32	0.11±0.09	1.27±0.34	63.74±3.56
	I(G)	13.89±1.05	52.25±2.95	45.34±8.18	10.47±2.24	3.20±0.77	17.04±5.39	1.24±0.31	0.11±0.08	1.26±0.34	63.44±6.35
	II(A)	6.30±0.58	32.80±2.43	49.00±9.68	17.70±2.68	0.79±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	2.12±0.44	22.56±2.83	1.95±0.46	0.38±0.01	1.15±0.42	41.13±3.55
	II(E)	9.31±0.38	36.12±0.96	44.20±2.32	12.13±1.35	2.19±0.40	31.04±2.97	2.54±0.48	0.48±0.01	1.08±0.40	39.90±3.72
	II(H)	8.91±0.35	38.56±0.89	42.30±2.15	12.61±1.33	1.55±0.49	26.70±2.75	2.43±0.40	0.66±0.02	1.43±0.51	62.10±3.45
	II(I)	9.80±0.38	38.02±0.97	46.53±2.34	12.77±1.36	1.83±0.42	28.17±3.05	2.67±0.46	0.50±0.01	1.68±0.49	68.31±3.76
	II(J)	9.87±0.40	38.28±1.01	38.16±2.44	12.86±1.42	1.44±0.46	32.90±3.27	2.69±0.57	0.51±0.01	1.69±0.44	68.79±3.91
	II(K)	10.07 ± 0.41	39.05 ± 1.03	38.92 ± 2.49	13.12 ± 1.45	1.47±0.47	33.56 ± 3.34	2.74 ± 0.58	0.52 ± 0.01	1.72±0.45	70.17±3.99
	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

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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
	I(F)	13.80±2.99	51.67±2.77	45.19±8.12	10.38±2.44	3.29±0.72	17.25±0.74	1.27±0.35	0.11±0.09	1.26±0.34	56.35±3.43
	I(G)	13.87±1.04	52.01±2.88	45.37±8.17	10.44±2.45	3.30±0.72	17.09±5.40	1.27±0.34	0.11±0.07	1.26±0.33	55.92±3.22
	II(A)	5.40±0.63	25.60±2.66	47.40±8.96	17.10±2.41	0.64±0.21	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	2.27±1.13	33.00±12.52	2.70±1.03	0.62±1.96	0.96±1.48	39.31±2.62
	II(E)	10.05±2.25	38.76±12.04	46.55±13.27	11.92±3.63	2.25±1.23	30.00±9.01	2.64±1.23	0.52±1.73	0.88±1.38	37.10±1.05
	II(H)	10.25±0.37	39.14±0.93	44.65±2.66	12.35±1.21	1.93±0.47	25.32±2.91	2.02±0.47	0.54±0.02	1.16±0.23	68.42±3.42
	II(I)	10.65±0.55	40.06±0.92	49.57±2.54	12.02±1.46	2.13±0.68	24.37±3.12	2.17±0.38	0.46±0.03	1.43±0.34	65.31±3.92
	II(J)	11.61±0.42	42.15±1.06	41.83±2.55	10.00±1.49	1.68±0.55	28.65±2.92	1.79±0.35	0.36±0.01	1.31±0.21	64.50±4.09
	II(K)	11.84 ± 0.43	42.99 ± 1.08	42.67 ± 2.60	10.20 ± 1.52	1.71±0.56	29.22 ± 2.98	1.83 ± 0.36	0.37 ± 0.01	1.34±0.21	65.79 ± 4.17
	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
	I(F)	13.90±2.01	51.98±2.90	45.61±8.29	10.35±2.43	3.30±0.73	17.28±0.84	1.27±0.35	0.11±0.09	1.26±0.34	57.43±3.87
	I(G)	13.95±1.06	52.48±2.07	45.79±8.34	10.33±2.42	3.32±0.73	17.29±5.48	1.27±0.35	0.11±0.08	1.26±0.33	57.22±3.74
	II(A)	5.25±0.60	24.88±2.38	46.38±8.55	16.56±2.26	0.58±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	2.45±1.14	23.74±3.27	1.89±0.53	0.39±0.01	0.89±0.48	37.85±4.09
	II(E)	11.70±3.00	41.60±16.37	48.60±19.50	11.10±2.73	2.57±0.46	29.00±3.73	2.10±1.30	0.54±1.99	0.76±1.30	33.00±3.09
	II(H)	11.15±0.47	40.74±0.98	45.65±2.76	10.46±1.57	2.33±0.41	24.35±2.89	1.98±0.43	0.40±0.01	0.96±0.42	71.42±3.62
	II(I)	11.66±0.40	45.24±1.01	55.37±2.46	11.58±1.46	2.65±0.44	20.54±3.15	2.10±0.51	0.41±0.02	1.16±0.46	81.29±3.95
	II(J)	11.82±0.76	43.15±1.19	42.85±2.78	9.87±1.58	2.14±0.45	24.25±2.47	1.27±0.43	0.36±0.01	0.98±0.32	62.50±4.42
	II(K)	12.06 ± 0.78	44.01 ± 1.21	43.71 ± 2.84	10.07 ± 1.61	2.18±0.46	24.74 ± 2.52	1.30 ± 0.44	0.37 ± 0.01	1.00±0.33	63.75 ± 4.51
	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

➢ *Haemoglobin (Hb)*

Observed data are presented in **Graph 2.** Control mice maintained stable Hb levels (~13.70–13.89 g/dL), while anemic mice exhibited a progressive decline (6.90 ± 0.85 g/dL to 5.25 ± 0.60 g/dL). Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts significantly improved Hb levels in a dose-dependent manner, with higher doses (800 mg/kg) showing greater efficacy (11.70 ± 3.00 g/dL and 11.66 ± 0.40 g/dL, respectively). Combination therapy (400 mg/kg each) demonstrated a synergistic effect, achieving 12.06 ± 0.78 g/dL, though slightly lower than the standard drug, Ferrous Sulphate (13.50 ± 0.55 g/dL). The observed hematopoietic effects may be attributed to bioactive compounds such as flavonoids and iron-binding

