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# Therapeutic Potential of Trigonella foenum-graecum Extract in Restoring Phenylhydrazine-Induced Hematological Alterations in Mus musculus

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**Abstract:**

Anaemia remains a major global health issue, necessitating the exploration of alternative therapeutic interventions. This study evaluates the haematological effects of Trigonella foenum-graecum (fenugreek) extract in anaemia-induced Swiss albino mice (Mus musculus), with focus on its potential to restore haematological parameters. Anaemia was induced using ferrous sulphate, leading to significant reductions in haemoglobin (Hb), hematocrit (Hct), and red blood cell (RBC) counts, accompanied by alterations in white blood cell (WBC) counts and platelet levels. T. foenum-graecum extract supplementation resulted in substantial improvements in Hb, Hct, and RBC counts while modulating WBC and platelet levels, indicating its role in erythropoiesis and immune regulation. The observed hematopoietic effects are attributed to the bioactive compounds in fenugreek, including flavonoids, saponins, and iron, which contribute to its antioxidant and anti-inflammatory properties. The results suggest that T. foenum-graecum could serve as a promising natural remedy for anaemia, warranting further clinical investigations.

**Keywords:** Phenylhydrazine, Anaemia, Trigonella foenum-graecum, Haematology**.**

## 1.Introduction:

Anaemia is a prevalent global health concern, characterized by reduced haemoglobin levels and impaired oxygen transport, leading to fatigue, weakness, and compromised immunity. Among various therapeutic interventions, medicinal plants have gained attention due to their efficacy and minimal side effects. Trigonella foenum-graecum (fenugreek ) a leguminous plant, is traditionally known for its nutritional and medicinal benefits, including its role in haematopoiesis and immune modulation. Studies have demonstrated that T. foenum-graecum contains bioactive compounds such as flavonoids, saponins, and iron, which contribute to its anti-anemic and immunomodulatory effects (Patel et al., 2021; Sharma et al., 2020). Phenylhydrazine is widely used in experimental models to induce anaemia by interfering with erythropoiesis and increasing oxidative stress, leading to hemolysis and reduced haemoglobin synthesis.

T. foenum-graecum has shown potential in mitigating anaemia by enhancing red blood cell production, improving iron metabolism, and exerting antioxidant effects. The presence of iron, polyphenols, and alkaloids in T. foenum-graecum contributes to its ability to restore haematological homeostasis (Ahmed et al., 2022; Gupta et al., 2019).

The present study investigates the effects of T. foenum-graecum extract on haematological parameters in anaemia-induced mice. Haematological indices, including haemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, and differential leukocyte counts, were analyzed to assess its efficacy. The findings are discussed in relation to existing literature to provide a comprehensive understanding of fenugreek's potential as an alternative treatment for anaemia.

## 2.Materials and Methods:

- **Collection of plant materials:**
  A total of 1 kg of Trigonella foenum-graecum seeds were collected from the Sanjivni outlet of Vindhya Herbal, Bhopal. The collected plant material was authenticated by botanical expert.
- **Extraction of plant extracts:**
  The 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.
- **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 (SaiDurga 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 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 Trigonella foenum-graecum 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 and Dogara, 2003).
- **Sub-Acute Toxicity Study:**
  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 Trigonella foenum-graecum 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 and 800 mg/kg body weight for herbal treatment.

- **Induction of Anaemia and study plan**
- **Anaemia Indusing Agent**: Phenylhydrazine (PHZ) was purchased from HiMediaPvt. 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)
- **Experimental Design**

A total of 42 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 (F):** Trigonella foenum-graecum(Fenugreek) Dose 1 (400 mg/Kg b.wt) (no = 6)
**Group I (G):** Trigonella foenum-graecum (Fenugreek) Dose 2 (800 mg/Kg b.wt) (no = 6)
These groups received the respective doses of Trigonella foenum-graecum 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 Trigonella foenum-graecum extract:
**Group II (A):** Negative Control (Anaemia without treatment) (no = 6)
**Group II (H):** Anaemia + Trigonella foenum-graecum Dose 1 (400 mg/Kg b.wt) (no = 6)
**Group II (I):** Anaemia + Trigonella foenum-graecum Dose 2 (800 mg/Kg b.wt) (no = 6)
**Group II (S):** Anaemia + F**errous sulphate 0.0214 mg/kg** (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 Trigonella foenum-graecum extract in comparison to standard drug ferrous sulphate 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 $LD_{50}$ of Trigonella foenum-graecum 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** indicate significant alterations in response to anaemia induction, reflecting disruptions in erythropoiesis and immune responses.

