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

Nargis Pirya[1], Ghulam Mustafa Dahri[2], Muhammad Azhar Mughal[3], Farzana Memon[4], Zaheera Yousif[5], Ayesha Asad Memon[5], Muhammad Zain ul Abdeen[7]

ABSTRACT

BACKGROUND: Fenugreek (*Trigonella foenum-graecum L.*) seeds have been known for its therapeutic properties, particularly its influence on male reproductive hormones. Despite extensive data from rodent and human models, limited studies have investigated its androgenic potential in lagomorphs, particularly rabbits, which share notable physiological homology with humans in steroidogenesis. **OBJECTIVE:** To evaluate the dose-dependent effects of fenugreek seeds extract on serum testosterone levels in healthy adult male rabbits under controlled experimental conditions. **METHODS:** A randomized controlled animal study was conducted using thirty adult male rabbits (aged 16-24 months), assigned into three groups, in each group (n=10). Group-A labeled as control, while Group- B and Group-C have received fenugreek seed extract at 500 mg/kg and 1000 mg/kg bodyweight, respectively, orally for 45 days. Blood samples were collected at Day 0, Day 23, and Day 45, and serum testosterone levels were analyzed using standard biochemical assays. Statistical comparisons were made by using SPSS version 25.0, ANOVA test, where $p<0.05$ was considered as significant. **RESULTS:** Testosterone levels remained stable in the control group (Group-A) across all time points ($p>0.05$). Group-B exhibited a statistically significant increase from 27.77 ± 3.27 ng/dl at baseline to
29.77 ± 3.67 ng/dl at Day 45 ($p=0.01$), while Group-C demonstrated a comparable elevation from 26.86 ± 3.67 ng/dl to 29.11 ± 3.83 ng/dl ($p=0.001$). The dose-dependent response was evident, with both treated groups showing sustained testosterone elevation. No major adverse events were noted except for mild lethargy in one subject from Group-B. **CONCLUSION:** Fenugreek seed powder extracts significantly enhanced serum testosterone levels in rabbits in a dose-dependent manner without adverse effects, highlighting its potential as a natural androgenic agent. These findings support its translational relevance and warrant further exploration in human clinical trials.

KEYWORDS: Fenugreek, *Trigonella foenum-graecum*, Testosterone, Rabbits, Androgenic activity,

1. Department of Pharmacology, Gambat Medical College, Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences, PAQSJIMS, Gambat, Sindh.
2. Department of Pharmacology, Peoples University of Medical & Health Sciences for Women, Shaheed Benazirabad, (Nawabshah), Sindh.
3. Department of Pharmacology, Jinnah Sindh Medical University, Karachi, Sindh.
4. Department of Pharmacology, Peoples University of Medical & Health Sciences for Women, Shaheed Benazirabad, (Nawabshah), Sindh.
5. Department of Pharmacology, Peoples University of Medical & Health Sciences for Women, Shaheed Benazirabad, (Nawabshah), Sindh.
6. Department of Pharmacology, Peoples University of Medical & Health Sciences for Women, Shaheed Benazirabad, (Nawabshah), Sindh.
7. Liaquat University of Medical & Health Sciences, Jamshoro.

Corresponding author: dr. Nargis pirya, Assistant Professor, Department of Pharmacology Gambat Medical College, Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences PAQSJIMS), Gambat, Sindh-Pakistan. Email: nargispriya0@gmail.com

