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Research Article

**ANTI-ANAEMIA AND HAEMOPOIETIC EVALUATION OF  
TRIGONELLA FOENUM GRAECUM IN RODENT MODEL**Radha Nath\*<sup>1</sup>, Anil Kumar<sup>2</sup><sup>1</sup>Millennium College of Pharmacy, Bhopal (M.P.)<sup>2</sup>Raghukul College of Pharmacy, Bhopal (M.P.)

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**Article Received:** January 2023**Accepted:** January 2023**Published:** February 2023**Abstract:**

Anaemia also defined as a decreased quantity of circulating red blood cells (RBCs), otherwise known as erythrocytes, is a major source of morbidity and mortality worldwide. The medicinal plants are always the good source to overcome many diseases across the world as they have enormous potential in the form of phytochemical (Phytomedicines) which are used in the treatment of various diseases. *Trigonella foenum-graecum* found to have Analgesic, Anti-inflammatory, neuropharmacological and cytotoxic activity. Thus, this study deals with the anti anemic activity of *Trigonella foenum-graecum* in rodent model. The plant material was collected & subjected to hydroalcoholic extraction followed by phytochemical screening & in vivo testing for various parameters in rat. The result showed that was found to be 9.43 %. Phytochemical analysis revealed the presence of large chemical groups that are: alkaloids, flavonoids, polyphenols, sterols, terpenes, glycosides and saponins. An increased number of red blood cells were observed after treatment with the extract of *Trigonella foenum-graecum*. The results show that the rats of the groups G1, G3, G4 and G5 have almost completely recovered RBC level & hemoglobin at the 13th day of study period ( $8.213 \pm 0.410$ ), ( $7.156 \pm 0.944$ ), ( $8.315 \pm 0.554$ ) and ( $8.281 \pm 0.459$ ) respectively. The rats of groups G3, G4, and G5 the increased of WBCs at day 13 was ( $8.2 \pm 0.548$ ), ( $8.431 \pm 0.447$ ), ( $8.638 \pm 0.572$ ) respectively. From the results obtained it can be concluded that *Trigonella foenum-graecum* exhibit potential to heal anaemia.

**Keywords:** RBC, WBC, Haemoglobin, *Trigonella foenum* phytochemical, Medicinal plants

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**INTRODUCTION:**

Anaemia stems from ancient Greek “Avbetaµibeta”, which means “without blood”. Anaemia is a reduction in the number of red blood cells (RBC) or the haemoglobin (Hb) content of blood, or a decreased ability of Hb to bind oxygen (Schnall, 2000). In clinical terms, anaemia is considered as an Hb concentration that is insufficient to meet the oxygen needs of the tissues. Anemia also defined as a decreased quantity of circulating red blood cells (RBCs), otherwise known as erythrocytes, is a major source of morbidity and mortality worldwide. Deficiencies of iron, vitamin A, riboflavin, vitamin B12 or folate can also affect iron handling and haematological status. There is mounting evidence that a substantial part of anaemia, particularly in the tropics, is due to infectious diseases such as malaria and helminthiasis (Vieth *et al.*, 2014; Dallman *et al.*, 1994).

Most forms of anemia within this class are monogenic disorders caused by inherited mutations. Diamond-Blackfan anemia (DBA) is a rare inherited anemia characterized by developmental anomalies and decreased RBC precursors.  $\beta$ -thalassemia is caused by mutations that inhibit the production of beta-globin chains. Inflammation-associated suppression of RBC. The proinflammatory cytokines, which stimulate hepcidin production to inhibit iron absorption and utilization block the differentiation of erythroid precursors/progenitors directly. Causes of cancer-associated anemia include chemotherapy-induced myelosuppression and the production of inhibitory cytokines (Vlachos *et al.*, 2010; Cao *et al.*, 2010; Dicato *et al.*, 2010).

The medicinal plants are always the good source to overcome many diseases across the world as they have enormous potential in the form of phytochemical (Phytomedicines) which are used in the treatment of various diseases (Peter *et al.*, 2014).