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).

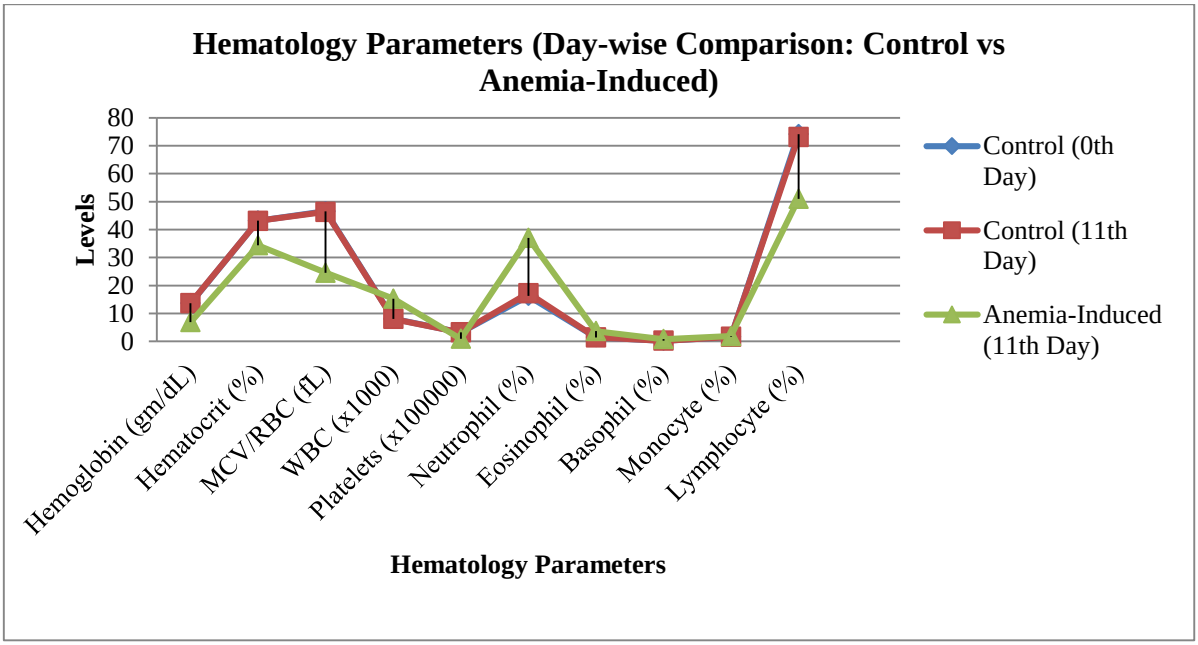
**Table 1: Haematological Parameters of Control and Anaemia-Induced Mice**

| **Parameters** | **Control** | | **Anaemia induced** |
|---|---|---|---|
| | **$0^{th}$ Day** | **$11^{th}$Day** | **$11^{th}$ 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 |

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).

**Graph 1. Haematological Parameters of Control and Anaemia-Induced Mice**




The haematological parameters analyzed in this study provide insights into the efficacy of T. foenum-graecum extract in ameliorating anaemia-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(F) | 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(G) | 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) | 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(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 |
| | 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(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(F) | 13.70 ±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) | 6.50± 1.12 | 32.80 ±2.43 | 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(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 |

