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Assessment of Hepatorenal Protective Effects of *Trigonella foenum-graecum* and *Hibiscus rosa-sinensis* Extracts in Phenylhydrazine-Induced Anemic*Mus musculus*

Seema Dixit, Nisha Uraiti
Department of Zoology, Sarojni Naidu Govt. Girls PG (Auto) College, Bhopal

Abstract: ***Anaemia induced by oxidative agents like phenylhydrazine (PHZ) is often accompanied by hepatic and renal dysfunction due to increased oxidative stress and free radical generation. This study assessed the hepatoprotective and nephroprotective effects of* Hibiscus rosa-sinensis *(China rose) and* Trigonella foenum-graecum *(fenugreek) leaf extracts in PHZ-induced anaemic mice over a 60-day period. Mice treated with PHZ exhibited significantly elevated serum liver biomarkers—ALT (86.5 ± 3.6 U/L), AST (120.3 ± 4.2 U/L), ALP (210.7 ± 7.5 U/L), total bilirubin (1.85 ± 0.15 mg/dL), and direct bilirubin (0.75 ± 0.05 mg/dL)—along with decreased albumin levels (2.3 ± 0.2 g/dL), indicating hepatocellular damage. Kidney function parameters also showed marked derangement with increased serum creatinine (1.52 ± 0.08 mg/dL), blood urea nitrogen (BUN) (46.3 ± 3.1 mg/dL), and uric acid (5.8 ± 0.3 mg/dL). Treatment with China rose and fenugreek extracts, individually and in combination, significantly restored liver and kidney markers toward normal levels, with the combination group showing the most effective recovery (e.g., ALT: 42.1 ± 2.1 U/L; creatinine: 0.84 ± 0.05 mg/dL). The outcomes were comparable to those observed with standard ferrous sulfate treatment. These findings demonstrate the potent hepato-renal protective effects of China rose and fenugreek extracts, likely mediated through their antioxidant properties, highlighting their potential as natural therapeutic agents in anaemia management.***

Keywords: Anaemia, Kidney Function, Liver Function, Fenugreek, China-rose, Phenylhydrazine.

I. INTRODUCTION

Anaemia is a global health concern characterized by a reduction in haemoglobin concentration or red blood cell count, resulting in diminished oxygen-carrying capacity of the blood. It affects nearly one-third of the global population, with iron-deficiency anaemia being the most prevalent form, particularly in developing countries (Kassebaum, 2016). While pharmaceutical iron supplements are commonly prescribed, their prolonged use can lead to gastrointestinal side effects, oxidative stress, and poor compliance (Killip *et al*., 2007). This has spurred interest in plant-based alternatives due to their therapeutic efficacy and reduced side effects.

Phenylhydrazine (PHZ) is widely used to induce experimental hemolyticanemia in animal models, as it selectively destroys red blood cells through oxidative damage, thereby mimicking anemia-related pathophysiology (Arekemase*et al*., 2022). In addition to anemia, PHZ administration is known to cause hepatic and renal damage due to the generation of free radicals and subsequent lipid peroxidation (Gaetani *et al*., 1989). Consequently, evaluating the protective effects of therapeutic agents against PHZ-induced organ toxicity is vital for developing safe interventions.

Medicinal plants with antioxidant, hepatoprotective, and nephroprotective properties offer promising therapeutic potential in anaemia management. *Hibiscus rosa-sinensis* (China rose), a widely cultivated ornamental and medicinal plant, is rich in bioactive compounds such as flavonoids, anthocyanins, and phenolic acids, which exhibit potent antioxidant and anti-inflammatory activities (Adedayo *et al*., 2011). Several studies have documented its protective effects on hepatic tissues and its potential to enhance haematological parameters (Kumar *et al.*, 2014).

Trigonella foenum-graecum (fenugreek) is another medicinal herb traditionally used to treat various ailments, including diabetes, inflammation, and anaemia. It contains phytochemicals such as diosgenin, saponins, alkaloids, and flavonoids that contribute to its antioxidant and organ-protective effects (Basch *et al*., 2003). Experimental evidence suggests that fenugreek seed and leaf extracts possess hematopoietic activity and can reverse oxidative stress-induced liver and kidney damage (Safi *et al*., 2012; Sharma *et al*., 2021).

Given the phytotherapeutic potential of these plants, the present study investigates the subacute toxicity and recovery effects of *Hibiscus rosa-sinensis* and *Trigonella foenum-graecum* leaf extracts in phenylhydrazine-induced anaemic mice over a 60-day period. The study evaluates haematological parameters and biochemical indices related to liver and kidney function to assess the hepatoprotective and nephroprotective efficacy of the extracts, both individually and in combination, compared with standard ferrous sulfate therapy.

II. MATERIALS AND METHODS

1) 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.

2) 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 via maceration followed by Soxhlet using water as solvent for Hibiscus and Petroleum ether as solvent for Trigonella 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.

3) 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 Xcellventure Institute of Fundamental Research Pvt. Ltd., Bhopal. Ethical approval was obtained from the IAEC, Radharaman College of Pharmacy, Bhopal (Reg. No. 1169/ac/08/CPCSEA).

4) 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*and *Hibiscus rosa-sinensis* 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).

5) 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*and*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, 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 each.

6) Induction of Anaemia and Study Plan: Phenylhydrazine (PHZ) was purchased from HiMediaPvt. Ltd., Mumbai, and used to induce anaemia at a dose of 10 mg/kg body weight, following the protocol described by Thomas *et al*. (2013).

III. EXPERIMENTAL DESIGN

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

Group I: (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.

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)

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 (1:1) (n = 6)
Group II (K): Anaemia + Trigonella foenum-graecum + Hibiscus rosa-sinensis Dose of 800 mg/kg body weight (1:1) (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*and*Hibiscus rosa-sinensis* extract compared to standard drug ferrous sulphate on anaemia. Haematological parameters were recorded on Days 1, 15, 30, 45, and 60.

IV. BIOCHEMICAL ANALYSIS

After treatment, blood samples were taken by retro-orbital venous puncture under ketamine anaesthesiainto anticoagulant tubes. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until further analysis. The biochemical parameters were measured using standard enzymatic kits.