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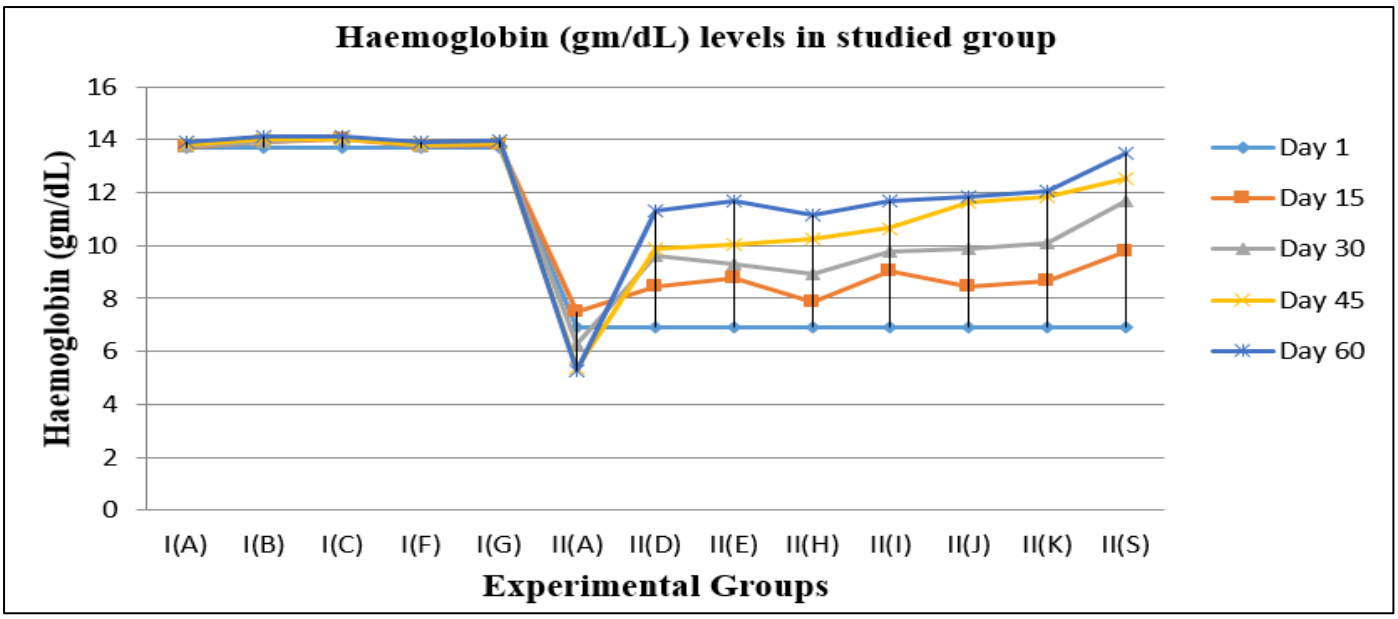
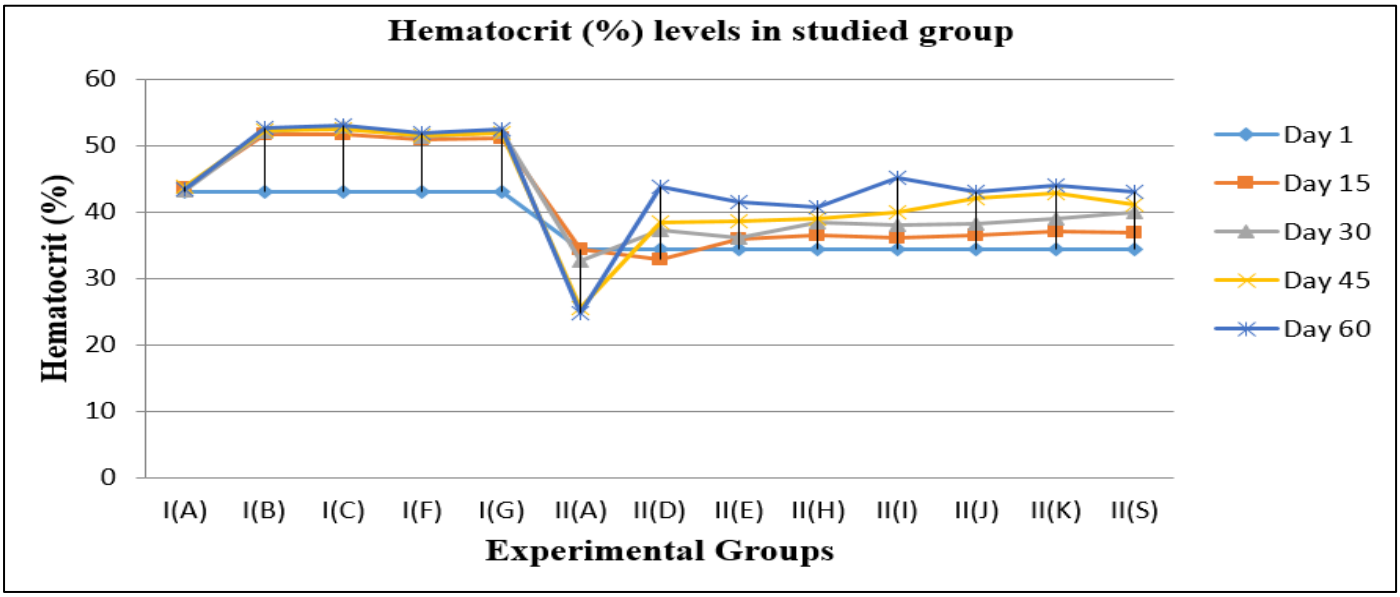
phytochemicals, which enhance erythropoiesis and reduce oxidative stress (Sowmya & Rajyalakshmi, 1999; Nissenson & Goodnough, 2003; García-Casal *et al.*, 2018).



