RESEARCH ARTICLE

Anti-anemic Activity of Seeds of *Trigonella Foenum-graecum* in Male Albino Rats

**ABSTRACT**

**Objective** To evaluate the anti-anemic activity of seeds of *Trigonella foenum-graecum* against phenyl hydrazine induced anemic rats.

**Methods** Rats were divided into 4 groups of 6 each. Group 1 was given normal saline and served as control and all other groups were given 40 mg/kg b.w. of phenyl hydrazine for 2 days to induce anemia. Group 3 was treated with Dexorange syrup (200 mg/kg) and served as the standard. Group 4 was treated with seeds of *Trigonella foenum-graecum* (200 mg/kg). All the treatments were given orally. On completion of the experimental period, all the test substance/vehicle-treated rats were sacrificed and the plasma separated was used for estimating various biochemical as well as hematological parameters as per standard procedures.

**Results** The experimental rats treated with seeds of *Trigonella foenum-graecum* at the dose level 200 mg/kg bw for 13 days revealed significant changes in biochemical and hematological parameters compared to phenyl hydrazine induced anemic rats.

**Conclusion** The present study concluded that the seeds of *Trigonella foenum-graecum* inhibits anemia induced by phenyl hydrazine in male albino rats.

**KEYWORDS** anemia, phenyl hydrazine, *trigonella foenum-graecum*, and dexorange

**INTRODUCTION**

Anemia is described as a reduction in Red Blood Cell (RBC) mass or blood hemoglobin (Hb) concentration resulting in a decrease in the oxygen-carrying capacity of the blood. The prevalence of anemia in the developing countries tends to be 3 to 4 times higher than in the developed countries. Anemia affects the physical and mental development of an individual leading to decreased working capacity, which in turn affects the development of the country. Since the technological advancement and economic development of a nation depend heavily on its trained human resources, the behavioral effects of anemia are highly relevant. Consequently, if anemia is highly prevalent in a country, it can substantially affect its intellectual and economic potential.

The World Health Organization (WHO) estimates the number of anemic people worldwide to be a staggering 2 billion (about 30% of the world’s population) and that approximately 50% of all anemias can be attributed to iron deficiency and in resource-poor areas, this is frequently exacerbated by infectious diseases, malaria, worm infestation and HIV/AIDS. Even though anemia is associated with nutritional deficiencies, acute or chronic disease, drug use or physiological states such as pregnancy, blood loss, impaired erythropoiesis and abnormal erythrocyte destruction are implicated.

Plant and plant products are being utilized as a source of medicine since long. Plant extracts are used as phytotherapeutics and are still a large source of natural antioxidants. Natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restored the optimal balance by neutralizing the reactive species. Particularly, flavonoids and phenolics are considered as potential therapeutic agents. A wide range of ailments and is widely distributed in the plant kingdom and, therefore, an integral part of the diet, with the significant amount reported in vegetables, fruits and beverages.

Fenugreek (*Trigonella foenum-graecum* Linn.) is an annual herb which has widely been consumed throughout the world as a food, a food additive and in the traditional remedies science civilizations. Fenugreek, being rich in photochemicals, has traditionally been used as a food, forage and medicinal plant. The active principles of many drugs found in plants are secondary metabolites.
The antimicrobial activities of plant extracts may reside in a variety of different components. Fenugreek seeds contain lysine and L-tryptophan rich proteins, mucilaginous fibre and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects. The steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) are thought to inhibit cholesterol absorption and synthesis and hence its potential role in arteriosclerosis. Locally, fenugreek seeds have traditionally and commonly been used to treat diabetes, coughs, congestion, bronchitis, fever, high blood pressure, headache, migraines, diarrhea, flatulence, anemia, irregular menstrual cycles and arthritis, to ease labor pains and menstruation pain, and as an appetite stimulant. Fenugreek has also been used as an external poultice to control inflammation and dandruff. Modern medicine is beginning to provide confirmation of many of the traditional medicinal applications of fenugreek seeds. To the best of our knowledge, no previous studies have been reported for anti-anemic activity of fenugreek. Thus, this study was aimed to evaluate the anti-anemic activity of fenugreek seeds in order to be used in anemia.