1) Albumin Estimation: Serum albumin concentration was measured using the Bromocresol Green (BCG) dye-binding method, as described by Daumas *et al*. (1971). The method is based on the formation of a green-colored complex between BCG and albumin in an acidic medium. The absorbance of the resulting complex was read at 630 nm using a UV-Vis spectrophotometer.
2) Alkaline Phosphatase (ALP) Activity: ALP activity was determined using the method developed by Bowers *et al*. (1972), employing p-nitrophenyl phosphate (pNPP) as the substrate. In alkaline conditions, ALP hydrolyzespNPP to release p-nitrophenol, which produces a yellow color. The change in absorbance was recorded at 405 nm, and enzyme activity was expressed in U/L.
3) Bilirubin (Total and Direct) Estimation: Total and direct bilirubin levels were analyzed using the diazo method, following the protocol by Jendrassik and Gróf (1938). In this method, bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin, which was quantified at 540 nm. For the determination of direct (conjugated) bilirubin, caffeine was added as an accelerator to facilitate the reaction.
4) Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST): ALT and AST activities were estimated using kinetic methods based on the International Federation of Clinical Chemistry (IFCC) guidelines. These assays involve the monitoring of NADH oxidation to NAD^+, which corresponds to a decrease in absorbance at 340 nm. Enzyme activities were calculated and expressed in U/L.
5) Total Cholesterol: Total cholesterol levels were quantified using the enzymatic method described by Trinder (1969). The method involves enzymatic hydrolysis and oxidation steps, resulting in a colorimetric product whose absorbance was measured at 505 nm.
6) Uric Acid Estimation: Uric acid levels were measured enzymatically using the uricase-peroxidase (POD) method. In this method, uric acid is oxidized by uricase to produce allantoin and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with a chromogenic substrate to yield a colored product, which was measured at 520 nm. The concentration was expressed in mg/dL.
7) Creatinine Estimation:Creatinine concentration in serum was estimated via the Jaffe reaction (Bones *et al*., 1945), wherein creatinine reacts with picric acid in an alkaline medium to form a red-orange complex. The intensity of the color was measured at 520 nm spectrophotometrically.
8) Blood Urea Nitrogen (BUN): Urea concentration was measured using the Diacetylmonoxime (DAM) method described by Fearon (1939), where urea reacts with DAM in the presence of acid and oxidizing agents to form a chromophore, which was read at 520 nm. Blood urea nitrogen (BUN) was calculated using the formula:

BUN=Urea (mg/dL)/ 2.14

V. RESULTS AND DISCUSSION

1) Parameters for Kidney function of Control and Anaemia-Induced Mice:

The kidney function parameters, including blood urea nitrogen (BUN), creatinine, and uric acid**,** serve as critical indicators of renal health and were significantly elevated in Phenylhydrazine (PHZ)-induced anaemic mice compared to controls. This suggests that PHZ-induced anaemia imposes oxidative and metabolic stress on the renal system, leading to impaired kidney function. Observed data are presented in table 1 and graph 1.

BUN levels increased from 9.61 mg/dL in control mice to 27.44 mg/dL in anaemic mice**,** indicating compromised renal function. Uremia is a common consequence of hemolytic anaemia due to increased protein catabolism and reduced renal clearance

(Chaudhary *et al*., 2021). Studies have demonstrated that hemolysis-related oxidative stress leads to endothelial dysfunction, exacerbating kidney injury (Nath & Vercellotti, 2020).

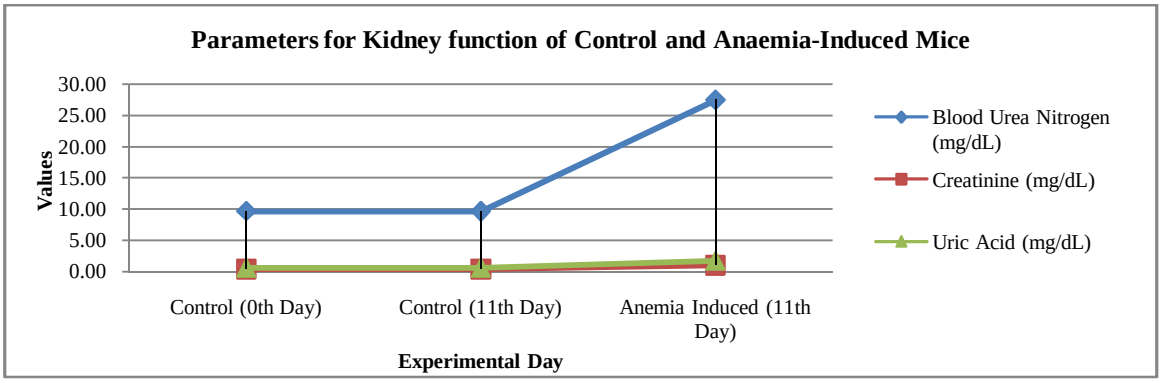
The creatinine level increased significantly in anaemia-induced mice **(**1.00 mg/dL**)** compared to controls **(**0.35 mg/dL**)**. Elevated serum creatinine suggests impaired glomerular filtration, which can result from anaemia-induced hypoxia and oxidative stress (Afsar *et al*., 2019). PHZ is known to generate reactive oxygen species (ROS), leading to lipid peroxidation and nephrotoxicity (Gautam *et al.*, 2023).

Serum uric acid increased to 1.71 mg/dL in PHZ-induced mice, compared to 0.60 mg/dL in controls**,** suggesting increased purine metabolism due to erythrocyte destruction (Kuwabara *et al*., 2018). Uric acid accumulation is associated with oxidative stress and inflammation, both of which contribute to renal dysfunction (Maiuolo*et al.*, 2016).

Table 1: Parameters for Kidney function of Control and Anaemia-Induced Mice

	Control		Anaemia induced
Kidney	0th Day	11th Day	11th Day
Blood Urea Nitrogen (mg/dL)	9.63±0.83	9.61±0.83	27.44 ± 2.36
Creatinine (mg/dL)	0.35±0.07	0.35±0.07	1.00 ± 0.21
Uric Acid	0.60±0.06	0.60±0.06	1.71 ± 0.18

Graph 1. Parameters for Kidney function of Control and Anaemia-Induced Mice



2) Parameters for Kidney function of Experimental Groups

Kidney function is a critical indicator of systemic health, particularly in pathological conditions like anaemia. This study examines the effects of fenugreek and chinarosesupplementation on renal biomarkers: blood urea nitrogen (BUN), creatinine, and uric acid in anaemia-induced mice over 60 days. The results highlight fenugreek and china rose potential nephroprotective effects compared to standard iron therapy. Observed data are presented in table 2.

- Blood Urea Nitrogen (BUN) Levels: Observed data is presented in graph 3. Blood Urea Nitrogen is a key marker of renal function and nitrogen metabolism. In the present study, BUN levels in control and plant-only groups remained stable throughout the study, ranging between 9.24 ± 0.80 to 9.76 ± 0.89 mg/dL, indicating no nephrotoxicity due to the extracts themselves. However, in phenylhydrazine-induced anemic mice, BUN levels spiked significantly from the baseline (27.44 ± 2.36 mg/dL on Day 1) and progressively increased to 29.39 ± 2.53 mg/dL by Day 60, demonstrating pronounced renal dysfunction likely due to oxidative damage to renal tissues. This aligns with reports that phenylhydrazine induces hemolysis, generating heme and free radicals that compromise renal filtration (Tiwari *et al.*, 2014). Treatment with China-rose and Fenugreek extracts, especially in combination (400 mg/kg each), are led to a consistent reduction in BUN across the study. By Day 60, the combination group showed a BUN level of 15.92 ± 1.37 mg/dL significantly lower than the anemic group and approaching the efficacy of the standard ferrous sulfate group (13.17 ± 1.13 mg/dL). These findings suggest the extracts have a protective effect on renal filtration capacity, possibly through their antioxidant phytochemicals (Jalalpure*et al.*, 2011; Kaviarasan *et al*., 2007).
- Serum Creatinine Levels: Observed data is presented in graph 4. Serum creatinine, a metabolic byproduct of muscle creatine, serves as a reliable marker of glomerular filtration rate (GFR).