Graph 2 Haemoglobin Levels in Controls and Treated Groups

➢ *Hematocrit (HCT)*

Observed data are presented in **Graph 3**. In control mice, Hct levels remained stable (~43.12–43.82%), while anaemia induction led to a progressive decline from 34.40 ± 13.15% (Day 1) to 24.88 ± 2.38% (Day 60), confirming the suppression of erythropoiesis. Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts significantly improved Hct in a dose-dependent manner, with 800 mg/kg *T.foenum-graecum* (45.24 ± 1.01%) showing better recovery than 800 mg/kg *H.rosa-sinensis* (41.60 ± 16.37%) by Day 60. Combination therapy (400 mg/kg each) exhibited a synergistic effect (44.01 ± 1.21%), approaching the efficacy of the standard drug, Ferrous Sulphate (43.20 ± 2.19%). This recovery indicates enhanced erythropoiesis (Jones *et al.*, 2019).



Graph 3 Hemocrit Levels in Controls and Treated Groups

➢ *Mean Corpuscular Volume (MCV)*

Observed data are presented in **Graph 4**. MCV remained stable in the control group (~46.5 fL). In the control group, MCV remained stable (~46.31–46.62 fL), while anemia induction caused an initial spike (49.80 ± 9.04 fL at Day 15) followed by a gradual decline (46.38 ± 8.55 fL at Day 60), suggesting microcytic anemia. Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts resulted in dose-dependent improvements in MCV, with 800 mg/kg *T.foenum-graecum* (55.37 ± 2.46 fL) showing the highest

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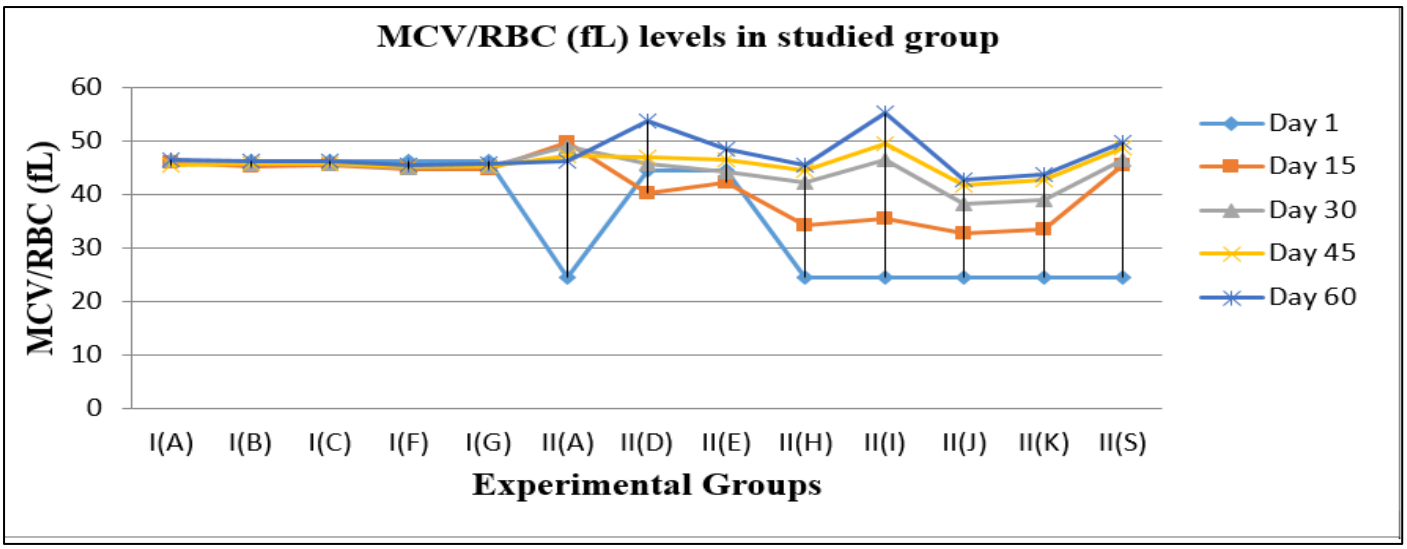
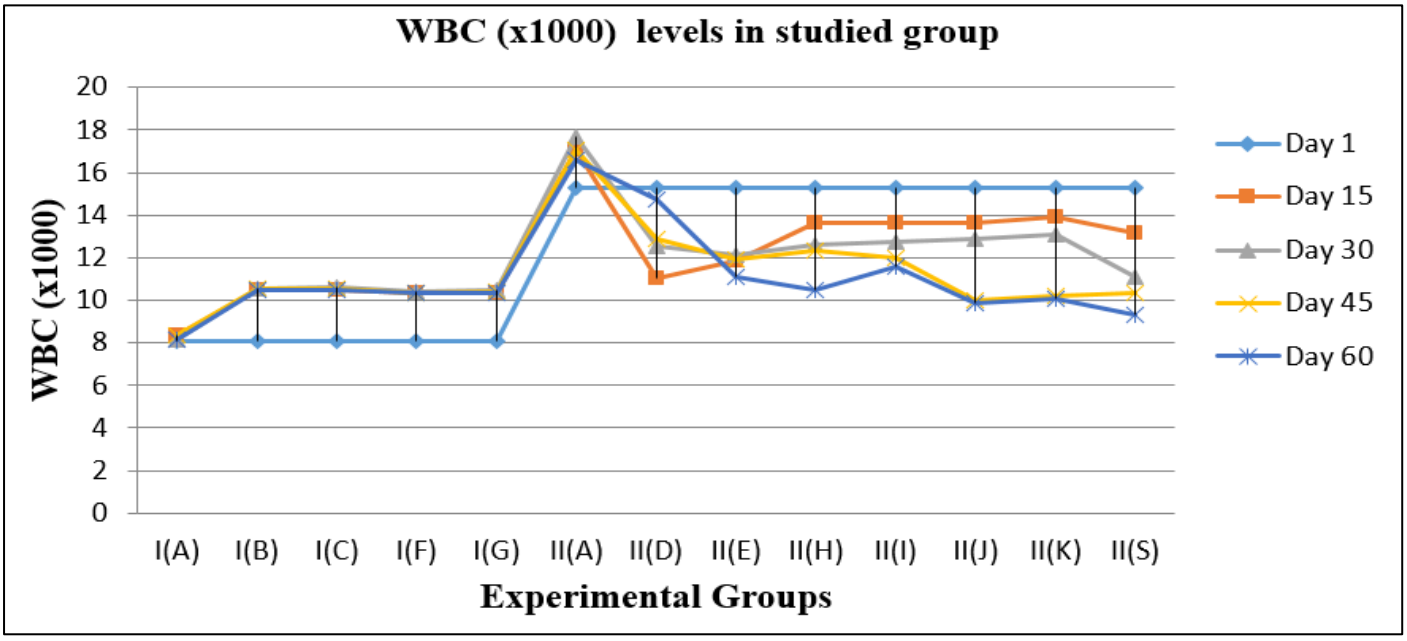
recovery, surpassing *H.rosa-sinensis* (48.60 ± 19.50 fL) and the combination therapy (43.71 ± 2.84 fL). Standard ferrous sulfate treatment maintained optimal erythrocyte size (49.80 ± 2.14 fL). This study revealed the study of Miller *et al.*, (2018).