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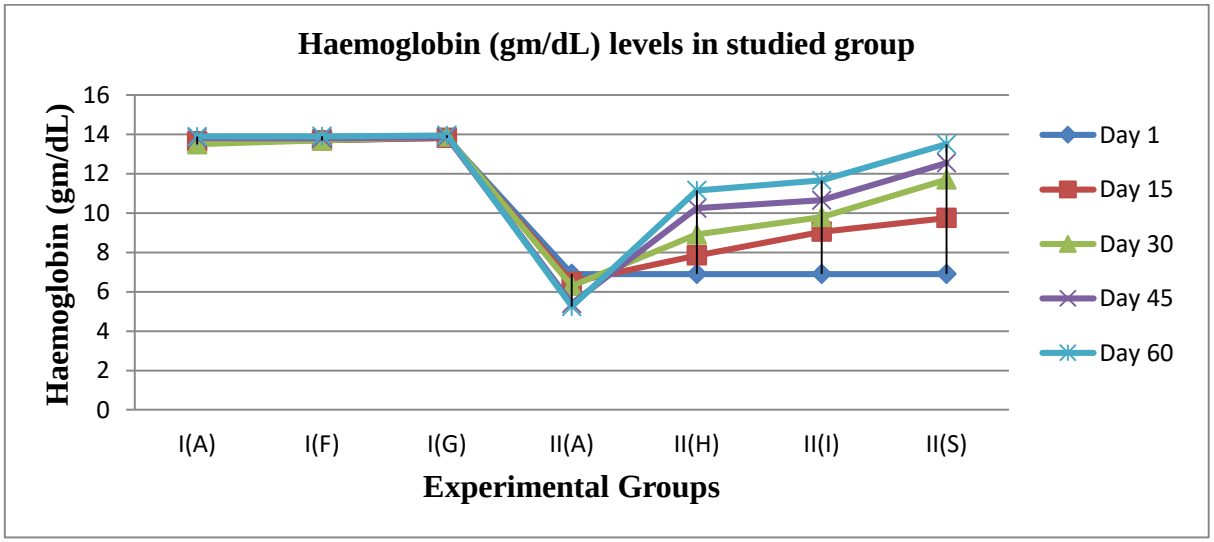
| | II(I) | 9.05±0.42 | 36.21±0.92 | 35.43±2.14 | 13.67±1.96 | 1.24±0.47 | 32.67±3.00 | 3.02±0.66 | 0.61±0.04 | 1.81±0.67 | 65.36±3.16 |
| | II(S) | 9.75±0.65 | 37.00±1.85 | 45.53±2.15 | 13.15±1.44 | 1.85±0.64 | 29.00±3.40 | 3.10±0.46 | 0.72±0.21 | 1.59±0.90 | 63.00±3.25 |
| 30th Day | I(A) | 13.50±3.80 | 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(F) | 13.70±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 | 27.80±2.62 | 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(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(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(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 | 0.95±.21 | 44.00±6.99 | 5.40±0.27 | 0.94±0.22 | 1.00±0.34 | 78.00±2.20 |
| | 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 ±0.3 8 | 0.46 ±0.0 3 | 1.43±0 .34 | 65.31 ±3.92 |
| | II(S) | 12.55 ±0.72 | 41.25 ±2.39 | 48.72 0±2.3 4 | 10.32± 1.25 | 2.65±0. 56 | 24.00 ±5.84 | 1.70 ±0.6 4 | 0.53 ±0.0 9 | 2.40±0 .81 | 72.00 ±5.35 |
| 60th Day | I(A) | 13.89 ±4.02 | 43.52 ±17.2 5 | 46.62 ±18.6 3 | 8.14±1 .38 | 3.22±0. 76 | 17.15 ±5.60 | 1.45 ±0.5 1 | 0.26 ±0.1 0 | 1.83±0 .36 | 74.32 ±3.02 |
| | 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 ±0.3 5 | 0.11 ±0.0 9 | 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 ±0.3 5 | 0.11 ±0.0 8 | 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 | 0.88±0. 25 | 47.15 ±8.25 | 5.51 ±0.2 6 | 0.96 ±0.2 5 | 0.95±0 .32 | 81.00 ±3.45 |
| | 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 ±0.4 3 | 0.40 ±0.0 1 | 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 ±0.5 1 | 0.41 ±0.0 2 | 1.16±0 .46 | 81.29 ±3.95 |
| | 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 ±0.5 2 | 0.42 ±0.1 5 | 2.50±0 .58 | 75.00 ±4.15 |