MATERIALS AND METHODS

Animals

Wistar strain male albino rats, weighing 100–120 g were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (22 ± 3°C, humidity 30–70%, 12 h light/dark cycle). The animals were allowed to have standard feed and water ad libitum. They were acclimated to the environment for one week prior to experimental use. The study protocol was carried out as per the rules and regulations of the Institutional Animal Ethical Committee (IAEC).

Chemicals

All chemicals and reagents were used of analytical grade, purchased from Shyam Brother Bhopal.

Plant material and preparation of extract

The seeds of *Trigonella foenum-graecum* were purchased from a departmental store during the month of August 2016. The seeds were coarsely powdered, weighed (240 g) and filled in soxhlet apparatus for extraction. The powdered drug was extracted with hydroalcoholic solvent (70:30) until the drug was completely extracted. % yield was calculated for each extract after drying under vacuum. (Kokate, 2000)

Experimental design

Anemia was induced by intra peritoneal injection of phenyl hydrazine at 40 mg/kg for 2 days, as described by Diallo et al. Following the injections, rats were divided into four groups of six rats each. Group I-Control rats received saline. Group II-Phenyl hydrazine treated rats (40 mg/kg per day for 2 days) Group III-Phenyl hydrazine treated rats with 40 mg/kg per day for 2 d and seeds of *Trigonella foenum-graecum* single dose (200 mg/kg) per day for 13 days. Group IV-Phenyl hydrazine treated rats with standard dexorange treated rats-a single dose (200 mg/kg) per day for 13 day. On completion of the experimental period, animals were anesthetized with thiopentone sodium (50 mg/kg, ip). The blood was collected with and without EDTA as an anticoagulant. Plasma was separated by centrifugation. Plasma was used for the estimation of various biochemical parameters. Hemoglobin was estimated by cyanomethaemoglobin method. WBC, RBC and platelet were counted by Ochel method, 2000.

Hematocrit or packed cell volume (PCV)

PCV% = Packed RBC column height/Total blood column height

Mean corpuscular hemoglobin (MCH)

This indicates the weight of hemoglobin in a single red blood cell and is expressed in picograms (pg) (1 pg = 10^-12 g).

MCH = Hemoglobin (g/dL)/RBC count Million per cu.mm

Mean corpuscular Hemoglobin concentration (MCHC)

This denotes the hemoglobin concentration per 100 ml of packed red blood cells and is related to the color of the red cells. This is expressed as the percentage of packed cells.

MCHC = Hemoglobin (g/dL)/PCV% × 100

This is expressed as the volume in cubic microns or femtoliters of an average red blood cell.

MCV = PCV%/RBC count/Million per cu.mm × 10

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust; reduced glutathione was estimated by the method of Moron et al., and serum GOT was estimated by the method of Reitman and Frankel.

Statistical analysis

The values were expressed as mean ± SD and statistically significant differences between mean values were determined by one-way analysis of variance (ANOVA) followed by the Tukey’s test for multiple comparisons. Statistical analysis carried out by MS-Windows based graph pad Instat software (Graph Pad software, San Diego, CA, USA) 3 version was used. A value of *P* < 0.05 was considered statistically significant.
RESULTS

The present study was carried out to assess the anti-anemic activity of seeds of *Trigonella foenum-graecum*. The observations made on different groups of experimental and control animals were compared as follows.

Table 1 represents the effect of seeds of *Trigonella foenum-graecum* on hemoglobin, WBC, RBC, and platelet count of male albino rats. Group II phenyl hydrazine intoxicated rats showed a significant (8.22 ± 0.87) decrease in the content of Hb when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with seeds of *Trigonella foenum-graecum* significantly (14.47 ± 0.93) increase in the level of Hb when compared to Group II. Group II phenylhydrazine induced rats showed a significant (2350 ± 236, 2.30 ± 0.18 and 18833.3 ± 2016) decrease in WBC, RBC and platelet levels respectively when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with seeds of *Trigonella foenum-graecum* significantly (3450 ± 278, 3.89 ± 0.04 and 219000 ± 1870) increase in WBC, RBC and platelet levels respectively when compared to group II.