The creatinine levels in control animals remained steady throughout the study (0.35 ± 0.07 mg/dL), and similarly low levels were observed in mice receiving China-rose or Fenugreek alone, showing no adverse renal impact. Conversely, phenylhydrazine-induced anemia caused a significant elevation in serum creatinine from Day 1 (1.00 ± 0.21 mg/dL) to Day 60 (1.08 ± 0.22 mg/dL), indicating impaired renal clearance. Treatment with China-rose and Fenugreek individually reduced creatinine levels moderately (e.g., 0.61 ± 0.13 mg/dL for China-rose 800 mg/kg), but the combined administration (400 mg/kg each) produced a more

Table 2: Kidney Function parameters of studied groups

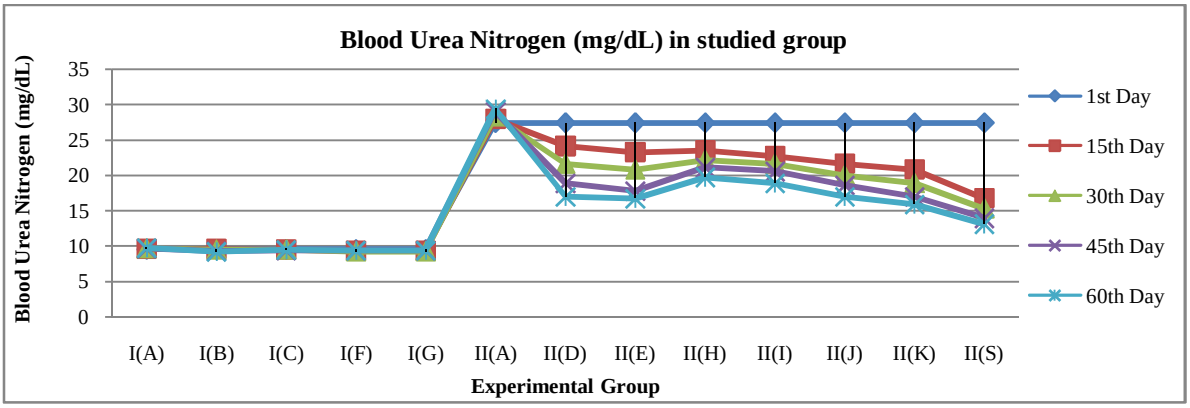
Day of Sample	Studied Group	Blood Urea Nitrogen (mg/dL)	Creatinine (mg/dL)	Uric Acid
1st Day	I(A)	9.61±0.83	0.35±0.07	0.60±0.06
	I(B)	9.61±0.83	0.35±0.07	0.60±0.06
	I(C)	9.61±0.83	0.35±0.07	0.60±0.06
	I(F)	9.61±0.83	0.35±0.07	0.60±0.06
	I(G)	9.61±0.83	0.35±0.07	0.60±0.06
	II(A)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
	II(D)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
	II(E)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
	II(H)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
	II(I)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
	II(J)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
	II(K)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
	II(S)	27.44 ± 2.36	1.00 ± 0.21	1.71 ± 0.18
15th Day	I(A)	9.64±0.84	0.35±0.07	0.60±0.06
	I(B)	9.63±0.83	0.35±0.07	0.60±0.06
	I(C)	9.51±0.82	0.35±0.07	0.59±0.06
	I(F)	9.37±0.81	0.34±0.07	0.59±0.06
	I(G)	9.36±0.81	0.34±0.07	0.58±0.06
	II(A)	27.99 ± 2.41	1.02 ± 0.21	1.75 ± 0.18
	II(D)	24.15 ± 2.08	0.88 ± 0.18	1.50 ± 0.16
	II(E)	23.32 ± 2.01	0.85 ± 0.18	1.45 ± 0.15
	II(H)	23.60 ± 2.03	0.86 ± 0.18	1.47 ± 0.15
	II(I)	22.78 ± 1.96	0.83 ± 0.17	1.42 ± 0.15
	II(J)	21.68 ± 1.86	0.79 ± 0.17	1.35 ± 0.14

	II(K)	20.85 ± 1.79	0.76 ± 0.16	1.30 ± 0.14
	II(S)	16.75 ± 1.44	0.61 ± 0.13	1.04 ± 0.11
30th Day	I(A)	9.68±0.86	0.35±0.07	0.60±0.06
	I(B)	9.53±0.82	0.35±0.07	0.59±0.06
	I(C)	9.53±0.82	0.35±0.07	0.59±0.06
	I(F)	9.27±0.80	0.34±0.07	0.58±0.06
	I(G)	9.30±0.80	0.34±0.07	0.58±0.06
	II(A)	28.37 ± 2.44	1.04 ± 0.21	1.77 ± 0.18
	II(D)	21.68 ± 1.87	0.79 ± 0.17	1.35 ± 0.14
	II(E)	20.85 ± 1.79	0.76 ± 0.16	1.30 ± 0.14
	II(H)	22.23 ± 1.91	0.81 ± 0.17	1.39 ± 0.15
	II(I)	21.68 ± 1.86	0.79 ± 0.17	1.35 ± 0.14
	II(J)	20.03 ± 1.72	0.73 ± 0.15	1.25 ± 0.13
	II(K)	18.93 ± 1.63	0.69 ± 0.14	1.18 ± 0.12
	II(S)	15.37 ± 1.32	0.56 ± 0.12	0.96 ± 0.10
45th Day	I(A)	9.72±0.87	0.35±0.07	0.60±0.06
	I(B)	9.34±0.80	0.34±0.07	0.58±0.06
	I(C)	9.42±0.81	0.34±0.07	0.59±0.06
	I(F)	9.44±0.82	0.34±0.07	0.59±0.06
	I(G)	9.41±0.81	0.34±0.07	0.59±0.06
	II(A)	29.02 ± 2.50	1.06 ± 0.22	1.81 ± 0.19
	II(D)	18.94 ± 1.63	0.69 ± 0.14	1.18 ± 0.12
	II(E)	17.84 ± 1.53	0.65 ± 0.14	1.11 ± 0.12
	II(H)	21.13 ± 1.82	0.77 ± 0.16	1.32 ± 0.14
	II(I)	20.58 ± 1.77	0.75 ± 0.16	1.28 ± 0.14
	II(J)	18.66 ± 1.60	0.68 ± 0.14	1.16 ± 0.12
	II(K)	17.01 ± 1.46	0.62 ± 0.13	1.06 ± 0.11
	II(S)	13.99 ± 1.20	0.51 ± 0.11	0.87 ± 0.09
	I(A)	9.76±0.89	0.36±0.07	0.61±0.06
	I(B)	9.24±0.80	0.34±0.07	0.58±0.06