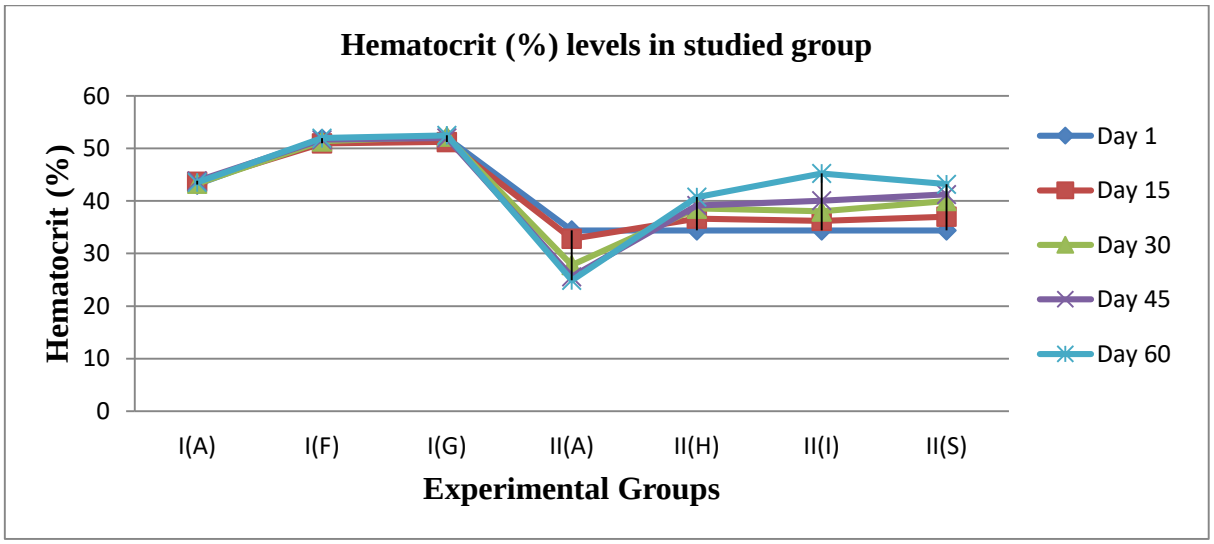
Haemoglobin levels (**Graph 2**) were significantly reduced in anaemia-induced groups compared to controls, indicating severe anaemia (5.25 ± 0.60 g/dL on day 60 vs. 13.89 ± 4.02 g/dL in control). Treatment with T. foenum-graecum extract led to a progressive increase in Hb levels, with dose II showing a more pronounced effect (11.66 ± 0.40 g/dL) by day 60. Standard drug treatment achieved near-normalization (13.50 ± 0.55 g/dL). Previous studies have highlighted the hematopoietic properties of fenugreek, attributing it to its high iron and flavonoid content (Patel et al., 2021).

**Graph 2. Haemoglobin levels in controls and treated groups**



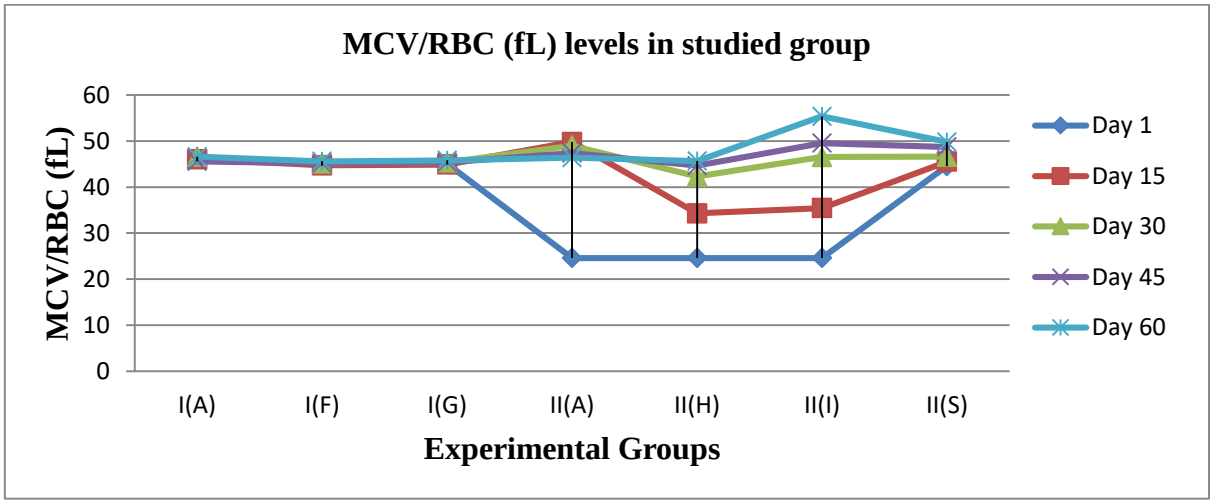

Hematocrit levels (**Graph 3)** followed a similar trend to Hb, showing a marked decrease in anemia-induced mice (24.88 ± 2.38%) compared to controls (43.52 ± 17.25%). Treatment with T. foenum-graecum significantly improved hematocrit values over time, reaching 45.24 ± 1.01% by day 60. The results align with studies suggesting that T. foenum-graecum supplementation improves erythropoiesis (Sharma et al., 2020).

