



EFFECTS OF FENUGREEK (TRIGONELLA FOENUM- GRAECUM) SEEDS EXTRACT ON THE TESTOSTERONE AND HEMOGLOBIN LEVELS IN RABBITS

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ABSTRACT

Background: Fenugreek (*Trigonella foenum-graecum*) is known as a medicinal plant; it is used for multiple therapeutic purposes since ancient times which include hormonal and hematologic modulation. Whereas, earlier studies have explored fenugreek's effect on the testosterone or hemoglobin concentrations alone, there is a lack of data about its pharmacological and physiological actions as dual actions.

Objective: To determine the effects of the orally administered fenugreek seed extract on the serum testosterone and hemoglobin levels on adult male rabbits.

Methods: A total of 30 male adult rabbits were randomized into three study groups, each group comprised of 10 rabbits. Group A was labeled as control, while Groups B and Group C received fenugreek seed extract at the doses of 500 mg/kg and 1000 mg/kg body weight respectively for a period of 45 days. Blood samples were taken periodically at the baseline, Day 23, and Day 45 to evaluate serum testosterone and hemoglobin levels. All data was analyzed by means of SPSS version 25.0, with an applied significance level of $p < 0.05$ at Day 45.

Results: Group B with a dose of 500 mg/kg of fenugreek's seed extract, was found to have a significant increase in the serum levels of testosterone from 27.77 ± 3.27 to 29.77 ± 3.67 ng/dL ($p < 0.01$) and also hemoglobin concentrations raised from baseline 12.06 ± 1.31 to 12.81 ± 1.23 g/dL ($p < 0.01$) by Day 45. Group C received a dose of 1000 mg/kg of fenugreek's seed extract, which

exhibited an even greater increase in the serum testosterone levels from 26.86 ± 3.67 to 29.11 ± 3.83 ng/dL, $p < 0.001$) and also hemoglobin raised from baseline 11.99 ± 0.97 to 12.59 ± 1.00 g/dL, $p < 0.001$ at Day 45. In the control group, statistically no significant changes were noted. While, mild lethargy was observed in only one animal belonging to Group B; otherwise, no any side effects or mortality were observed.

Conclusion: Fenugreek seed extract increases serum testosterone as well as hemoglobin levels in the adult male rabbits in a dose dependent fashion. The dual biological effects and its safety profile observed, suggest its potential use as a natural therapeutic agent in the clinical management of hypogonadism and iron-deficiency anemia.

Keywords: *Trigonella foenum-graecum*, Fenugreek, Testosterone, Hemoglobin, Rabbit, Phytoandrogens, Herbal Supplement, Erythropoiesis, Dose-Response Effects, Androgen Deficiency.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum*) is a widely studied medicinal herb/plant traditionally used across the Asia, Middle East, and North Africa for its diverse therapeutic properties [1]. In the recent years, role of fenugreek in enhancing serum testosterone levels and improving hemoglobin concentration has gained growing interest in experimental and clinical research.

Fenugreek (*Trigonella foenum-graecum*) as a leguminous plant with traditional herbal medical and dietary supplement usage has a variety of bioactive compounds, including: saponins (including diosgenin), alkaloid, flavonoid, and amino acids. These phytochemicals are behind several therapeutic effects such as androgenic effects, hypoglycemic, and antioxidant. A number of preclinical studies have demonstrated the possible effect of fenugreek seed extracts as positive in influencing the actions of testosterone and this may be acting through the inhibition of 5- α reductase and aromatase enzymes and thereby improving the activity of endogenous testosterone. In rodents, fenugreek supplementation resulted in increased sexual activity, spermatogenesis as well as androgen levels. [2,3]

The study conducted by Sharma and Sharma (2023) have evaluated the biochemical features of fenugreek (*Trigonella foenum-graecum*) based on the possible effect on the hematopoietic and endocrine system [2]. It has been reported that the steroidal saponins are found in the fenugreek seeds, i.e., diosgenin and protodioscin, both have been reported to affect the steroidogenic enzyme expression in Leydig cells. [3] Moreover, the fenugreek seeds are good source of iron-chelating compounds, and have bioactive polysaccharides, that could have a stronger erythropoiesis. [4]

Testosterone is a highly essential androgenic hormone that regulates a series of physiological mechanisms like the formation of proteins of muscle, bones density, and also the process of spermatogenesis [5].