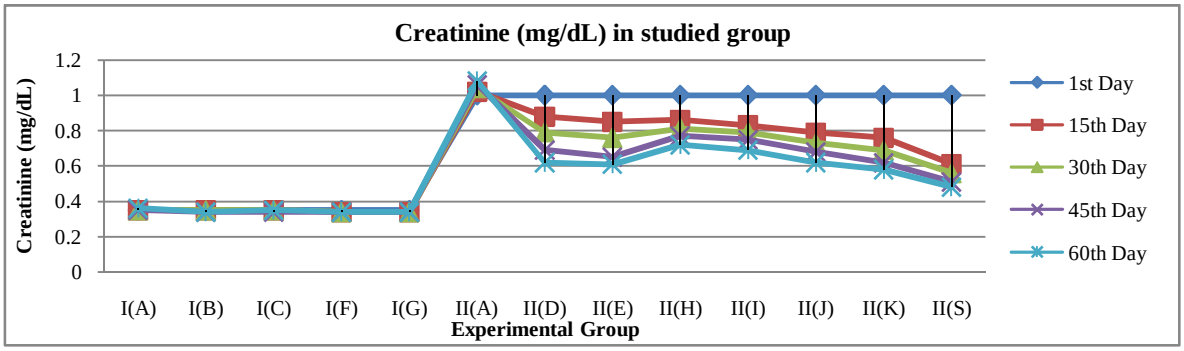
60th Day	I(C)	9.53±0.82	0.35±0.07	0.59±0.06
	I(F)	9.42±0.81	0.34±0.07	0.59±0.06
	I(G)	9.42±0.81	0.34±0.07	0.59±0.06
	II(A)	29.39 ± 2.53	1.08 ± 0.22	1.83 ± 0.19
	II(D)	17.01 ± 1.46	0.62 ± 0.13	1.06 ± 0.11
	II(E)	16.74 ± 1.44	0.61 ± 0.13	1.04 ± 0.11
	II(H)	19.76 ± 1.70	0.72 ± 0.15	1.23 ± 0.13
	II(I)	18.94 ± 1.63	0.69 ± 0.14	1.18 ± 0.12
	II(J)	17.01 ± 1.46	0.62 ± 0.13	1.06 ± 0.11
	II(K)	15.92 ± 1.37	0.58 ± 0.12	0.99 ± 0.10
	II(S)	13.17 ± 1.13	0.48 ± 0.10	0.82 ± 0.09

pronounced effect, reducing creatinine to 0.58 ± 0.12 mg/dL by Day 60. This value closely matched the ferrous sulfate group (0.48 ± 0.10 mg/dL), suggesting synergistic nephroprotection. The improvements may result from the anti-inflammatory and membrane-stabilizing properties of the plant constituents that protect renal tubules from oxidative stress (Patel & Goyal, 2016).

Graph 3. Blood Urea Nitrogen (mg/dL) in studied group



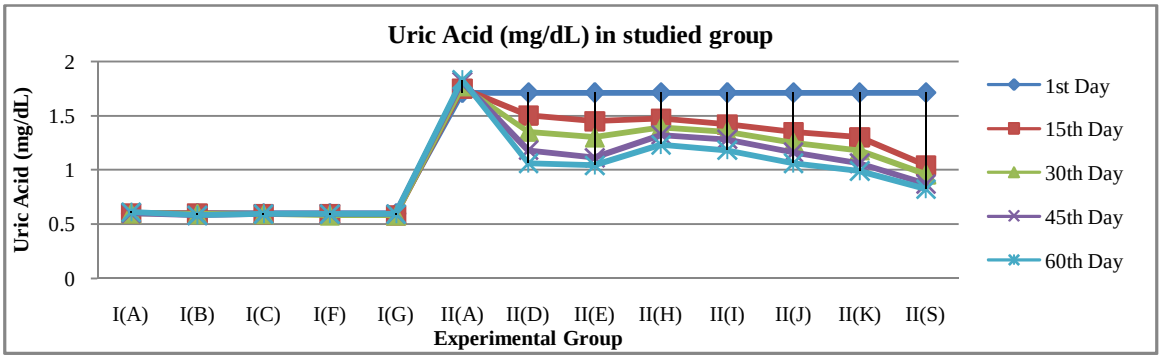
Graph 4. Creatinine (mg/dL) in studied group



Uric Acid Levels: Observed data is presented in graph 5. Uric acid is a final product of purine metabolism and serves as a secondary renal function biomarker.

Control and extract-only groups maintained uric acid levels around 0.58–0.61 mg/dL throughout, signifying normal renal excretion. In anaemic mice, uric acid levels escalated significantly due to impaired excretion and increased purine degradation from hemolysis, rising from 1.71 ± 0.18 mg/dL on Day 1 to 1.83 ± 0.19 mg/dL by Day 60. This hyperuricemia could contribute to renal oxidative stress, further damaging the nephrons (Kumari & Kakkar, 2011). Intervention with China-rose and Fenugreek gradually decreased uric acid levels, with the best effect seen in the combination group (400 mg/kg), where uric acid reduced to 0.99 ± 0.10 mg/dL by Day 60. This reduction parallels the ferrous sulfate group (0.82 ± 0.09 mg/dL), supporting the extract's role in modulating purine metabolism and promoting uric acid excretion. The observed effects are likely due to polyphenols and flavonoids that enhance xanthine oxidase inhibition and support renal recovery.

Graph 5. Uric Acid (mg/dL) in studied group



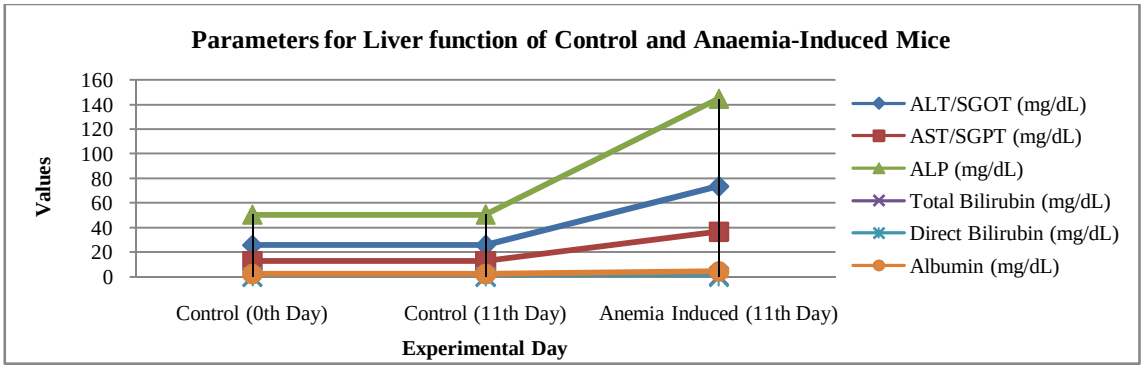
*3) Parameters for Liver function**of Control and Anaemia-Induced Mice:***

Liver function markers, including ALT, AST, ALP, bilirubin, and albumin, play a crucial role in evaluating hepatic health. In PHZ-induced anaemia, a significant increase in these markers indicates hepatic damageand potential oxidative stress-related hepatotoxicity. Observed data are presented in table 3 and graph 6.