The effect of seeds of *Trigonella foenum-graecum* on PCV, MCH, MCHC and MCV count in male albino rats (Table 2). Group II phenylhydrazine intoxicated rats showed a significant (16 ± 0.01) decrease in PCV when compared to Group I rats. Group III rats treated with seeds of *Trigonella foenum-graecum* significantly (29 ± 0.11) increase in PCV when compared to group II. Group II phenyl hydrazine induced rats showed a significant increase in MCH, MCHC, MCV when compared to Group I rats. Group III rats treated with seeds of *Trigonella foenum-graecum* significantly (33 ± 0.35, 38 ± 0.24 and 48 ± 0.97) decreases in MCH, MCHC, and MCV levels respectively when compared to group II.

Table 3 represents the levels of MDA, GSH, and SGOT in male albino rats. Group II phenyl hydrazine intoxicated rats showed a substantial (7.4 ± 1.24) increase in the level of MDA when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with seeds of *Trigonella foenum-graecum* significantly (4.2 ± 1.02) a decrease in the level of MDA when compared to group II. Group II phenyl hydrazine intoxicated rats showed a significant (2.1 ± 0.54) decrease in the level of GSH when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with seeds of *Trigonella foenum-graecum* significantly (4.0 ± 0.67) increase in the level of GSH as compared to Group II. Group II phenyl hydrazine intoxicated rats showed a significant increase (205.3 ± 72.5) in the activity of SGOT when compared to Group I rats. Group III phenyl hydrazine intoxicated rats treated with significantly (79.2 ± 22.8) a decrease in the content of SGOT when compared to group II.

DISCUSSION

Anemia is a disease characterized by a reduction in the concentration of hemoglobin, circulating red blood cell and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient. The present study aimed to evaluate the anti-anemic activity of seeds of *Trigonella foenum-graecum* against phenyl hydrazine-induced anemic rats. Phenyl hydrazine is recognized for its capacity to cause hemolysis both *in vitro* and *in vitro* by the formation of aryl and hydroxyl radicals, which have been demonstrated to be associated with its interaction with erythrocytes.

Oxidative stress in erythrocytes is considered as an important mechanism of hemolysis. Disruption of membrane integrity arises from fragility, dehydration as well as increased production of reactive oxygen species. Chronic hemolysis leads to loss of hemoglobin. These metabolic changes lead to the depletion of essential nutrients and micronutrients which are required for proper cell function. The accumulation of hydrogen peroxide in addition to the detoxifying capacity of the red cell may lead to the oxidation of essential cellular constituents including membrane phospholipids. Such alterations presumably contribute to the eventual hemolysis of affected cells.

The intoxication of rats with phenyl hydrazine (4 mg/kg for 2 d) resulted in a marked hemolytic anemia characterized by decreased RBC, hemoglobin and PCV. Similar results were obtained in our study when experimental rats were administered phenyl hydrazine in order to induce anemia. Phenyl hydrazine altered the function of RBC by hemolysis characterized by decreased levels of RBC,
hemoglobin and PCV. In addition, Ferrali et al. observed an increased reticulocytosis, methaemoglobinemia and hemocoelithrosis in phenyl hydrazine intoxicated rats.

This study is intended to evaluate the effect of the seeds of *Trigonella foenum-graecum* on the hemolytic anemia induced by phenyl hydrazine. It has been demonstrated previously that intraperitoneal administration of phenyl hydrazine decreases the hemoglobin concentration, red blood cell number, and hematocrit in rats. This anemia which resulted from the early lysis of the red blood cells was naturally reversed 12 days later by the regeneration of those blood cells due to the increase of the reticulocytes.

The seeds of *Trigonella foenum-graecum* could stimulate erythropoiesis process. The increase in the number of young red blood cells (reticulocytes) explains the strong osmotic resistance of the red blood cells in rats treated with the extract. The number of circulating reticulocytes coincided with the increase in MCV, thus suggesting that erythrocyte precursors become enucleated at a more differentiated stage of erythropoiesis. On the other hand, the increase in MCH observed during the experimental period could be indicative of a certain degree of intravascular hemolysis.

