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Chemical Profiling of Fenugreek *(Trigonella foenum-graecum L.)* Seeds and Their Hematological Modulatory Effects in Adult Male Rabbits: A Dose-Response Study

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Abstract: This study investigated the phytochemical and nutritional composition of *Trigonella foenum-graecum* (fenugreek) seeds and evaluated their effects on hematological parameters in male rabbits. The phytochemical analysis revealed appreciable levels of bioactive compounds, including total saponins (6.25 ± 0.42%), diosgenin (1.47 ± 0.08%), total flavonoids (37.84 ± 2.16 mg QE/g), total polyphenols (76.92 ± 4.35 mg GAE/g), and trigonelline (0.31 ± 0.02%). In addition, the seeds exhibited a high crude protein content (27.85 ± 1.12%) with moderate levels of fiber, moisture, and ash. Hematological evaluation showed that fenugreek supplementation significantly improved red blood cell count, hemoglobin concentration, and packed cell volume in treated groups compared with the control group, with dose-dependent variations. Mean corpuscular indices (MCV, MCH, and MCHC) remained relatively stable across all groups. Overall, fenugreek seed supplementation demonstrated a positive influence on hematological status in male rabbits, indicating its potential nutritional and physiological benefits.

Keywords: Fenugreek, *Trigonella foenum-graecum*, phytochemicals, hematology, rabbits.

Introduction

Fenugreek (*Trigonella foenum-graecum*) is an important medicinal plant widely used in traditional medicine and as a functional food ingredient due to its rich composition of bioactive compounds. The seeds are particularly known for their high content of phytochemicals such as saponins, flavonoids, polyphenols, and alkaloids, which contribute to their diverse pharmacological properties. In addition, fenugreek seeds contain valuable nutritional components, including proteins, dietary fiber, minerals, and essential metabolites that support various physiological functions [1]. Several studies have highlighted the potential biological activities of fenugreek, including antioxidant, anti-inflammatory, hypoglycemic, and hematopoietic effects. These activities are mainly attributed to compounds such as diosgenin and trigonelline, which are believed to play a role in modulating metabolic and hematological processes [2-10]. Due to these properties, fenugreek has gained increasing attention as a natural dietary supplement with potential health-promoting effects. Hematological parameters are important indicators of physiological and health status, reflecting oxygen transport capacity and overall blood health [11-20]. Alterations in red blood cell indices, hemoglobin concentration, and packed cell volume can provide valuable insights into the effects of dietary supplementation on systemic function[21-25]. Therefore, this study aimed to evaluate the phytochemical and nutritional composition of fenugreek seeds and to investigate their effects on selected hematological parameters in male rabbits, in order to better understand their potential biological and nutritional significance.

Materials and methods

Fenugreek (*Trigonella foenum-graecum*) seeds were obtained from a certified local supplier. The seeds were cleaned to remove impurities, air-dried under room temperature, and then finely ground into a uniform powder using an electric grinder. The powdered samples were stored in airtight containers at 4°C until further analysis Standard analytical procedures were used to determine the phytochemical and nutritional composition of fenugreek seeds. Total saponins, flavonoids, polyphenols, diosgenin, and trigonelline were quantified using spectrophotometric and chromatographic methods. Nutritional parameters, including crude protein, crude fiber, moisture, and ash content, were assessed according to established proximate analysis techniques. Adult male rabbits were used in this study and maintained under controlled laboratory conditions with standard temperature, humidity, and light cycles. The animals were randomly divided into three groups: a control group and two treatment groups receiving fenugreek supplementation at low (500 mg/kg) and high (1000 mg/kg) doses. At the end of the experimental period, blood samples were collected from all animals under aseptic conditions. Hematological parameters, including red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), were determined using an automated hematology analyzer. Data were expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using appropriate statistical tests, and differences were considered significant at $p < 0.05$.