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INTRODUCTION

Fenugreek (Trigonella foenum graecum) has been recognized for its medicinal and nutritional properties since very long in the South Asian and Middle-Eastern traditional systems. Recent research studies have focused on its influence on the endocrine system, especially its effects on the testosterone regulation and reproductive physiology. It is believed that fenugreek seeds are known to have rich in steroidal saponins, such as protodioscin, which modulates the androgenic activity (Bagchi et al; 2017)[1] Multiple research studies have concluded that fenugreek extract has the potential to increase testosterone levels in animal and human models. In the human clinical studies, it was reported that standardized fenugreek extract has raised serum testosterone and also beneficially improved sperm parameters in the healthy male subjects (Steels et al, 2011)[2] and Wankhede et al., 2016)[3]. Whereas, animal studies in particular (Rabbit models) showed different findings (Haba MK et al, 2012)[4]. Testosterone is a crucial androgen hormone that controls important physiological processes in the male mammals, including bone mineral density, muscle mass, libido and spermatogenesis (Nassar & Leslie, 2023)[5]. The modulation of testosterone created significant interest for managing conditions like hypogonadism and infertility. Fenugreek (Trigonella foenum graecum L. seeds) have a long history in the ethanopharmacological reputation as galactagouge and a potential aphrodisiac (Wani and Kumar, 2018)[6]. The contemporary research conducted on the fenugreek has increasingly focuses on the bioactive constituents, especially steroidal saponins (e.g., diosgenin derivatives and alkaloids (e.g., trigonelline), suggested to have influence on endocrine mechanisms which are relevant to androgen synthesis and its bioavailability (Sharma et al., 2021)[7].

Testosterone deficiency remains a meaningful clinical concern, with its implications for reproductive health, musculoskeletal integrity and metabolic function (Salonia et al, 2023)[8]. Fenugreek seeds have drawn attention as phytoandrogen due to their historical use in Ayurvedic and Unani medicine for enhancing the male vitality (Gupta & Sharma, 2024)[9]. Whereas, in vitro studies suggested that fenugreek extracts cause upregulation of StAR protein expression and 17β-hydroxysteroid dehydrogenase activity in Leydig cells, potentiating testosterone synthesis (Singh et al., 2023)[10]. The results of studies conducted on rodent reported that 20-35% increases in the serum testosterone levels following the standardized fenugreek seed extract administration (Rathore et al., 2022)[11]; (Mandal et al., 2024)[12]. However, a translational limitation prevails due to metabolic differences between rodents and higher mammals. Rabbits (Oryctolagus cuniculus) are known to be a physiologically relevant animal model, sharing more than 90% homology in steroidogenic enzymes with humans and have similar pharmacokinetics mechanisms (El-Sabrout et al., 2023)[13], despite of this fact, very few controlled studies have evaluated dose-dependent effects of fenugreek seed extract on the endocrinology of lagomorphs. Concerning to the potential hepatotoxicity reported from chronic fenugreek alkaloids e.g.,

trigonelline have also necessitated safety validation in the animal models (Patel et al., 2024)[14].

Unbelievingly, specific dose-response effects and safety profiles in controlled mammalian models, particularly lagomorphs, are notably sparse. Rabbits (Oryctolagus cuniculus) serve as valuable translational models for reproductive endocrinology because of their similarities in the regulation by hypothalamic-pituitary-gonadal axis and metabolic responses (Alagawany et al., 2022)[15]. However, rigorous, controlled investigations on fenugreek's direct physiological impact on the testosterone homeostasis are lacking.

Therefore, we have designed this study to see the effects of orally administered dose and duration dependent effects of fenugreek (*Trigonella foenum-gracium* L.) seeds extract on the testosterone levels and this study also aims to clarify fenugreek's tolerability and safety profile in rabbits under the controlled conditions, contributing to the understanding of its androgenic effects in translational research.

MATERIAL AND METHODS

This experimental animal study was conducted on rabbits at the Department of Pharmacology, Peoples University of Medical & Health Sciences, Nawabshah. Thirty (30) adult male rabbits having age ranging from 16 to 24 months, with weight 1.5 to 3.5 kilograms were used. The total duration of the study was six months. Simple random sampling technique was used to select the animals, whereas, the female rabbits having age less than 16 months or more than 24 months or underweight or over weight did not include in the study.

The written permission was granted for conducting this experimental study from Ethical Review Committee of Peoples University of Medical & Health Sciences, Nawabshah. The animal house of Gambat Medical College at Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences, Gambat, district Khairpur Mirs, Sindh-Pakistan was used with the permission of this Institute. Animals were kept in a neat and clean, well-ventilated environment maintaining at day and night with a room temperature between 20°C-30°C during the whole study period at Rabbits were separated from other animals of the animal house. Five rabbits were housed in each cage, each cage was sufficient for living of rabbits. During the whole study, it the properly ventilated, fresh water and hay food was provided to all the experimental animals.