Table 3: Parameters for Liver function of Control and Anaemia-Induced Mice

Liver	Control		Anaemia induced
	0th Day	11th Day	11th Day
ALT/SGOT (mg/dl)	25.88±8.16	25.85±8.15	73.75 ± 23.31
AST/SGPT (mg/dl)	12.94±2.45	12.92±2.44	36.88 ± 7.00
ALP (mg/dl)	50.72±9.47	50.67±9.45	144.55 ± 26.99
Total BILIRUBIN (mg/dL)	0.34±0.09	0.34±0.09	0.97 ± 0.27
Direct BILIRUBIN (mg/dL)	0.11±0.04	0.11±0.04	0.32 ± 0.12
ALBUMIN (mg/dL)	2.50±0.89	2.49±0.89	4.72 ± 1.68

Graph 6. Parameters for Liver function of Control and Anaemia-Induced Mice



Serum ALT and AST levels increased significantly in PHZ-treated mice, suggesting liver injury. ALT, primarily found in hepatocytes, increased from 25.85 mg/dL (control) to 73.75 mg/dL (anemia-induced), indicating hepatocellular damage.

Similarly, AST increased from 12.92 mg/dL (control) to 36.88 mg/dL (anemia-induced), reflecting mitochondrial dysfunction due to oxidative stress (Tiwari *et al.*, 2020). PHZ-induced hemolysis leads to excessive heme release, which generates reactive oxygen species (ROS), contributing to liver damage (Zou *et al.*, 2021).

The ALP level surged from 50.67 mg/dL (control) to 144.55 mg/dL (anemia-induced), indicating bile duct dysfunction. Elevated ALP is a marker of cholestasis and hepatobiliary damage, commonly observed in oxidative stress-induced hepatic injury (Kumar *et al.*, 2019). Hemolysis leads to bilirubin overload, which may cause liver congestion and hinder bile excretion, further elevating ALP levels.

Bilirubin levels increased in anaemic mice **(**total bilirubin: 0.34 to 0.97 mg/dL; direct bilirubin: 0.11 to 0.32 mg/dL**)**, indicating excessive haemoglobin breakdown and impaired hepatic clearance. In hemolytic anaemia, red blood cells rupture, releasing haemoglobin, this is broken down into bilirubin. An increase in direct bilirubin suggests hepatic dysfunction, as the liver struggles to conjugate and excrete bilirubin efficiently (Johnson *et al.*, 2022).

Albumin levels were significantly higher in anaemic mice (4.72 mg/dL compared to 2.49 mg/dL in controls), which could be a compensatory response to hepatic stress. Normally, albumin levels decrease in chronic liver disease, but acute hemolysis and oxidative stress can lead to transient hepatic hyperactivity, increasing albumin production (Zhao *et al.*, 2021).

4) Parameters for Liver function of Experimental Groups

Liver function is critical for overall metabolic homeostasis, particularly in conditions such as anemia, which can induce oxidative stress and hepatic injury. Liver biomarkers, including ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), ALP (Alkaline Phosphatase), bilirubin (total and direct), and albumin, serve as indicators of hepatic integrity and function. This study evaluates the effect of fenugreek and chinarose(400 mg/kg and 800 mg/kg) compared to ferrous sulfate on liver function in anaemia-induced mice over 60 days.Observed data is presented in table 4.

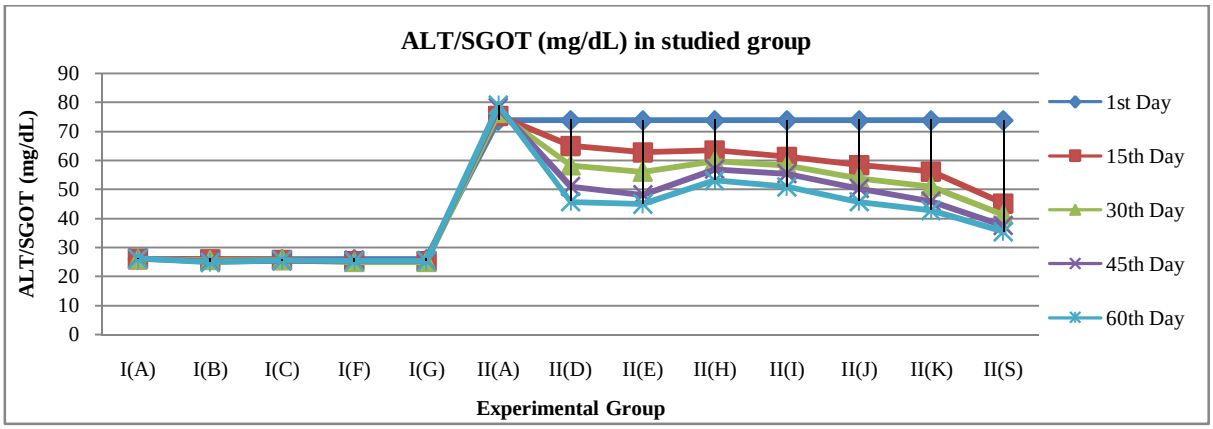
Table 4: Liver Function parameters of studied groups.