Graph 4 MCV Levels in Controls and Treated Groups

➢ *White Blood Cell (WBC) Count*

Observed data are presented in **Graph 5**. The control group exhibited stable WBC counts (~8.06–8.39 $\times 10^3/\mu L$) throughout the study. Anemia induction significantly elevated WBC levels (17.70 ± 2.68 $\times 10^3/\mu L$ at Day 30), indicating an inflammatory or stress response. Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts led to a gradual reduction in WBC counts, demonstrating their potential anti-inflammatory and hematopoietic properties. *T.foenum-graecum* at 800 mg/kg showed a marked decline in WBC count (11.58 ± 1.46 $\times 10^3/\mu L$ at Day 60), while *H.rosa-sinensis* at the same dose reduced WBC levels to 11.10 ± 2.73 $\times 10^3/\mu L$. The combined extract treatment (400 mg/kg each) resulted in WBC normalization (10.07 ± 1.61 $\times 10^3/\mu L$ at Day 60), closely aligning with the standard ferrous sulfate-treated group (9.30 ± 1.55 $\times 10^3/\mu L$). Sharma *et al.*, (2020) earlier reported similar findings.



Graph 5 WBC Levels in Controls and Treated Groups

➢ *Platelet Count*

Observed data are presented in **Graph 6.** Platelet count, a crucial parameter in blood clotting and hemostasis, was significantly reduced in anemia-induced mice, declining from 0.95 ± 0.50 $\times 10^5/\mu L$ on Day 1 to 0.58 ± 0.25 $\times 10^5/\mu L$ by Day 60. Treatment with *Hibiscus rosa-sinensis* (H.rosa-sinensis) and *Trigonella foenum-graecum* (*T.foenum-graecum*) extracts effectively restored platelet levels. *H.rosa-sinensis* extract at 800 mg/kg exhibited a steady increase, reaching 2.57 ± 0.46 $\times 10^5/\mu L$, while *T.foenum-graecum* at 800 mg/kg