A total of 30 rabbits were included in this study which was divided in three groups, each group comprised of 10 rabbits. All the animals were labeled with proper numbering with their respective study group. **Group-A** was labeled as control group in which normal physiological atmosphere of living was provided and they were fed with Hay and fresh water only, whereas, **Group-B** and **Group-C** animals were given different doses of *Trigonella foenum graecum* seeds powder along with Hay and fresh water. Rabbits were hosed in specially manufactured steel cages that were divided in blocks. The size of each cage was 48 inches in length while height and width were 24 inches having self-cleaning floor to maintain hygiene and a tray under mesh as shown in **(Figure-1)**.

Figure-1: Housing of Rabbits in animal cages for experiment (Photography from the Animal House of PAQSJIMS, Gambat, Sindh)

The seeds of *Trigonella foenum-graecum* **(Figure-2)** were purchased from the certified supplier which was verified by the qualified and experienced pharmacognostist of university. The seeds were properly washed with water and dried in direct sunlight foe a week and grinded into coarsely powdered using electric grinder. The powder was weighed by using electronic digital scale weight balance model SF 400. The powder was packed and weight according to dose into small cellophane plastic air tight bags for use. The viability of powder was considered for 30 days only. The process of powder sample preparation was repeated on 23rd day and 45th day. The experimental procedures were performed by adopting all the precautionary and aseptic techniques. The blood samples were collected from marginal ear vein of rabbits on the scheduled days i.e., at baseline and subsequent samples were also drawn at day 23rd and 45th day respectively after the start of *Trigonella foenum-graecum* daily calculated dose which was administered early in the morning.

Figure-2: Fenugreek (*Trigonella foenum-graecum*) Seeds and Leaves

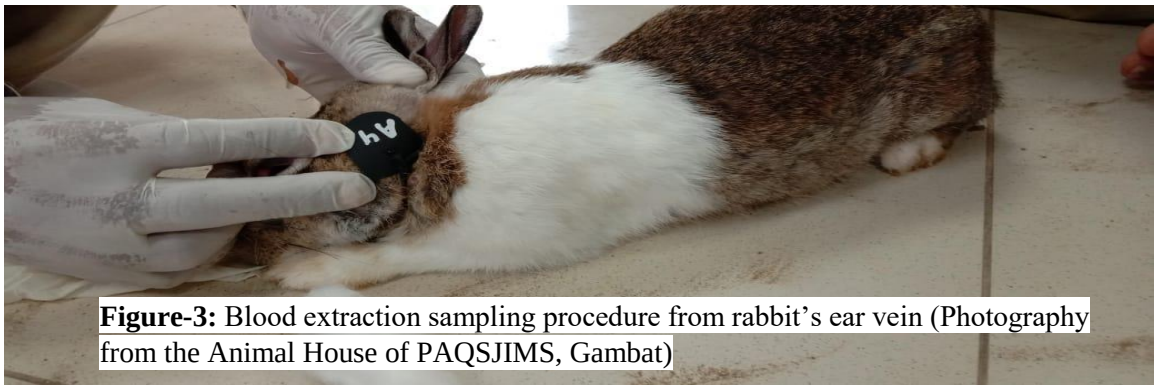

Figure-3: Blood extraction sampling procedure from rabbit's ear vein (Photography from the Animal House of PAQSJIMS, Gambat)

Trigonella foenum-graecum seeds powder 500 mg/kg bodyweight was given to group-B and 1000 mg/kg bodyweight was given to group-C, while group-A was considered as control group. The calculated dose was mixed in 5 ml of milk and was given by oral route with the help of butterfly needle-free feeding syringe. The blood samples were kept in the collection test tubes with pre-defined labeling which containing prefilled Ethylene diamine tetra acetic acid (EDTA) as an anticoagulant and the samples were sent to diagnostic laboratory of Gambat

Medical College for the test of serum Testosterone levels.