The effects of fenugreek in male reproductive health are well demonstrated in rats and mice, but there is a few research being carried-out on it on the rabbits. As rabbits exhibit similar physiological and hematological characteristics with human subjects which make a good example of translational biomedical research. A result of a study conducted by Abd SKA et al., (2025) suggested that the oral administration of aqueous fenugreek seed extract with a dose of 650 mg/kg and 1,300 mg/kg showed significant changes in the testosterone level and semen characteristics in the male rabbits which could be an evidence of its androgenic capacity [6].

The results of a recent in-vitro research indicates that saponins of fenugreek stimulate expression of cytochrome P450scc which is also known as CYP11A1 enzyme, that is a major pathway in the testosterone biosynthesis [7]. Despite of these promising findings, a research gap remains certainly there concerning the dual effect of fenugreek (*Trigonella foenum-graecum*) on both testosterone and hemoglobin levels in a controlled rabbit model.

To our knowledge, no prior study has systematically been evaluated locally on these two parameters together. Therefore, the present study was planned to investigate the effect of fenugreek seeds extract on the serum testosterone and hemoglobin levels in adult male rabbits, findings from this current study

may provide further insight into the plant's potential as a natural modulator of testosterone hormone as well as on the hematological parameter.

MATERIAL AND METHODS

The current experimental animal study was carried out at the Department of Pharmacology, Peoples University of Medical & Health Sciences, Nawabshah. Ethical Review Committee of Peoples University of Medical & Health Sciences, Nawabshah reviewed the study protocol and gave formal permission to carry out this experimental research study. An approval was also obtained from the administration of the Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences, Gambat, for the use of the animal house facility at Gambat Medical College, District, Khairpur Mirs, Sindh, Pakistan.

Thirty adult male rabbits of age 16 to 24 months and weight 1.5 to 3.5 kilograms were selected in this study. The duration of study was six months. The animal selection was done through simple random sampling method. Female rabbits and age of rabbits either less than 16 months or more than 24 months and those who were underweight or overweight were not included in the investigation.

The selected animals were kept in the animal house in a neat and clean, well-ventilated environment which was maintained at day and night with a room temperature of 20°C-30°C throughout the duration of the study. Rabbits were kept in specially manufactured steel cages which were partitioned in blocks. The size of each cage was 48 inches in length while height and width were 24 inches having self-cleaning floor to keep it hygienic and also it had a tray under the mesh. Rabbits were isolated from the other animals of the animal house. Five rabbits were kept into each cage that was adequate for living of five rabbits. All experimental animals were provided with fresh water and hay.

The seeds of fenugreek (*Trigonella foenum-graecum*) were purchased from the local market which were checked and verified by a qualified and experienced pharmacognostist of the university. The seeds were properly washed and dried up in direct sunlight for a week and grinded into coarse powder using the electric grinder. Weighing of the powder was performed with the electronic digital scale weight balance model SF-400. The powder was weighed and packed into small cellophane plastic air tight bags (according to selected dose) that was labelled accordingly and was made ready to be used. The viability of the seed extract powder was taken into consideration for only 30 days. The process of preparation of powder samples was repeated on the 23rd and 45th day respectively. The experimental procedures were carried-out using all the precautionary and aseptic techniques. The blood samples were drawn from marginal ear vein of the rabbits according to the scheduled days i.e., at the baseline and on continuous days, i.e., the 23rd day and 45th day respectively after administering the calculated dose of fenugreek (*Trigonella foenum graecum*) seeds powder given daily early in the morning.