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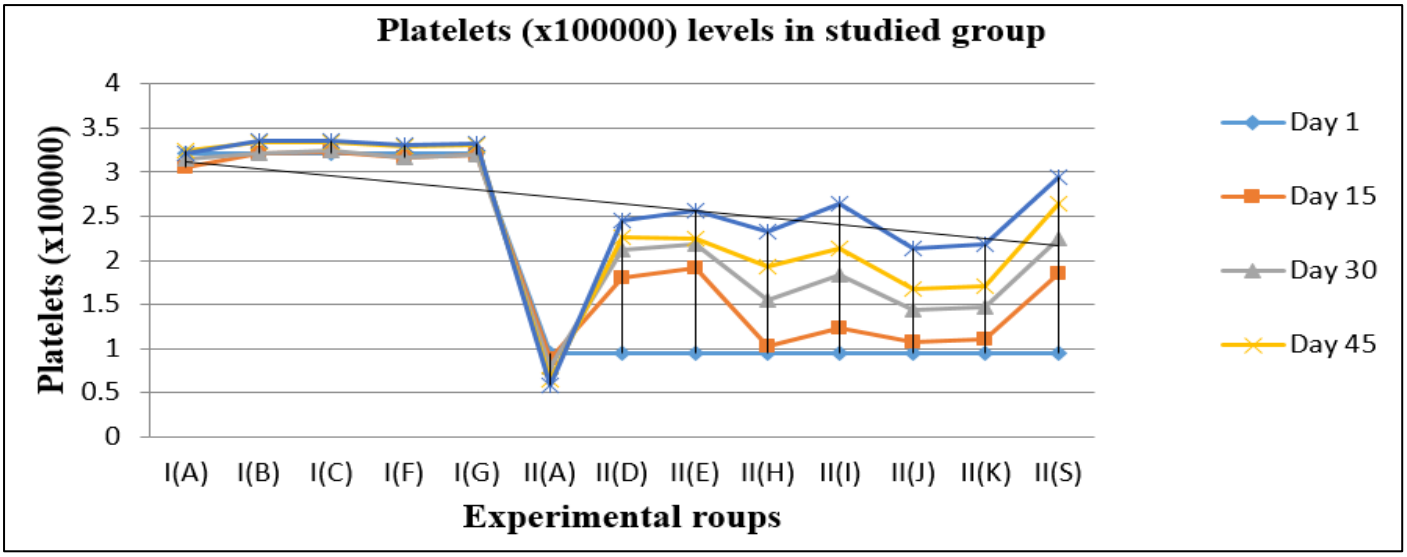
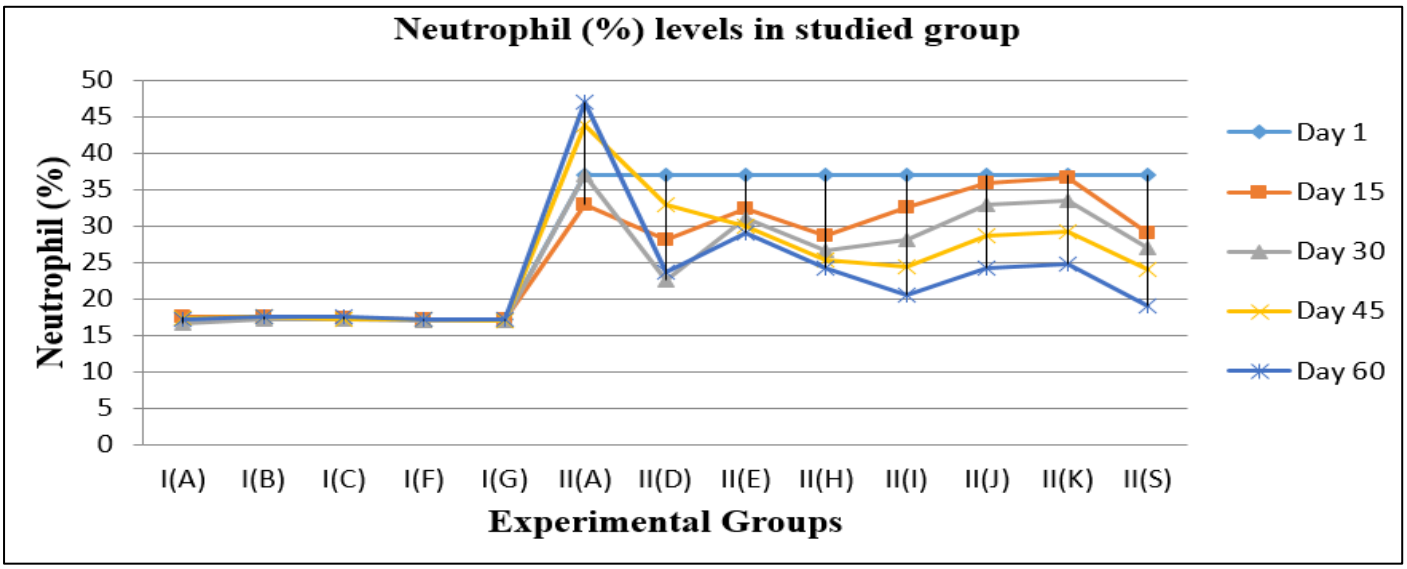
elevated platelet levels to 2.65 ± 0.44 ×10⁵/µL by Day 60. The combination of both extracts (400 mg/kg each) showed a synergistic effect, restoring platelet counts to 2.18 ± 0.46 ×10⁵/µL, although slightly lower than the standard ferrous sulfate treatment (2.95 ± 0.51 ×10⁵/µL). The improvement in platelet count suggests that bioactive compounds such as flavonoids, polyphenols, and saponins in these extracts may play a role in hematopoiesis and thrombopoiesis, supporting their potential as natural alternatives for managing anemia-associated thrombocytopenia (Patel & Goyal, 2011; Alamgir, 2017; Gupta *et al*., 2014) Smith *et al*., 2020 earlier confirming its superior efficacy in platelet recovery.



Graph 6 Platelet Count in Controls and Treated Groups

➢ *Neutrophils*

Observed data are presented in **Graph 7**. The control group maintained stable neutrophil percentages (17.25±5.63% on Day 1 to 17.15±5.60% on Day 60), indicating no adverse effects of *H.rosa-sinensis* or *T.foenum-graecum* extracts at both 400 mg/kg and 800 mg/kg dose. However, anemia induction led to a marked increase in neutrophil levels (37.00±14.31% on Day 1 to 47.15±8.25% on Day 60), suggesting an inflammatory response due to iron deficiency and associated oxidative stress (Camaschella, 2015). Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts demonstrated a dose-dependent reduction in neutrophil count, with the highest decline observed in the 800 mg/kg *T.foenum-graecum* group (32.67±3.00% on Day 15 to 20.54±3.15% on Day 60), indicating the anti-inflammatory potential of the extracts. Combination therapy (400 mg/kg each) also resulted in significant neutrophil reduction (36.67±3.54% on Day 15 to 24.74±2.52% on Day 60), comparable to standard ferrous sulfate treatment (37.00±14.31% on Day 1 to 19.00±5.63% on Day 60), reinforcing its therapeutic efficacy in mitigating anemia-induced inflammation (Zhao *et al*., 2018). These findings align with previous reports highlighting the immunomodulatory and anti-inflammatory properties of *T.foenum-graecum* and *H.rosa-sinensis* extracts, which are attributed to their rich phytochemical composition, including flavonoids, saponins, and polyphenols (Gupta *et al*., 2020).