*Trigonella foenum-graecum* is an erect, sometimes ascending, loosely-branched annual plant growing 40 - 80cm tall. A fairly common food, condiment and medicinal plant, it is widely cultivated for its edible seed in warm temperate to tropical regions, there are some named varieties. leaves contain steroidal saponin (diosgenin, yamogenin, tigogenin and neo tigogenin); sapogenins, furostanol saponins, alkaloids, flavonoids, salicylate, graecunin- B,C,D,E and G. Vitamin k has also been reported in leaves. The plant found to have Analgesic, Anti-inflammatory, neuropharmacological and cytotoxic activity (Srinivasan *et al.*, 2006; Yadav and Baquer, 2004). Thus, this study deals with the anti

anemic activity of *Trigonella foenum-graecum* in rodent model.

**MATERIALS AND METHODS:****Collection of plant material**

The plant *Trigonella foenum-graecum* will be authenticated by Dr. Saba Khan, Botanist, Dept. of Botany, Safia Science College, Bhopal.

**Experimental animals**

Male albino Wistar rats (150-200g) were selected at random from animal house of Pinnacle Biomedical Research Institute. Animals were further randomly divided into various treatment groups and kept in propylene cages (38×23×10cm) with sterile husk as bedding. Animals were housed in relative humidity of 44-56%, at 22 ± 20 C and 12:12 light and dark cycle. Animals were fed with standard pellets and water was available ad libitum. The study conducted will be approved by the Institutional Animal Ethical Committee (IAEC) of PBRI. The standard gastric cannulas will be used for oral drug administration in experimental animals.

**METHODS:****Extraction of plant material**

1kg of seeds of *Trigonella foenum-graecum* will be collected & cleaned to remove dirt. The dried material was powdered by using a blender to obtain a coarse powder and then passed through 40 mesh sieve. The powdered material will be subjected to extraction using hydroalcoholic solvent (70:30) by soxhlet extraction method (López-Bascón, 2020).

**Phytochemical screening**

The extract obtained from successive Soxhlet extraction method will be subjected to preliminary phytochemical analysis. Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents.

**Acute oral toxicity**

All animals fed with standard rat pellet diet had free access to tap water ad libitum. The doses selected for the study were 2000 mg/Kg, 300 mg/Kg, 50 mg/Kg, 5 mg/Kg. Animals were observed for mortality for next 72 hours after sample administration till 14 days. On the basis of acute toxicity study, two test were selected for the pharmacological screening on the basis of maximum tolerated dose limit (MTD), as there was no lethality observed up to 2000 mg/Kg. Finally selected doses (2000 mg/Kg) were chosen for further pharmacological studies.

**Anaemia induction (Droucoula *et al.*, 2017)**

Anaemia was induced in rats by intraperitoneal administration of 40 mg / kg / day of phenylhydrazine (PHZ) for two days (Day 0 and Day 1). The treated rats with phenylhydrazine with haemoglobin concentration < 13 g/dl were considered as anaemic and included in the study.

#### Experimental design:

The animals were randomly distributed according to weight in five groups each of six rats.

**Group I (Normal):** Normal control received Normal saline.

**Group II (PHZ control):** Anaemic control received phenylhydrazine (40 mg/kg) at day 0 and day 1 then distilled water daily during 13 days.

**Group III (TFG 200):** In this group rats were treated with phenylhydrazine (40 mg/kg) at day 0 (D0) and day 1 (D1) then treated with *Trigonella foenum-graecum* extract (200 mg/kg) daily during 13 days.

**Group IV (TFG 500):** In this group rats were treated with phenylhydrazine (40 mg/kg) at day 0 (D0) and day 1 (D1) then treated with *Trigonella foenum-graecum* extract (500 mg/kg) daily during 13 days.

**Group V (Vit B12):** Standard control received phenylhydrazine (40 mg/kg) at days 0 (D0) and 1 (D1) and dexorange single dose 200 mg/kg. Blood samples were collected on day 0, 2 and 13 from all the rats from the orbital plexus of eye in EDTA vials and evaluated for hematological parameters such as RBC, Hb, and WBCs.

#### RESULTS & DISCUSSION:

The plant material was extracted and the percentage yield calculated by the following formula was found to be 9.43 %. Phytochemical analysis revealed the presence of large chemical groups that are: alkaloids, flavonoids, polyphenols, sterols, terpenes, glycosides and saponins. An increased number of red blood cells was observed after treatment with the extract of *Trigonella foenum-graecum*. The results show that the rats of the groups G1, G3, G4 and G5 have almost completely recovered RBC level & hemoglobin at the 13th day of study period ( $8.213 \pm 0.410$ ), ( $7.156 \pm 0.944$ ), ( $8.315 \pm 0.554$ ) and ( $8.281 \pm 0.459$ ) respectively. The rats of groups G3, G4, and G5 the increased of WBCs at day 13 was ( $8.2 \pm 0.548$ ), ( $8.431 \pm 0.447$ ), ( $8.638 \pm 0.572$ ) respectively.