Indicators of anemia are reduced hemoglobin concentration (Hb), red blood cell count (RBC), WBC and PCV. Animals are similar to humans in that reduction in Hb, RBC and PCV are indicative of anemia. Mean Corpuscular Hemoglobin Concentration (MCHC—the amount of hemoglobin per unit erythrocyte volume) often reduces in hemolytic anemia or increased in the case of massive intravascular hemolysis. Mean Corpuscular Volume (MCV—average volume of the erythrocyte) is often increased in hemolytic anemia as the result of reticulocytosis. Mean corpuscular hemoglobin (MCH—the average amount of hemoglobin per cell) often increases in hemolytic anemia. In the present study, phenyl hydrazine intoxicated rats decrease hemoglobin levels, RBC, WBC, platelet count and PCV whereas; it induces an increase in MCV, MCH, and MCHC. Our results with earlier reports suggested that reduced the oxidative damage. Supplementations of seeds of *Trigonella foenum-graecum* to phenyl hydrazine intoxicated rats restored the altered hematological parameters.

Lipid peroxidation and the resultant perturbation of the structural integrity of the plasma membrane have long been considered to be capable of initiating the hemolytic response, though how the generalized destruction of membrane lipids could stimulate a selective macrophage response was not clear. The recent reports that lipid peroxidation in nucleated cells correlates with the accumulation of Phosphatidylserine (PS) on the outer leaflet of the lipid bilayer. ROS production was associated with extensive binding of oxidized and denatured hemoglobin to the membrane cytoskeleton. Thus, phenyl hydrazine induced hemolytic injury seems to be derived from oxidative alterations to red blood cell membrane lipids. In the present study, increased lipid peroxidation products, as MDA were observed on phenyl hydrazine, intoxicated rats. Supplementations of seeds of *Trigonella foenum-graecum* restored the MDA content suggested that reduced the oxidative damage.

In the present study, a marked decrease in the concentration of GSH was observed in phenyl hydrazine intoxicated rats when compared to control rats. Administration of the seeds of *Trigonella foenum-graecum* significantly increases the levels of GSH in phenyl hydrazine intoxicated rats. Enzymes catalyze specific biochemical reactions in the body. Changes in their levels and of cellular damage, the intracellular concentration of the enzymes and the mass properties alter the functional ability of an organism. The diagnosis of organ disease/damage is aided by measurement of a number of non-functional plasma enzymes characteristic of that tissue or organ. The amount of enzyme released depends on the degree of the affected tissue. The concentration of the enzymes released reflects the severity of the damage. SGOT and SGPT are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. When liver cells are damaged or die transaminases is released into the bloodstream, where they can be measured they are therefore the index of liver injury. The hepatocellular damage indicated by increased activity of SGOT in serum was observed in this study. Supplementation of the seeds of *Trigonella foenum-graecum* to phenyl hydrazine intoxicated rats restored the SGOT activity.

Herbal medicine is increasingly gaining greater recognition from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life. Several plant products are known to exhibit creditable medicinal properties for the treatment of various ailments and need to be explored to identify their potential application in prevention and therapy of human ailments. Keeping in view the present study has evaluated the anti-anemic activity of sprouted seeds of *Trigonella foenum-graecum* Phenyl hydrazine, an alkyl hydrazine was chosen to induce hemolytic anemia. Phenyl hydrazine induces the destruction of red blood cells by oxidation stress and many changes in cellular levels resulting in hemolytic anemia. Supplementation of the seeds of *Trigonella foenum-graecum* to phenyl hydrazine intoxicated rats shows the following results.

- Improved the Hb content.
- Restored the WBC, RBC and platelet count.
Secondary parameters of erythrocytes such as PCV, MCH, MCHC, and MCV were restored.

Reduced oxidative damage confirmed by the decreased MDA content.

Improved the detoxification mechanism by increased GSH content.

Normalized liver functions as evidenced by SGOT activity.

In developing countries, anemia is one of the major health problems and in India, Lauha bhasma, an iron-based herbo-metallic preparation, is prescribed for treating anemia.

CONCLUSION

The results of the present study accomplished that the seeds of *Trigonella foenum-graecum*. Inhibits anemia induced by phenyl hydrazine model similar to those induced by parasites such as *Plasmodium falciparum*. This result supports at least partially the traditional use of seeds of *Trigonella foenum-graecum* in the treatment of anemia. Further investigations are needed to understand the mechanism involved in the anti-anemic action of seeds of *Trigonella foenum-graecum*.

REFERENCES