Day of Sample	Studied Group	ALT/SGOT (mg/dl)	AST/SGPT (mg/dl)	ALP (mg/dl)	T.BIL (mg/dL)	D. BIL (mg/dL)	ALB (mg/dL)
1st Day	I(A)	25.85±8.15	12.92±2.44	50.67±9.45	0.34±0.09	0.11±0.04	2.49±0.89
	I(F)	25.85±8.15	12.92±2.44	50.67±9.45	0.34±0.09	0.11±0.04	2.49±0.89
	I(G)	25.85±8.15	12.92±2.44	50.67±9.45	0.34±0.09	0.11±0.04	2.49±0.89
	II(A)	73.75 ± 23.31	36.88 ± 7.00	144.55 ± 26.99	0.97 ± 0.27	0.32 ± 0.12	4.72 ± 1.68
	II(H)	73.75 ± 23.31	36.88 ± 7.00	144.55 ± 26.99	0.97 ± 0.27	0.32 ± 0.12	4.72 ± 1.68
	II(I)	73.75 ± 23.31	36.88 ± 7.00	144.55 ± 26.99	0.97 ± 0.27	0.32 ± 0.12	4.72 ± 1.68
	II(S)	73.75 ± 23.31	36.88 ± 7.00	144.55 ± 26.99	0.97 ± 0.27	0.32 ± 0.12	4.72 ± 1.68
15th Day	I(A)	25.92±8.18	12.96±2.46	50.82±9.50	0.34±0.09	0.11±0.04	2.51±0.89
	I(F)	25.19±7.96	12.60±2.38	49.91±9.22	0.33±0.09	0.11±0.04	2.43±0.87
	I(G)	25.18±7.94	12.58±2.38	49.38±9.21	0.33±0.09	0.11±0.04	2.43±0.87
	II(A)	75.25 ± 23.75	37.63 ± 7.14	147.49 ± 27.54	0.99 ± 0.27	0.33 ± 0.12	4.86 ± 1.73
	II(H)	63.42 ± 20.05	31.72 ± 6.02	124.31 ± 23.21	0.83 ± 0.23	0.28 ± 0.10	4.06 ± 1.44
	II(I)	61.21 ± 19.35	30.62 ± 5.81	119.98 ± 22.41	0.81 ± 0.22	0.27 ± 0.10	3.92 ± 1.39
	II(S)	44.99 ± 14.22	22.50 ± 4.27	88.18 ± 16.47	0.59 ± 0.16	0.20 ± 0.07	2.88 ± 1.02

30th Day	I(A)	26.03±8.22	13.02±2.49	51.00±9.55	0.34±0.09	0.11±0.04	2.54±0.90
	I(F)	24.93±7.86	12.46±2.35	48.86±9.11	0.33±0.09	0.11±0.04	2.40±0.86
	I(G)	25.02±7.89	12.51±2.36	49.07±9.15	0.33±0.09	0.11±0.04	2.41±0.86
	II(A)	76.25 ± 24.10	38.13 ± 7.23	149.45 ± 27.93	1.01 ± 0.28	0.34 ± 0.12	4.96 ± 1.76
	II(H)	59.74 ± 18.88	29.87 ± 5.67	117.09 ± 21.86	0.79 ± 0.22	0.26 ± 0.10	3.82 ± 1.36
	II(I)	58.26 ± 18.42	29.13 ± 5.53	114.20 ± 21.32	0.77 ± 0.21	0.25 ± 0.09	3.73 ± 1.33
	II(S)	41.30 ± 13.05	20.65 ± 3.92	81.35 ± 15.11	0.54 ± 0.14	0.18 ± 0.07	2.64 ± 0.94
45th Day	I(A)	26.10±8.24	13.06±2.50	51.06±9.57	0.35±0.09	0.11±0.04	2.55±0.91
	I(F)	25.41±8.00	12.70±2.40	49.84±9.29	0.33±0.09	0.11±0.04	2.45±0.88
	I(G)	25.31±7.98	12.65±2.39	49.63±9.26	0.33±0.09	0.11±0.04	2.44±0.87
	II(A)	78.00 ± 24.63	39.00 ± 7.39	152.88 ± 28.54	1.03 ± 0.28	0.34 ± 0.13	5.13 ± 1.86
	II(H)	56.79 ± 17.95	28.40 ± 5.39	111.30 ± 20.78	0.75 ± 0.21	0.25 ± 0.09	3.63 ± 1.29
	II(I)	55.31 ± 17.48	27.66 ± 5.25	108.41 ± 20.24	0.73 ± 0.20	0.24 ± 0.09	3.54 ± 1.26
	II(S)	37.41 ± 11.89	18.81 ± 3.57	73.72 ± 13.76	0.49 ± 0.14	0.16 ± 0.06	2.41 ± 0.86
60^{th} Day	I(A)	26.19±8.28	13.11±2.52	51.24±9.62	0.35±0.09	0.12±0.04	2.57±0.92
	I(F)	25.35±7.98	12.67±2.39	49.71±9.27	0.33±0.09	0.11±0.04	2.44±0.87
	I(G)	25.37±8.00	12.68±2.40	49.71±9.28	0.33±0.09	0.11±0.04	2.44±0.87
	II(A)	79.00 ± 24.94	39.50 ± 7.48	154.84 ± 28.93	1.04 ± 0.29	0.35 ± 0.13	5.23 ± 1.86
	II(H)	53.10 ± 16.78	26.55 ± 5.04	104.08 ± 19.43	0.70 ± 0.19	0.23 ± 0.09	3.40 ± 1.21
	II(I)	50.89 ± 16.08	25.45 ± 4.83	99.74 ± 18.62	0.67 ± 0.19	0.22 ± 0.08	3.26 ± 1.16
	II(S)	35.40 ± 11.19	17.71 ± 3.32	69.38 ± 12.95	0.47 ± 0.13	0.15 ± 0.06	2.27 ± 0.81

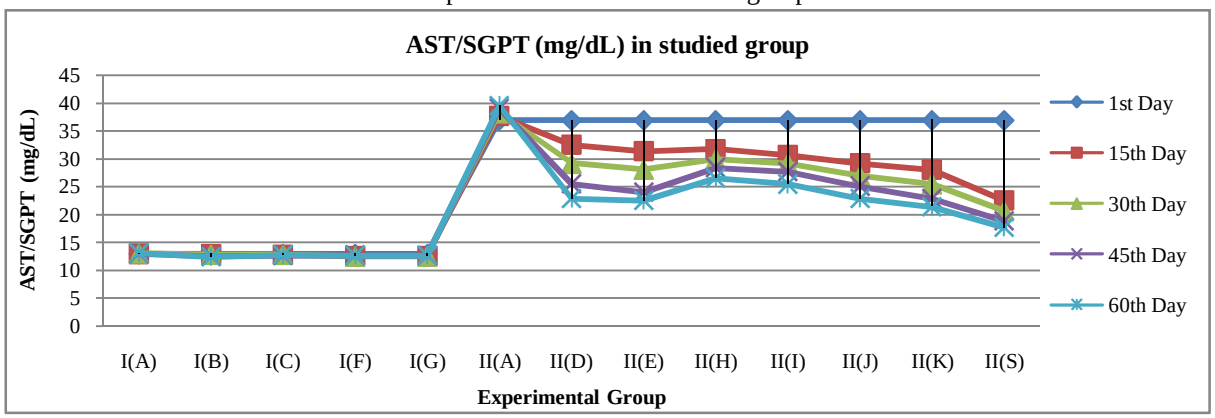
Alanine Aminotransferase (ALT) Levels: Observed data is presented in graph 7. In the anaemia-induced group, ALT levels were significantly elevated throughout the study period (73.75 ± 23.31 on Day 1 to 79.00 ± 24.94 on Day 60), indicating hepatic injury due to oxidative stress caused by phenylhydrazine (PHZ), which is known to induce hemolytic anaemia and hepatotoxicity via free radical generation (Uddin et al., 2016). In contrast, the normal control and extract-only groups (China rose or fenugreek at 400 and 800 mg/kg) showed ALT levels within normal physiological limits (~25 mg/dL), indicating non-toxicity of the herbal extracts. Treatment of anaemic mice with China rose and/or fenugreek extracts led to progressive improvement in ALT levels, with the combination at 400 mg/kg showing notable efficacy (42.77 ± 13.52 by Day 60), possibly due to synergistic antioxidant effects (Bukhari et al., 2020; Joshi et al., 2021). Ferrous sulphate treatment also led to a significant decline in ALT (35.40 ± 11.19), though marginally less effective than the polyherbal combination.