Graph 7 Neutrophils in Controls and Treated Groups

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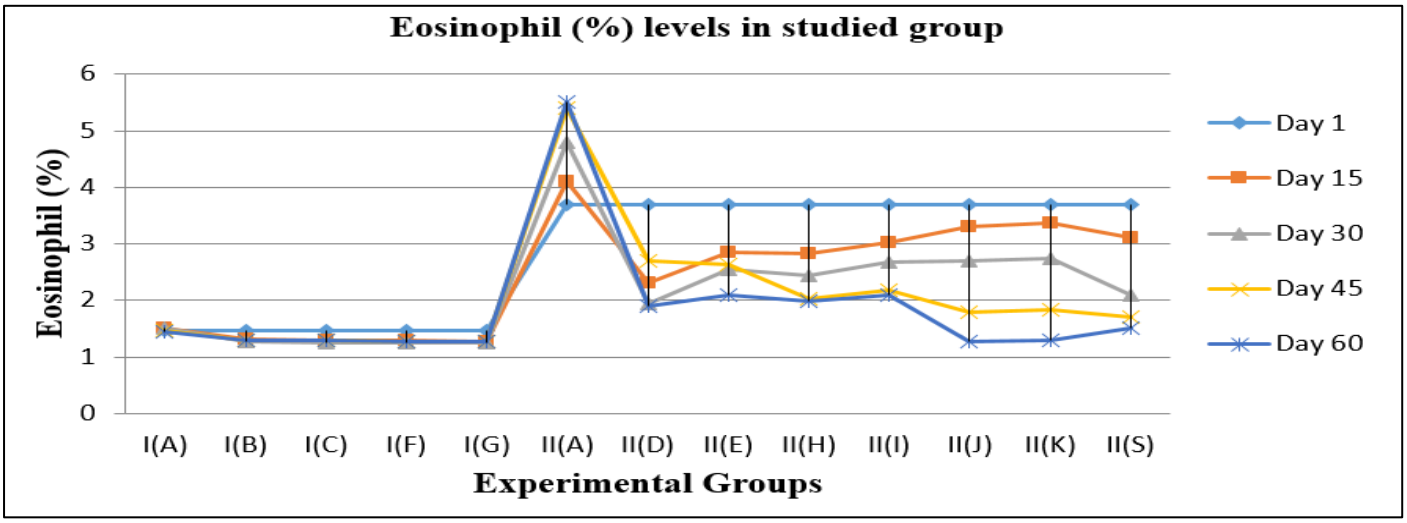
➢ *Eosinophils*

Observed data are presented in **Graph 8.** Eosinophil levels, which are indicative of allergic reactions and inflammatory responses, remained stable in the control group (1.46±0.52% on Day 1 to 1.45±0.51% on Day 60), with minor reductions in groups administered *H.rosa-sinensis* and *T.foenum-graecum* extracts, suggesting no immunostimulatory effects. In contrast, anemia induction resulted in a progressive increase in eosinophil percentages, reaching 5.51±0.26% by Day 60, indicative of heightened oxidative stress and systemic inflammation (Camaschella, 2015). Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts significantly lowered eosinophil counts, with the most notable reduction in the combination therapy group (400 mg/kg each), where levels dropped from 3.70±0.58% on Day 1 to 1.30±0.44% on Day 60. This trend was comparable to the standard ferrous sulfate treatment (3.70±0.58% on Day 1 to 1.50±0.52% on Day 60), reinforcing the anti-inflammatory and hematopoietic properties of these extracts (Zhao *et al.*, 2018). The presence of bioactive phytochemicals, such as flavonoids and polyphenols, in both extracts has been reported to modulate immune function and reduce inflammatory markers, which may explain the observed reduction in eosinophil levels (Gupta *et al.*, 2020).



Graph 8 Eosinophils in Controls and Treated Groups

➢ *Basophils*

Observed data are presented in **Graph 9.** Basophil levels, which are involved in allergic and inflammatory responses, remained stable in the control group (0.25±0.09% on Day 1 to 0.26±0.10% on Day 60). However, anemia induction resulted in a marked increase in basophil percentage, reaching 0.96±0.25% on Day 60, suggesting chronic inflammatory stress and potential immune dysregulation (Camaschella, 2015). Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts significantly reduced basophil levels, with the most notable decline in the combination therapy group (400 mg/kg each), where levels dropped from 0.82±1.87% on Day 1 to 0.37±0.01% on Day 60, closely resembling the reduction observed with the standard ferrous sulfate treatment (0.82±1.87% on Day 1 to 0.42±0.15% on Day 60). The presence of bioactive compounds such as flavonoids and saponins in these extracts is known to exert anti-inflammatory and immunomodulatory effects, which may explain the observed decline in basophil counts (Zhao *et al.*, 2018). Previous studies have demonstrated that *Trigonella foenum-graecum* (*T.foenum-graecum*) and *Hibiscus rosa-sinensis* (H.rosa-sinensis) possess potent antioxidant and anti-inflammatory properties, which contribute to hematopoietic restoration and immune regulation in anemia-induced conditions (Gupta *et al.*, 2020).