Thirty rabbits were divided into three groups, consisting of 10 rabbits in each group. Each of the animals was numbered accordingly with their study group. Group-A was referred to as the control group where they were fed only with hay and fresh water, while group-B and group-C animals received different dosages of fenugreek (*Trigonella foenum graecum*) seeds powder which was added to hay and fresh water and normal physiological atmosphere of living was maintained throughout the study period. Group-B was administered fenugreek seeds extract powder at the dose of 500 mg/kg bodyweight and group-C was administered 1000 mg/kg bodyweight. The calculated dose was mixed in 5 ml of milk and administered through oral route using butterfly needle-free feeding syringe. The collection test tubes were labelled properly and the blood samples were stored in the test tubes which containing prefilled Ethylenediaminetetra acetic acid (EDTA) as an anticoagulant, till they were sent to diagnostic laboratory of Gambat Medical College for assessing serum testosterone and hemoglobin levels.

The data on age, body weight, serum testosterone, hemoglobin levels and the side effects were recorded on structured study proforma. Each experimental group receiving a defined dose of fenugreek (*Trigonella foenum-graecum*) seed powder was assigned a specific identification code.

The serum concentration of testosterone and hemoglobin was measured using periodically taken blood samples thrice i.e., at day-0, day-23 and day-45.

Statistical analysis of the data was done by using SPSS version 25.0, and the confidence interval was set at 95%, and the significance level was taken to be $p < 0.05$. The comparison between all the groups was done in terms of the change in the levels of serum testosterone and hemoglobin among other parameters of the study.

RESULTS

A total of 30 rabbits were included in this study, which were equally divided into three groups. Group A (Control), Group B treated with (500 mg/kg fenugreek seed extract), and Group C treated with (1000 mg/kg fenugreek seed extract). All the animals remained alive throughout the experimental period of study, and no mortality was seen amongst selected animals. Among the recorded adverse effects, only one subject in Group B manifested lethargy, while all other animals in the three study groups showed no any signs of distress or clinical abnormalities (Table-1).

The baseline demographic data of the animals showed comparable age and weight distributions within the groups (Table). The mean age in the control group was 18.10 ± 1.60 months, while Group B and C had mean ages of 17.20 ± 1.58 months, respectively. The average body weights at baseline were 2.10 ± 0.15 kg (Group A), 2.05 ± 0.29 kg (Group B), and 2.10 ± 0.23 kg (Group C), which showed that no any statistically significant differences between the groups at the beginning of the experiments. Serum testosterone and hemoglobin levels were measured at the baseline, Day 23, and Day 45 to assess the physiological effect of fenugreek seed extract (Table 3). Both the parameters in the control group, have not shown any significant changes during the study over time ($p > 0.05$) for both variables. Conversely, animals treated with 500 mg/kg fenugreek extract (Group B) had a profound rise in serum testosterone levels from 27.77 ± 3.27 ng/dL at baseline to 29.77 ± 3.67 ng/dL ($p < 0.01$) at Day 45. Whereas, a concurrent rise in the levels of hemoglobin was also noted, increasing from baseline 12.06 ± 1.31 g/dL to 12.81 ± 1.23 g/dL ($p < 0.01$) at Day 45.

The strongest effects were seen in the testosterone levels of Group C animals, who had received the higher dose of fenugreek seed extract (1000 mg/kg) which rose significantly from a baseline of 26.86 ± 3.67 ng/dL to 29.11 ± 3.83 ng/dL on the Day 45 ($p < 0.001$). The levels of hemoglobin also improved significantly, which increased from 11.99 ± 0.97 g/dL at baseline to 12.59 ± 1.00 g/dL ($p < 0.001$) at Day 45.

These findings of our study suggest a dose-related enhancement of both testosterone and hemoglobin levels following the oral administrations of fenugreek seed extract over a period of Day 45.

Table 1: Demographic Characteristics and Adverse Effects of Study Subjects

Sr. No.	Sample ID	Experimental Group	Age (months)	Body Weight (kg)	Adverse Effect Noted
1	A-1	Group A (Control)	16	1.9	No
2	A-2	Group A (Control)	18	2.1	No
3	A-3	Group A (Control)	20	2.0	No
4	A-4	Group A (Control)	17	2.2	No
5	A-5	Group A (Control)	16	1.9	No
6	A-6	Group A (Control)	18	2.2	No
7	A-7	Group A (Control)	18	2.1	No
8	A-8	Group A (Control)	19	2.1	No
9	A-9	Group A (Control)	18	2.4	No
10	A-10	Group A (Control)	21	2.1	No
11	B-1	Group B (500 mg/kg)	16	2.0	No
12	B-2	Group B (500 mg/kg)	17	2.1	No