Graph 7. ALT/SGOT in studied group



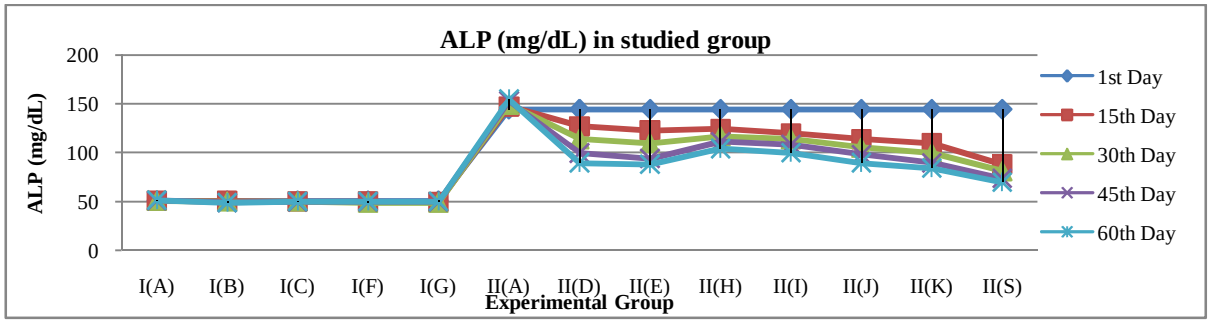
Aspartate Aminotransferase (AST) Level: AST levels mirrored ALT trends, increasing significantly in the anaemic group (up to 39.50 ± 7.48 by Day 60), reflecting hepatocellular injury. The herbal treatments demonstrated a dose-dependent reversal, with the combination extract (400 mg/kg) showing a marked improvement (21.39 ± 4.06), nearly comparable to ferrous sulphate (17.71 ± 3.32). The hepatoprotective effect may be attributed to the phenolic compounds and flavonoids in the extracts, which scavenge reactive oxygen species (ROS) and stabilize cellular membranes (El-Hawary et al., 2017).

Graph 8. AST/SGPT in studied group



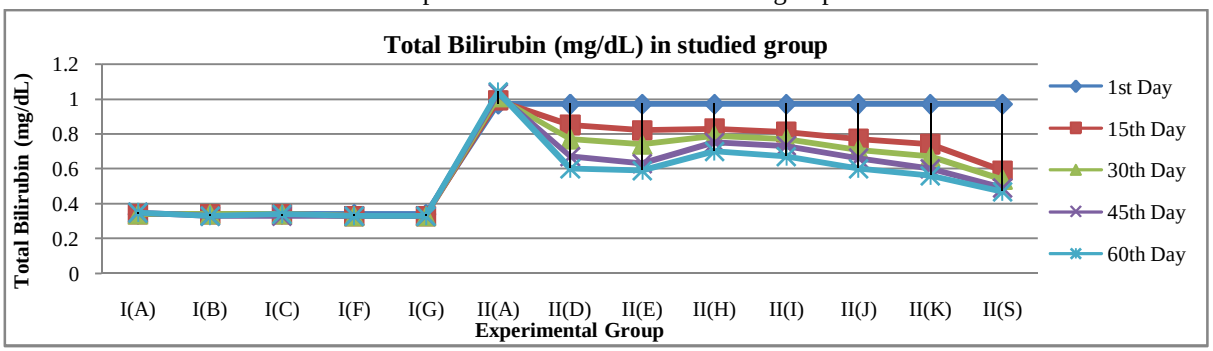
Alkaline Phosphatase (ALP) Levels: ALP is an indicator of bile duct function and hepatobiliary integrity. Elevated ALP levels are associated with liver dysfunction and hemolysis-induced hepatic stress (Sharma *et al.*, 2023). Observed data is presented in graph 9. The anaemia group displayed elevated ALP levels (154.84 ± 28.93), likely due to cholestatic stress or hepatobiliary dysfunction (Sakr et al., 2014). Treatment with China rose and fenugreek significantly restored ALP values, especially with the 400 mg/kg combination (83.84 ± 15.65 by Day 60), highlighting their potential in reversing hepatobiliary impairment. The ALP-lowering effect was slightly superior in the ferrous sulphate group (69.38 ± 12.95), which serves as the standard of care.

Graph 9. ALP in studied group

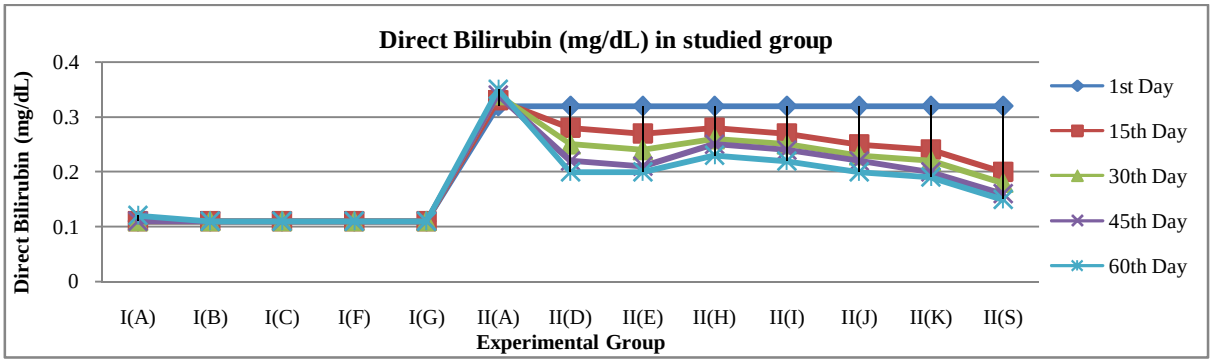


Bilirubin (Total and Direct) Levels: Bilirubin is a breakdown product of haemoglobin and a key marker of liver function and erythrocyte turnover. Increased bilirubin suggests **ineffective erythropoiesis and hepatic dysfunction** (Garg *et al.*, 2023). Observed data is presented in graph 10& 11. Elevated bilirubin in the anaemic group (Total: 1.04 ± 0.29; Direct: 0.35 ± 0.13) confirmed hepatic dysfunction and ineffective erythrocyte clearance. Polyherbal and individual treatments showed a reduction in bilirubin levels over time, with the combination at 400 mg/kg yielding a significant decrease (Total: 0.56 ± 0.16; Direct: 0.19 ± 0.07), demonstrating restoration of hepatic excretory function (Thakur et al., 2022). Ferrous sulphate exhibited comparable efficacy.