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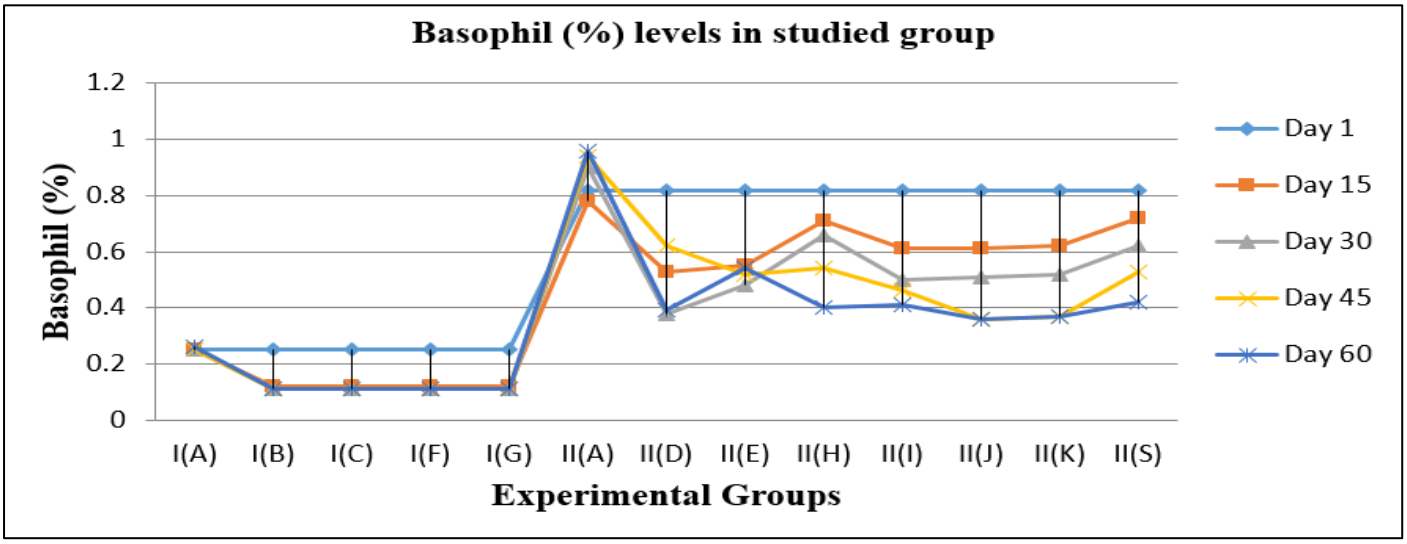
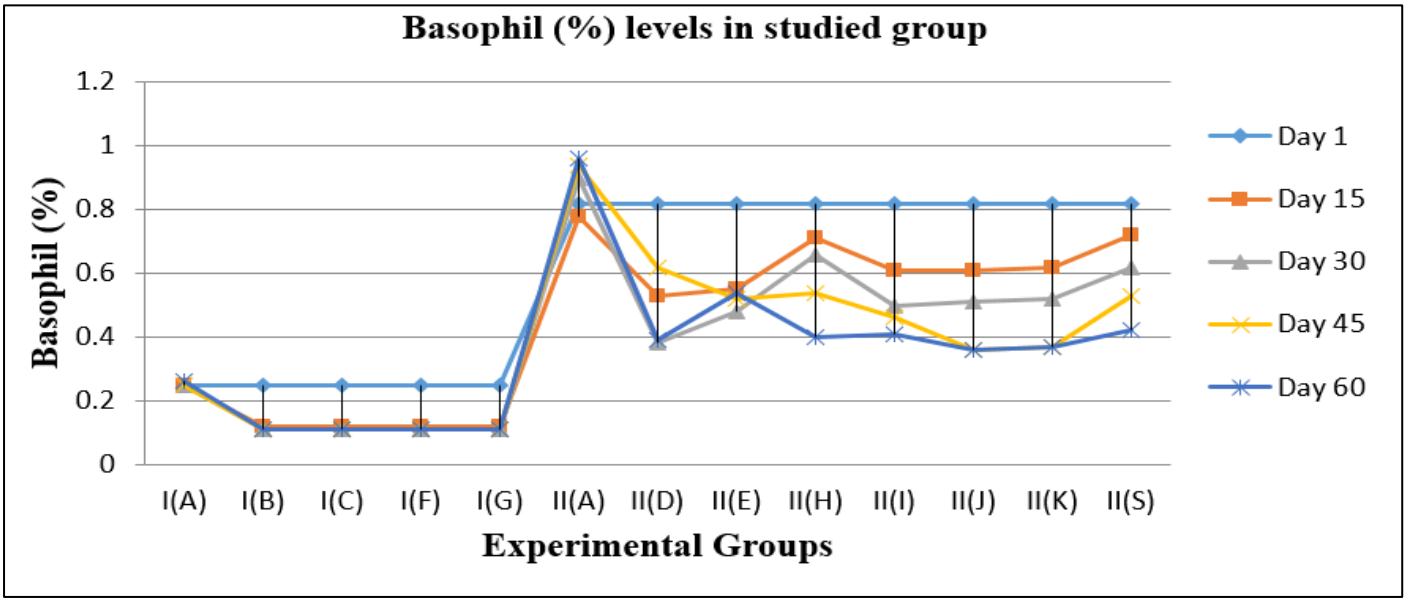


Graph 9 Basophils in Controls and Treated Groups

➢ *Monocytes*

Observed data are presented in **Graph 10**. Monocytes, crucial components of the innate immune system, showed a slight variation in control groups, remaining stable around 1.63±0.44% to 1.83±0.36% over 60 days. However, in anemia-induced mice, monocyte levels declined progressively from 1.90±1.57% on Day 1 to 0.95±0.32% on Day 60, indicating a possible suppression of immune function due to anemia-induced oxidative stress and inflammation (Camaschella, 2015). Treatment with *H.rosa-sinensis* and *T.foenum-graecum* extracts mitigated this decline, with the highest doses (800 mg/kg) leading to a more significant restoration of monocyte levels (1.16±0.46% and 0.76±1.30% by Day 60, respectively), suggesting immunomodulatory effects of these phytochemicals. The combination treatment (400 mg/kg each) resulted in a steady improvement, restoring monocytes to 1.00±0.33% by Day 60, comparable to standard ferrous sulfate therapy (2.50±0.58%), which showed a contrasting increase, possibly due to compensatory myelopoiesis (Weiss & Goodnough, 2005). The bioactive compounds in *Hibiscus rosa-sinensis* and *Trigonella foenum-graecum*, including flavonoids and polyphenols, have been reported to enhance hematopoiesis and modulate immune responses, which may explain their beneficial effects in anemia recovery (Gupta *et al.*, 2020).