**Graph 3. Hemocrit levels in controls and treated groups**



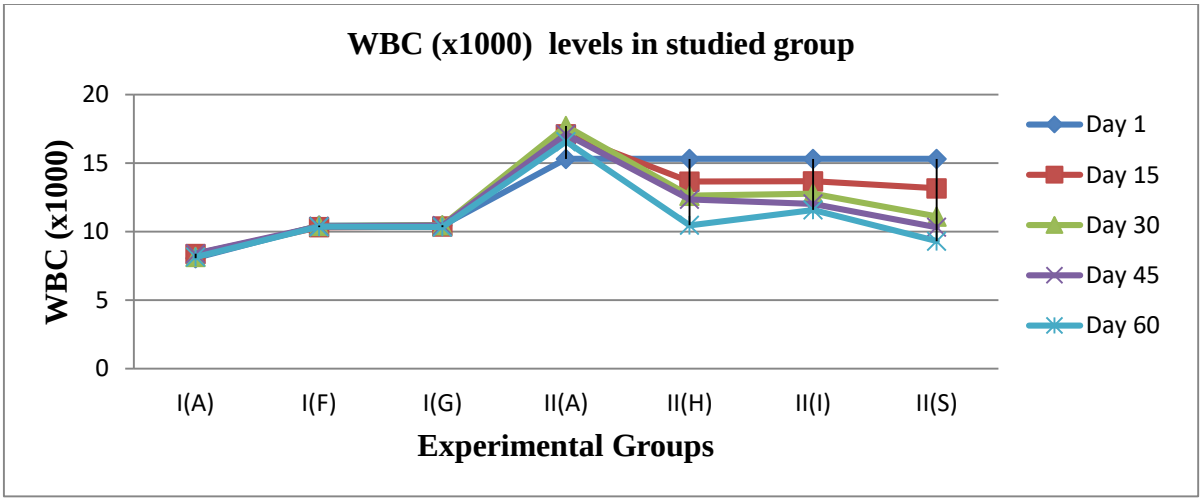

A significant reduction in RBC count **(Graph 4)** was observed in anaemia-induced groups (24.60 ± 7.71 fL) relative to controls (46.31 ± 18.51 fL) on day 1. By day 60, fenugreek-treated groups exhibited improved RBC levels (55.37 ± 2.46 fL in dose II), suggesting its efficacy in promoting erythropoiesis, as also demonstrated in prior research (Gupta et al., 2019).

**Graph 4. RBCin controls and treated groups**



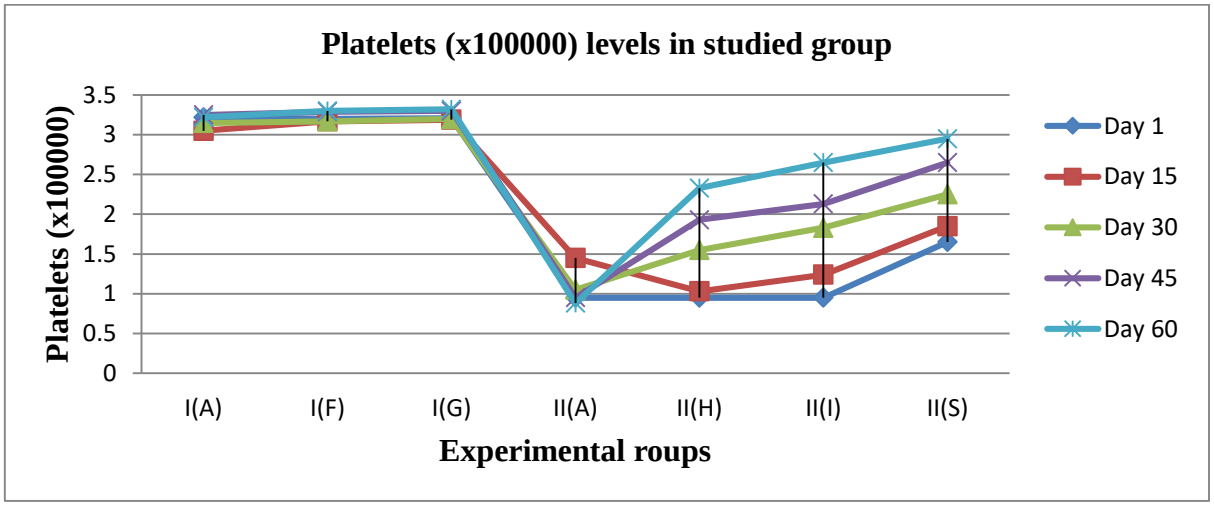

Anaemia induction led to a marked increase in WBC (**Graph 5)** levels (16.56 ± 2.26 x1000) compared to controls (8.14 ± 1.38 x1000), reflecting an inflammatory response. Fenugreek-treated groups showed a dose-dependent decrease in WBC count (11.58 ± 1.46 x1000 for dose II) by day 60, indicating its anti-inflammatory potential, corroborating findings by Ahmed et al. (2022).