**Table 1: Phytochemical testing of extract**

S. No.	Experiment	Methanolic extract	
		Present	Absent
1	<b>Alkaloids</b>		
1.1	Mayer's reagent test	✓	-
1.2	Wagner's reagent test	✓	-
1.3	Hager's reagent test	✓	-
2.	<b>Carbohydrates</b>		
2.1	Molish's test	✓	-
2.2	Fehling's test	✓	-
2.3	Benedict's test	✓	-
2.4	Barfoed's test	✓	-
3	<b>Proteins and Amino Acids</b>		
3.1	Biuret test	✓	-
4.	<b>Flavonoids</b>		
4.1	Alkaline reagent test	✓	-
4.2	Lead Acetate test	✓	-

5.	<b>Glycoside</b>		
5.1	Borntrager test	-	✓
5.2	Legal's test	-	✓
5.3	Killer-Killiani test	-	✓
6.	<b>Tannin and Phenolic Compounds</b>		
6.1	Ferric Chloride test	✓	-
6.2	Lead Acetate test	✓	-
6.3	Gelatin test	✓	-
7.	<b>Saponin</b>		
7.1	Foam test	✓	-
8.	<b>Test for Triterpenoids and Steroids</b>		
8.1	Salkowski's test	✓	-
8.2	Libermann-Burchard's test	✓	-

**Table 2: Acute oral toxicity**

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg Bodyweight	0/3
2.	50 mg/kg Bodyweight	0/3
3.	300 mg/kg Bodyweight	0/3
4.	2000 mg/kg Bodyweight	0/3

**Table 3: Effect of administration of *Trigonella foenum-graecum* extract on RBCs of iron deficient rats.**

S. No.	Treatment Groups	Day 0 (10 <sup>6</sup> /μl)	Day 1 (10 <sup>6</sup> /μl)	Day 13 (10 <sup>6</sup> /μl)
1	Normal Control	8.17±0.302	7.16±0.046	8.21±0.410
2	Positive control (PHZ 40 mg/kg)	8.11±0.501	3.83±0.329	4.37±0.226
3	TFG treated group 200mg/kg	8.05±0.581	4.12±0.489	7.15±0.944*
4	TFG treated group 400mg/kg	8.11±0.291	4.00±0.431	8.31±0.554**
5	Standard (200 mg/kg)	8.12±0.325	3.72±0.537	8.28±0.459**

**Table 4: Effect of administration of *Trigonella foenum-graecum* extract on haemoglobin of iron deficient rats.**

S. No.	Treatment Groups	Day 0 (gm/dL)	Day 1 (gm/dL)	Day 13 (gm/dL)
1	Normal Control	14.75±0.377	14.3±0.201	14.5±0.071
2	Positive control (PHZ 40 mg/kg)	14.32±0.528	8.11±0.540	12.2±1.639
3	TFG treated group 200mg/kg	14.70±0.381	8.12±0.155	12.44±0.551*
4	TFG treated group 400mg/kg	14.31±0.308	8.25±0.247	14.04±0.355**
5	Standard (200 mg/kg)	14.55±0.322	8.19±0.684	14.54±0.411**

**Table 5 : Effect of administration of *Trigonella foenum-graecum* extract on WBCs of iron deficient rats.**

S. No.	Treatment Groups	Day 0 ( $10^3/\mu\text{l}$ )	Day 1 ( $10^3/\mu\text{l}$ )	Day 13 ( $10^3/\mu\text{l}$ )
1	Normal Control	8.13±0.186	7.55±0.533	8.54±0.297
2	Positive control (PHZ 40 mg/kg)	7.37±0.705	4.16±0.823	5.87±0.674
3	TFG treated group 200mg/kg	8.08±0.622	5.3±0.648	8.2±0.548*
4	TFG treated group 400mg/kg	8.05±0.368	5.00±0.700	8.43±0.447**
5	Standard (200 mg/kg)	8.01±0.289	3.72±0.537	8.63±0.572**

**CONCLUSION:**

The anti-anemia potential and haemoglobin restoring effect of *Trigonella foenum-graecum* extract as suggested by the data in this study could be attributed in part to its phytochemical constituents.

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