Graph 10. Total Bilirubin in studied group

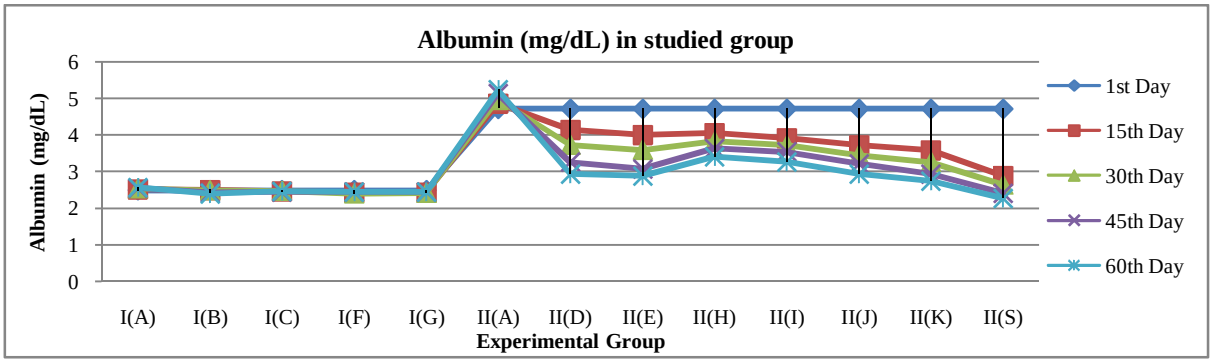


Graph 11. Direct Bilirubin in studied group



Albumin Levels: Serum albumin is a marker of liver synthetic function and nutritional status. Reduced albumin levels are often observed in chronic anemia due to oxidative damage and impaired protein synthesis (Singh *et al.*, 2021). Observed data is presented in graph 12. Albumin was abnormally high in the anaemic group (5.23 ± 1.86), potentially due to hemoconcentration or compensatory hepatic response. Treatment groups exhibited gradual normalization, with the combination extract at 400 mg/kg achieving near-control values (2.74 ± 0.97), in line with ferrous sulphate (2.27 ± 0.81). Restoration of albumin synthesis further supports hepatic recovery and improved metabolic function (Ibrahim et al., 2019).

Graph 12. Albumin in studied group



VI. CONCLUSION

The present study demonstrates that *Hibiscus rosa-sinensis* (China rose) and *Trigonella foenum-graecum* (fenugreek) leaf extracts exhibit significant hepatoprotective and nephroprotective effects in phenylhydrazine-induced anemic mice. The extracts effectively ameliorated elevated liver enzymes (ALT, AST, ALP), bilirubin levels, and kidney function biomarkers (creatinine, BUN, uric acid), indicating restoration of hepatic and renal function. Among the treatment groups, the combination of both extracts showed the most pronounced recovery, comparable to standard ferrous sulfate therapy. These protective effects are likely attributed to the antioxidant potential of the plant extracts. Thus, China rose and fenugreek may serve as promising natural adjuvants for managing anemia-associated organ toxicity.

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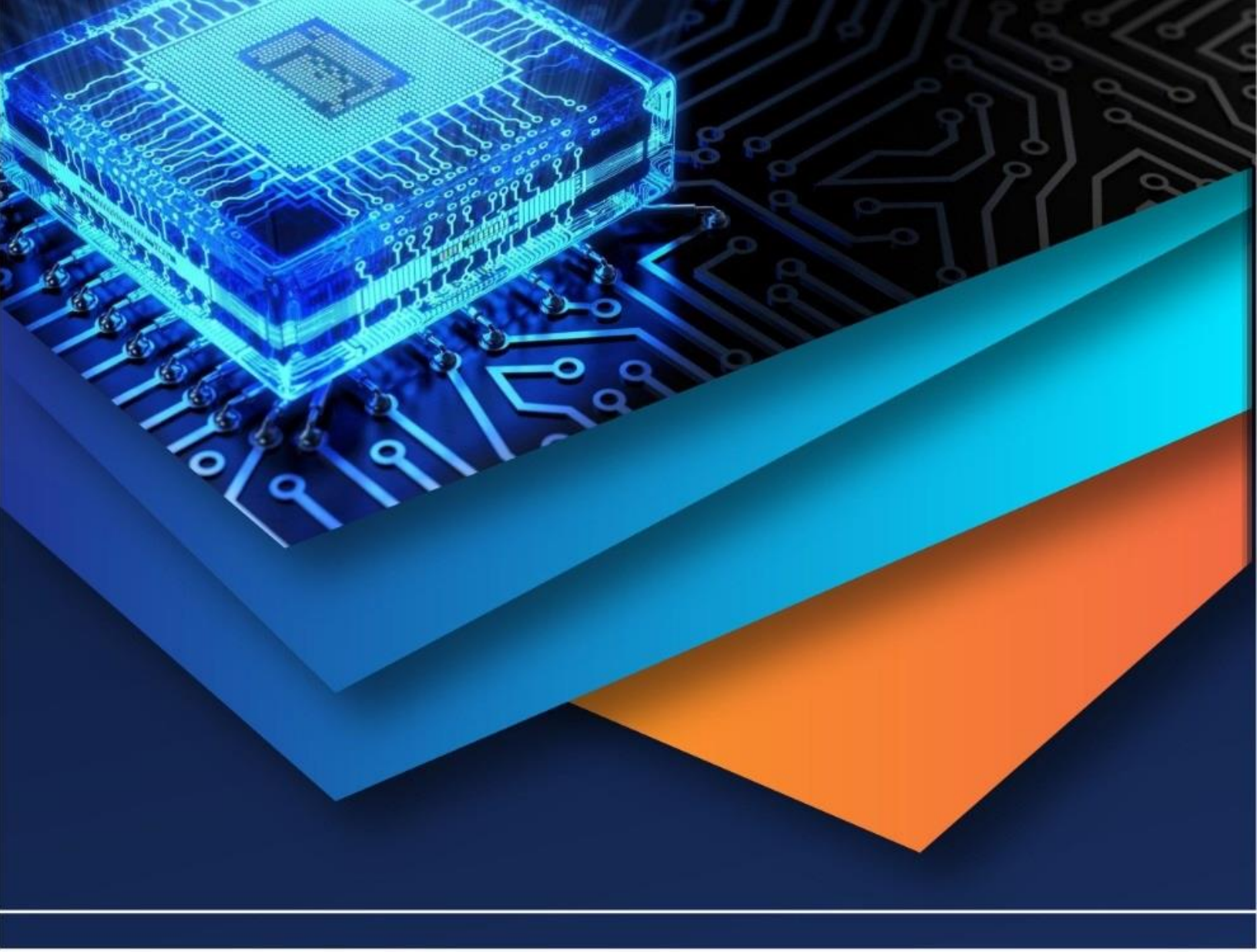

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