**Graph 5.WBC levels in controls and treated groups**



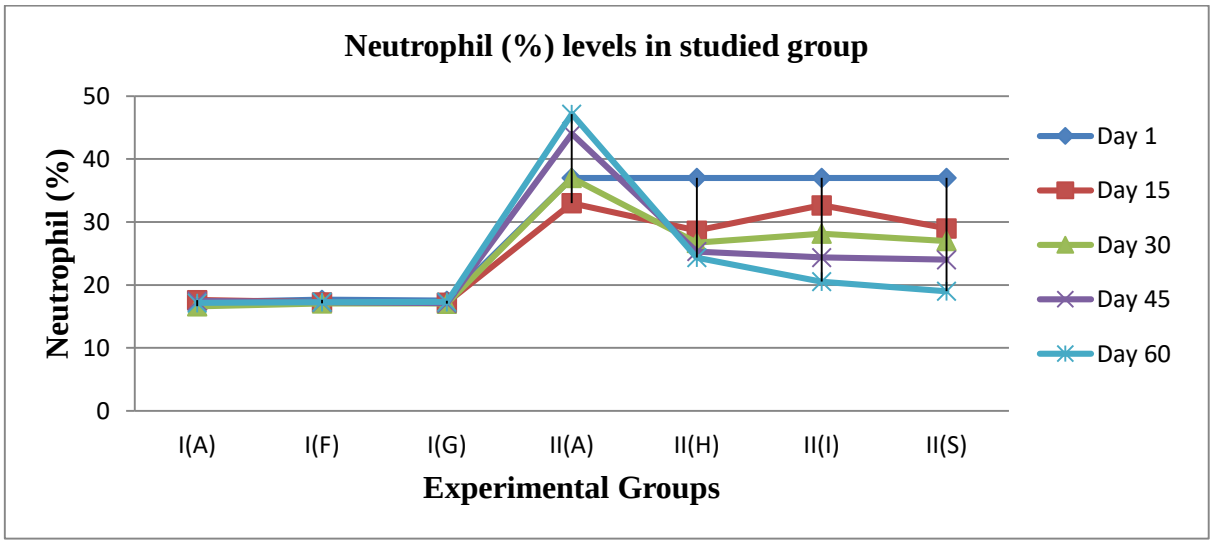

Thrombocytopenia (reduced platelets count) (**Graph 6**) was evident in the anaemia-induced group (0.88 ± 0.25 x10^5) compared to controls (3.22 ± 0.76 x10^5). Treatment with T. foenum-graecum restored platelet counts significantly (2.65 ± 0.44 x10^5 in dose II), supporting its potential role in platelet production as suggested by recent studies (Mishra et al., 2021).