13	B-3	Group B (500 mg/kg)	16	1.9	No
14	B-4	Group B (500 mg/kg)	19	2.4	No
15	B-5	Group B (500 mg/kg)	20	2.7	Lethargic
16	B-6	Group B (500 mg/kg)	16	1.8	No
17	B-7	Group B (500 mg/kg)	17	2.0	No
18	B-8	Group B (500 mg/kg)	16	1.9	No
19	B-9	Group B (500 mg/kg)	18	1.8	No
20	B-10	Group B (500 mg/kg)	17	1.9	No
21	C-1	Group C (1000 mg/kg)	16	1.9	No
22	C-2	Group C (1000 mg/kg)	17	1.9	No
23	C-3	Group C (1000 mg/kg)	20	2.5	No
24	C-4	Group C (1000 mg/kg)	16	2.0	No
25	C-5	Group C (1000 mg/kg)	16	2.1	No
26	C-6	Group C (1000 mg/kg)	17	1.9	No
27	C-7	Group C (1000 mg/kg)	19	2.3	No
28	C-8	Group C (1000 mg/kg)	19	2.4	No
29	C-9	Group C (1000 mg/kg)	16	1.9	No
30	C-10	Group C (1000 mg/kg)	19	2.1	No

Note: Group A = Control; Group B = Received Fenugreek seed extract (500 mg/kg); Group C = Received Fenugreek seed extract (1000 mg/kg). Adverse effects were monitored throughout the study period.

Table 2: Baseline Characteristics of Study Subjects (Age and Weight)
(n = 10 per group)

Group	Age (months) Mean \pm SD	Age Range (min–max)	Weight (kg) Mean \pm SD	Weight Range (min–max)
Group A (Control)	18.10 \pm 1.60	16-21	2.10 \pm 0.15	1.9 – 2.4
Group B (500 mg/kg)	17.20 \pm 1.40	16-20	2.05 \pm 0.29	1.8 – 2.7
Group C (1000 mg/kg)	17.50 \pm 1.58	16-20	2.10 \pm 0.23	1.9 – 2.5

Values are shown as Mean \pm SD

Table 3: Effects of Fenugreek Seeds Extract on Serum Testosterone and Hemoglobin Levels in Rabbits Over 45 Days

Study Group and Interventions	Time Point	Testosterone (ng/dl) Mean \pm SD	Hemoglobin (g/dl) Mean \pm SD
Group A Control (No Treatment)	Baseline	27.80 \pm 3.07	11.70 \pm 1.09
	Day 23	27.73 \pm 3.06	11.72 \pm 1.08
	Day 45	27.52 \pm 3.25	11.94 \pm 0.95
		(p >0.05)	(p >0.05)
Group B Fenugreek (500 mg/kg)	Baseline	27.77 \pm 3.27	12.06 \pm 1.31
	Day 23	28.65 \pm 3.41	12.26 \pm 0.99
	Day 45	29.77 \pm 3.67	12.81 \pm 1.23
		(p <0.01)	(p <0.01)
Group C Fenugreek (1000 mg/kg)	Baseline	26.86 \pm 3.67	11.99 \pm 0.97
	Day 23	28.01 \pm 3.51	12.26 \pm 1.00
	Day 45	29.11 \pm 3.83	12.59 \pm 1.00
		(p <0.001)	(p <0.001)

P-values indicate comparisons between baseline and Day 45 levels (paired sample t-test).

Reference ranges: Testosterone \geq 0.26 ng/dl (normal), <0.25 ng/dl (low); Hemoglobin: 12-15 g/dl (normal).

DISCUSSION

In this experimental study, it was revealed that the oral administration of fenugreek (*Trigonella foenum-graecum*) seed extract in the adult male rabbits (*Oryctolagus cuniculus*) caused a significant rise in serum testosterone and hemoglobin concentrations in a dose-dependent manner. Such data provides a significant evidence of the dual biological effect of fenugreek on the androgen and hematopoiesis, besides its safety profile as we did not observe severe effect with the exception of a single subject in which lethargy was observed with a dose of 500 mg/kg of fenugreek group.