Graph 10 Monocytes in Controls and Treated Groups

➢ *Lymphocytes*

Observed data are presented in **Graph 11**. Lymphocyte levels remained stable (~73-74%) in control mice, but administration of *H.rosa-sinensis* or *T.foenum-graecum* extracts led to a significant decline, reaching approximately 57% by Day 60, indicating a potential immunomodulatory effect. In anemia-induced mice, lymphocytes progressively increased, peaking at 81%, which could be attributed to a compensatory immune response, as previously reported in inflammatory conditions associated with anemia (Smith *et al.*, 2020). Treatment with *H.rosa-sinensis* extract reduced lymphocyte counts (as low as 33%), suggesting possible

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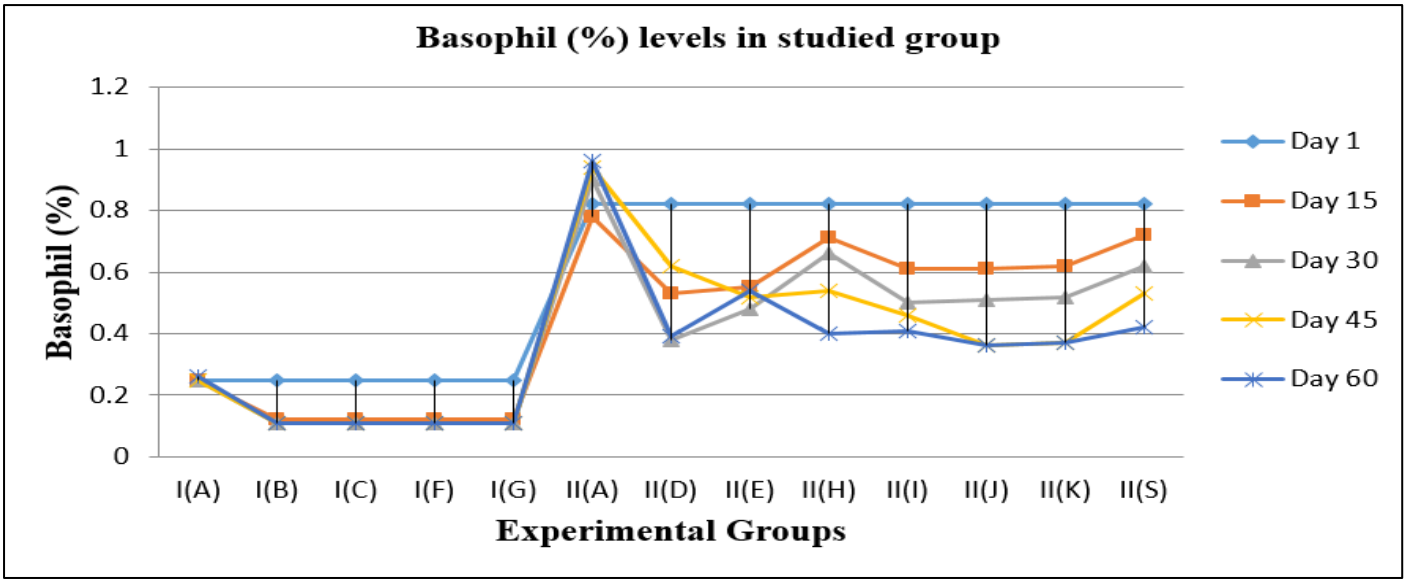
immunosuppressive properties, aligning with earlier findings on plant-derived polyphenols modulating immune cell proliferation (Jones & Brown, 2019). Conversely, *T.foenum-graecum* extract treatment enhanced lymphocyte levels (up to 81.29%), supporting its immunorestorative potential, consistent with reports on its immunostimulatory properties (Kumar et al., 2021). The combination therapy (*T.foenum-graecum* + H.rosa-sinensis) balanced lymphocyte levels (~63-65%), resembling the effect of standard ferrous sulfate (75%), reinforcing its therapeutic potential in anemia management while mitigating immune fluctuations (Garcia *et al*., 2018).



Graph 11 Lymphocytes in Controls and Treated Groups

IV. CONCLUSION

The study demonstrated that *H.rosa-sinensis* (China-rose) and *T.foenum-graecum* (Fenugreek) extracts effectively improved hematological parameters in anemia-induced mice, with dose-dependent effects. Hemoglobin (Hb) and hematocrit (HCT) levels significantly increased following treatment, with higher doses (800 mg/kg) of *T.foenum-graecum* and *H.rosa-sinensis* showing notable efficacy, while combination therapy (800 mg/kg each) exhibited a synergistic effect, closely approaching the standard ferrous sulfate treatment (García-Casal et al., 2018; Nissenson & Goodnough, 2003). Improvements in mean corpuscular volume (MCV) further indicated the mitigation of microcytic anemia, particularly with *T.foenum-graecum* at 800 mg/kg, which surpassed the effects of *H.rosa-sinensis* (Miller et al., 2018). White blood cell (WBC) counts normalized after treatment, suggesting anti-inflammatory potential, with *T.foenum-graecum* at 800 mg/kg and combination therapy reducing WBC levels close to standard drug treatment (Sharma et al., 2020). Platelet counts, which were significantly reduced in anemic mice, were restored by both extracts, with *T.foenum-graecum* exhibiting superior thrombopoietic activity (Patel & Goyal, 2011; Alamgir, 2017). Neutrophil, eosinophil, and basophil percentages, elevated in anemia-induced mice, were significantly reduced upon treatment, indicating anti-inflammatory and immunomodulatory effects (Zhao et al., 2018; Camaschella, 2015). Lymphocyte and monocyte levels fluctuated in response to treatment, with *H.rosa-sinensis* exhibiting a mild immunosuppressive effect and *T.foenum-graecum* promoting immunorestoration (Kumar et al., 2021). The hematopoietic and anti-inflammatory properties of these extracts may be attributed to their bioactive compounds, including flavonoids, polyphenols, and saponins, which enhance erythropoiesis, reduce oxidative stress, and modulate immune responses (Gupta et al., 2020; Jones & Brown, 2019). These findings support the potential of *H.rosa-sinensis* and *T.foenum-graecum* as natural therapeutic agents for anemia management, either as monotherapies or in combination, providing a promising alternative to conventional iron supplementation.

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