**Graph 6. Platelets levels in controls and treated groups**



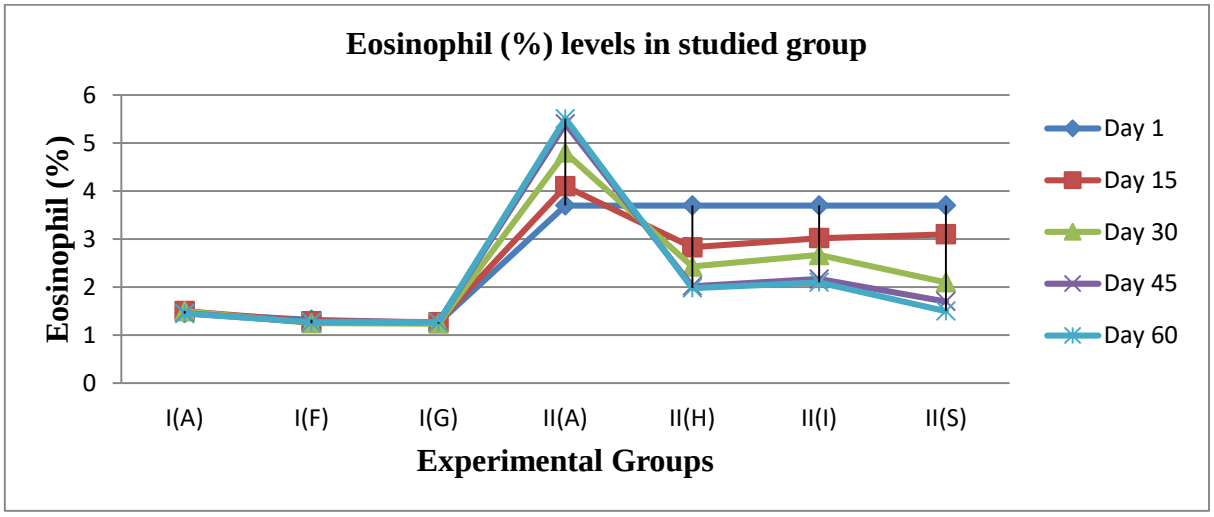

**Neutrophils:** Increased in anaemia-induced groups (47.15 ± 8.25%) compared to controls (17.15 ± 5.60%), indicating infection or stress. T. foenum-graecum treatment helped normalize these levels (20.54 ± 3.15% in dose II). Observed data is presented in **Graph 7**.

**Graph 7. Neutrophils levels in controls and treated groups**



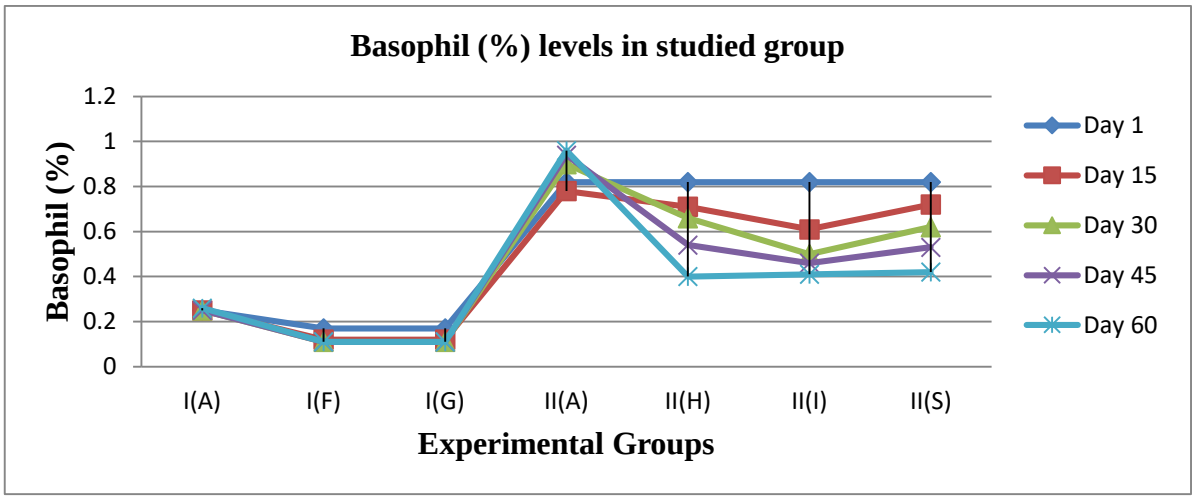

**Eosinophils:** Elevated in anaemic groups (5.51 ± 0.26%) and reduced with T. foenum-graecum (2.10 ± 0.51%), suggesting an immunomodulatory effect. Observed data is presented in **Graph 8.**