The noted increase in testosterone level is in line with the earlier studies, which included Roberts et al. (2022), who reported that saponins as activators of CYP11A1, an important enzyme in the production of steroid hormones [8]. Similar results were noted in another study which was conducted on the rodent by Mokhtari et al. (2008) and El-Hak et al. (2022) and also in a recently conducted study on rabbits by Abd et al. (2025) [11-13]. Nevertheless, co-analysis of hematological parameters, especially hemoglobin concentration is not well reflected in the literature. The current findings support the previous studies conducted by Mehta and Devaraj (2021) that fenugreek do possess iron-chelating constituents which cause erythropoietic effect. [9]

Our findings are congruent with other studies as Wankhede et al. (2016) had reported an increased hemoglobin concentration that is indicative of its impact on the iron metabolism and erythropoiesis. [10] Our study findings are indicating a distinct dose-response effect that has not been expressed to a greater extent in the previously conducted studies.

The strengths of our study include a well experimental design, stratified dosing, a standardized environment, and sequential biochemical surveillance. Notably, the rabbit model, has significant physiologic similarity with human beings as far as hematopoietic and endocrine indicators are concerned. These increase the translational validity of the findings of our research study.

However, the constraints need to be discussed as this experiment lasted only for 45 days and no analysis of long-term hormonal and hematological patterns was carried-out. Moreover, molecular level mechanistic comprehension was not studied.

In the context of previously published research studies, the findings of our study extended beyond the range of recent studies conducted by Sharma and Sharma (2023) and Prakash and Majeed (2022), which were only limited to access either reproductive or biochemical outcomes. [16,17] To the contrary, the present study exhibits a comprehensive physiological influence of the fenugreek compound, encompassing both the endocrine regulation as well as hematopoietic modulation.

Our results indicate that fenugreek could have some potential use as a natural supplement in the clinical management of testosterone deficiency as well as in the borderline anemia particularly where there exists a need to avail cost-effective treatment. To the policymakers, this study serves as an eye-opener to the importance of exploring tested indigenous conventional cures in the form of fenugreek seed extract in well-established experimental models. On the contrary, many studies could not establish a significant difference of testosterone concentration with fenugreek supplementation [18,19]. This inconsistency could be due to variation in the study design, species (human vs. animal models) or the content of the extract of the fenugreek used. The availability of a controlled animal model enabled us to accurately administer a dose and limit confounders that could potentially provide some explanation as to why more meaningful results were observed and also the fact that no mortality was recorded which makes this herb is to be considered as safe at the doses used.

Further studies are certainly needed to look at the long-term safety, efficacy in both the sexes (male and female rabbits), as well as to investigate the molecular mechanism(s) like as erythropoietin production and androgen receptor signaling activity. [11] To ensure its clinical applicability, randomized control trials on the human population are recommended.

Conclusion

The fenugreek seed extract is an effective supplementation that enhances the synthesis of testosterone and hemoglobin in a rabbit model, and its benefit can be utilized in hormonal and hematologic health. Our study results suggest that fenugreek seed extract can act as a natural phyto- supplement to the conditions of hypogonadism and iron-deficiency anemia and provide clinicians with an additional alternative, especially in an era of people who tend to go plant-based food.

Conflict of Interest

The authors comply with the statement of the lack of competing interests concerning the publication of this study. All the authors took part in the study without the influence of an institution in its conceptualization, design, conducting of the study, data analysis and writing up. Despite of the authors affiliated with different institutions, which did not impact on the objectivity, transparency and integrity of the research. Moreover, there was no funding received, in the course of carrying out this research; the authors covered all the expenses incurred on the research work.

Ethical Approval

This study was reviewed by People University of Medical & Health Sciences, Nawabshah Committee of Research Ethics. All the animal procedures have been conducted in line with the institutional policies and in the framework of the use of laboratories animals based on the national ethics guidelines

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