The laboratory tests were performed for evaluating serum testosterone levels within 2 hours of sample collection. The data was collected and recorded on the specially designed study Proforma in which baseline parameters regarding age, bodyweight and serum testosterone levels with particular dose of *Trigonella foenum-graecum* seeds powder received to the individual experimental animal who was assigned particular numbering. The periodic blood samples were drawn for the measurement of serum testosterone levels on three occasions i.e., at day-0, day-23 and day-45. The data was statistically analyzed using SPSS version 25.0, confidence interval was set at 95% and the significance level was considered at $p<0.05$. All the groups were compared with respect any change in serum testosterone levels and other study parameters.

RESULTS

This study was conducted on 30 healthy rabbits (*Oryctolagus Cuniculus*) that were randomized into three experimental equal groups (n=10 animals per group), with no dropouts occurred. Samples were systemically assigned (A-1 to A-10, B-1 to B-10, and C-1 to C-10. **Table-1** is showing demographics and adverse effects of individual subjects. **Table-2** is showing animal group baseline characteristics (age and weight) of study samples.

Group-A (Control, no intervention by fenugreek treatment)

Group-B (Received fenugreek seed extract at the dose of 500 mg/kg)

Group-C (Received fenugreek seed extract at the dose of 1000mg/kg)

Table-2 described the baseline characteristics of subjects which were found comparable across the groups. The age of the rabbits ranged from 16 to 21 months across all groups. The mean ages in months were comparable between the groups as follow:-

Group-A: 18.10 ± 1.60 (Range: 16-21)
Group-B: 17.20 ± 1.40 (Range: 16-20)
Group-C: 17.50 ± 1.58 (Range: 16-20)

The weights of subjects were measured in (kg) as follow:-

Group-A: 2.10 ± 0.15 (Ranging from 1.9 to 2.4)
Group-B: 2.05 ± 0.29 (Ranging from 1.8 to 2.7)
Group-C: 2.10± 0.23 (ranging from 1.9 to 2.5)

Out of all study subjects, the adverse event occurred in only one rabbit (Group-B, B-5) which was exhibited as lethargic, whereas, no any immediate adverse effects were observed in any study group A or C and all other subjects tolerated the intervention without any complication, following the blood samples extraction from 29 rabbits (96.6%) that were performed via the ear vein, ensuring the minimal invasiveness **(Figure-3)**. Overall, the baseline demographic data (weight and age) indicated generally comparable groups at the study outset, as the blood sampling was done uniformly with minimal adverse events recorded in the study groups.

Table-3 is showing longitudinal testosterone dynamics across the study groups. We have noted minimal fluctuations in the longitudinal serum testosterone levels in the control Group-A, while measuring testosterone levels at Day-0 (baseline), Day-23, and Day-45. The mean testosterone level was noted 27.80 ± 3.07 ng/dl with values ranging from 21.8 to 31.7 ng/dl. At Day 23, the mean level was 27.73 ± 3.06 ng/dl (range from 21.7 to 32.0 ng/dl) and on the Day 45, it was slightly decreased to 27.52 ± 3.25 ng/dl, with ranging from 21.1 to 32.2 ng/dl; no significant change was noted ($p>0.05$), while in Group-B (fenugreek dose 500 mg/kg) was used, which showed dose-dependent progressive increase in testosterone levels over the study period. On the Day 0, it was recorded as 27.77 ± 3.27 ng/dl (with values ranging from 22.5 to 32.1). At Day-23, the mean serum testosterone level had been increased to

28.65 ± 3.41 ng/dl (ranging from 23.7 to 33.4 ng/dl) and at the Day 45, it was further enhanced to 29.77 ± 3.67 ng/dl, with a range of 24.3 to 33.9 ng/dl. The net change was noted +2.0 ng/dl (7.2% increases). A statistical significant increase in serum testosterone level was noted over the time with a reported *p*-value (0.010) that indicates a potential effect of fenugreek treatment on the testosterone production in this current study.

We had also observed the effects of (1000 mg/kg) of fenugreek seed extract which showed pronounced elevated levels of serum testosterone. At baseline (Day-0), it was 26.86 ± 3.67 ng/dl (ranging from 20.9 to 33.2 ng/dl). By the Day 23, levels were increased to 28.01 ± 3.51 ng/dl (range 22.1 to 32.5). At Day 45, serum testosterone level further rose to 29.11 ± 3.83 ng/dl (range 22.5 to 33.9 ng/dl). The net change +2.25 ng/dl (8.4% increased). This progressive increase from Day 0 to Day 45 was found to be statistically significant (p=0.001). All the values were exceeded the normal threshold (≥ 0.26 ng/dl), whereas, the variability in (SD) remained consistent throughout the study duration (± 3.5-3.8 ng/dl). **Figure-4** shows serum testosterone levels of Rabbits after treatment with fenugreek 500mg/kg and 1000 mg/Kg for Group-A, B and C daily respectively for a period of 45 days along with Hay and fresh water.