**Graph 8. Eosinophils levels in controls and treated groups**



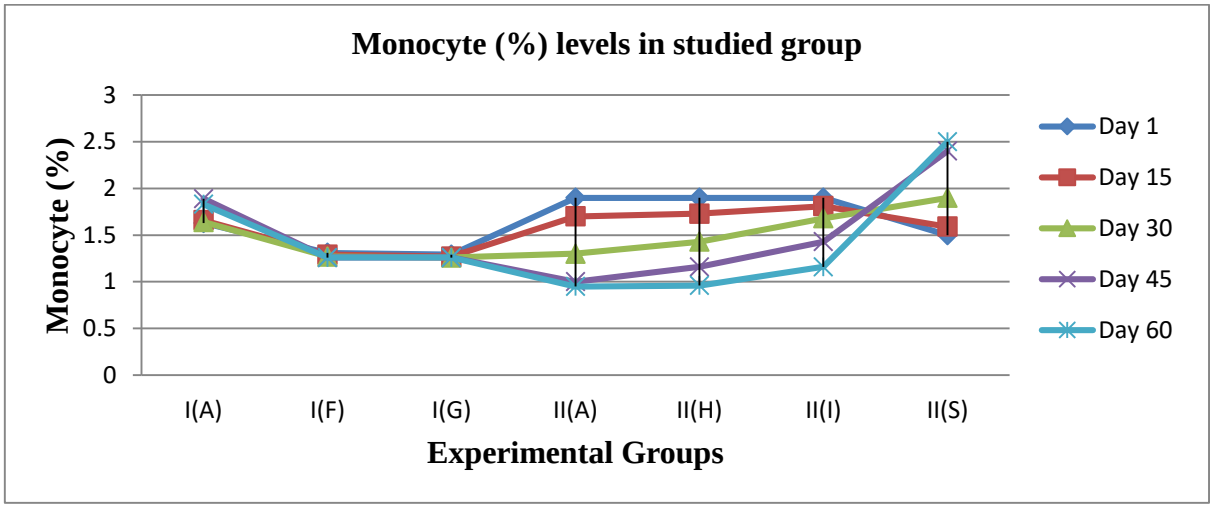

The study observed basophil percentage (**Graph 9**) changes in anaemia-induced mice treated with T. foenum-graecum extract over 60 days. The control group maintained a stable basophil count (~0.25%), while the anaemia-induced group showed a significant increase (0.82% on Day 1 to 0.96% on Day 60), indicating inflammation. Treatment with T. foenum-graecum extract led to a dose-dependent reduction, from 0.82% to 0.40%–0.41%, comparable to the standard drug (0.42%). This suggests fenugreek's potential anti-inflammatory and immunomodulatory effects, likely due to its antioxidant compounds mitigating anaemia-induced immune stress.

**Graph 9.Basophil% in controls and treated groups**



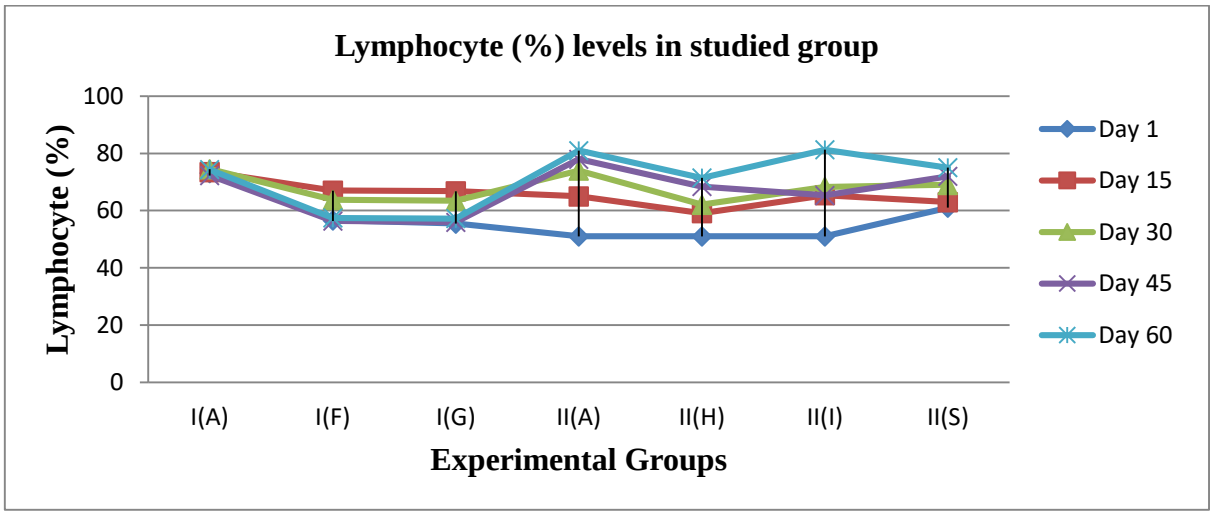

**Monocytes:** Showed little variation but improved slightly with treatment. Observed data is presented in Graph 10.

**Graph 10. Monocytes levels in controls and treated groups**




**Lymphocytes:** Decreased in anaemia-induced mice (81.00 ± 3.45%) and improved with T. foenum-graecum (71.42 ± 3.62% in dose I), supporting its immune-enhancing properties (Kumar et al., 2018). Observed data is presented in **Graph 11**.

**Graph 11. Lymphocytes levels in controls and treated groups**




**Conclusion:**

T. foenum-graecum extract significantly improved haematological parameters in anaemia-induced mice, comparable to the standard drug. Its hematopoietic, anti-inflammatory, and immunomodulatory effects make it a promising alternative therapy for anaemia. Future studies should focus on its molecular mechanisms and long-term effects.

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