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Results

The phytochemical and nutritional profile of *Trigonella foenum-graecum* seeds showed that total saponins were 6.25 ± 0.42%, diosgenin 1.47 ± 0.08%, total flavonoids 37.84 ± 2.16 mg QE/g, total polyphenols 76.92 ± 4.35 mg GAE/g, trigonelline 0.31 ± 0.02%, crude protein 27.85 ± 1.12%, crude fiber 9.14 ± 0.56%, moisture 8.72 ± 0.41%, and ash 3.48 ± 0.19%. In hematological parameters of male rabbits, RBC values increased from 5.5 ± 0.3 $\times 10^6/\mu L$ in the control group to 6.2 ± 0.4 and 6.5 ± 0.3 in the low- and high-dose groups, respectively. Hemoglobin levels were 11.5 ± 1.0 g/dL in the control, rising to 12.8 ± 1.2 and 13.2 ± 1.1 g/dL in the treated groups. PCV values increased from 35.0 ± 2.5% to 39.5 ± 3.0% and 41.0 ± 2.8%. MCV remained relatively stable at 63.0 ± 3.0, 64.0 ± 2.5, and 63.5 ± 2.8 fL across groups. MCH values were 21.0 ± 1.5, 21.5 ± 1.2, and 20.8 ± 1.4 pg, while MCHC values were 33.0 ± 1.5, 33.5 ± 1.2, and 32.8 ± 1.3 g/dL for control, low-dose, and high-dose groups, respectively, with several parameters showing statistical significance across treated groups compared with control.

Table 1. Phytochemical and Nutritional Composition of Fenugreek (Trigonella foenum-graecum) Seeds

Constituent	Value (Mean ± SD)
Total Saponins (%)	6.25 ± 0.42
Diosgenin (%)	1.47 ± 0.08
Total Flavonoids (mg QE/g)	37.84 ± 2.16
Total Polyphenols (mg GAE/g)	76.92 ± 4.35
Trigonelline (%)	0.31 ± 0.02
Crude Protein (%)	27.85 ± 1.12
Crude Fiber (%)	9.14 ± 0.56
Moisture (%)	8.72 ± 0.41
Ash (%)	3.48 ± 0.19

Values are expressed as Mean ± SD. The reported values fall within the normal phytochemical and nutritional ranges documented for fenugreek seeds in the scientific literature.

Table 2. Effect of Fenugreek Supplementation on Hematological Parameters in Male Rabbit

Parameter	Control Group	Low-Dose (500 mg/kg)	High-Dose (1000 mg/kg)
RBC ($\times 10^6/\mu L$)	5.5 ± 0.3	6.2 ± 0.4*	6.5 ± 0.3**
Hb (g/dL)	11.5 ± 1.0	12.8 ± 1.2**	13.2 ± 1.1***
PCV (%)	35.0 ± 2.5	39.5 ± 3.0**	41.0 ± 2.8***
MCV (fL)	63.0 ± 3.0	64.0 ± 2.5	63.5 ± 2.8
MCH (pg)	21.0 ± 1.5	21.5 ± 1.2	20.8 ± 1.4
MCHC (g/dL)	33.0 ± 1.5	33.5 ± 1.2	32.8 ± 1.3

*Values are expressed as Mean ± SD. Significant differences were determined compared with the control group (*p < 0.05, **p < 0.01, ***p < 0.001).*

Discussion

The present study demonstrated that fenugreek (*Trigonella foenum-graecum*) seeds possess a rich spectrum of bioactive phytochemicals, including saponins, flavonoids, polyphenols, diosgenin, and trigonelline, in addition to a notable nutritional composition characterized by high protein content and moderate dietary fiber [26-30]. These constituents collectively reflect the functional and nutritional value of fenugreek as a plant with significant biological relevance [31-39]. The hematological findings indicated that fenugreek supplementation exerted a positive influence on blood parameters in male rabbits. The treated groups showed an overall improvement in red blood cell-related indices, including hemoglobin concentration and packed cell volume, suggesting an enhancement in hematopoietic activity and oxygen-carrying capacity [40-45]. This response appeared more evident with higher levels of supplementation, indicating a dose-related biological effect. In contrast, the red blood cell indices remained largely unchanged across experimental groups, suggesting that fenugreek primarily influenced the quantity of circulating erythrocytes rather than their morphological characteristics or hemoglobin content per cell [46-50]. This stability reflects a balanced physiological response without evidence of hematological disruption. The observed effects may be attributed to the bioactive compounds present in fenugreek seeds, which are known to support metabolic and physiological processes associated with blood formation and systemic antioxidant balance. These properties collectively contribute to the overall improvement in hematological status observed in the treated animals [51-62].

In conclusion, fenugreek supplementation demonstrated a beneficial role in enhancing hematological health, supporting its potential application as a natural functional feed additive with physiological and nutritional advantages.

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