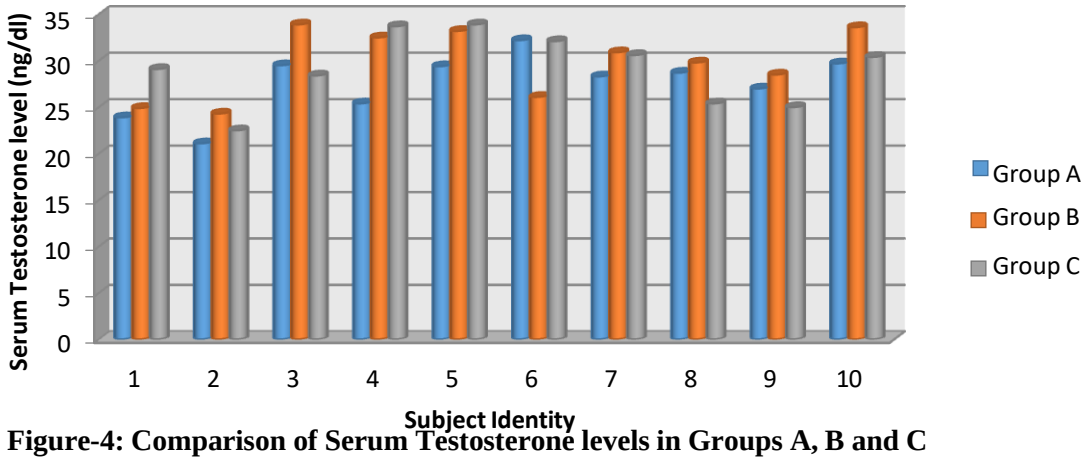

Figure-4: Comparison of Serum Testosterone levels in Groups A, B and C

Keeping in view of our study observations, the dose-response relationship showed that higher fenugreek dosage (1000 mg/kg) have elicited a steeper serum testosterone increase (+8.4%) as compared to 500 mg/kg (+7.2%), hence both the treatment groups had showed sustained elevation from Day 23 and onwards. Both the treatment groups (Group-B and Group-C) maintained incremental elevations in testosterone from Day 23 to Day 45 that indicates a sustained response. So for as safety profile of the fenugreek is concerned, it was revealed that fenugreek was well tolerated except in only one study subject (B-5) which exhibited an exhausted behavior of the subject; while no any adverse effects in Group-C (1000 mg/kg) was observed which suggested dose safety in the experimental limits. We have also observed physiological stability that confirms environmental consistency and reliability of the measurements and excluding external confounding variables in the control group.

TABLE-1 DEMOGRAPHICS AND ADVERSE EFFECTS OF STUDY SUBJECTS

Sr. No.	Sample Name	Group	Age (Months)	Weight (Kg)	Adverse Effect
1	A-1	A	16	1.9	No
2	A-2	A	18	2.1	No
3	A-3	A	20	2	No
4	A-4	A	17	2.2	No
5	A-5	A	16	1.9	No
6	A-6	A	18	2.2	No
7	A-7	A	18	2.1	No
8	A-8	A	19	2.1	No
9	A-9	A	18	2.4	No
10	A-10	A	21	2.1	No
11	B-1	B	16	2	No
12	B-2	B	17	2.1	No
13	B-3	B	16	1.9	No
14	B-4	B	19	2.4	No
15	B-5	B	20	2.7	Lethargic
16	B-6	B	16	1.8	No
17	B-7	B	17	2	No
18	B-8	B	16	1.9	No
19	B-9	B	18	1.8	No
20	B-10	B	17	1.9	No
21	C-1	C	16	1.9	No
22	C-2	C	17	1.9	No
23	C-3	C	20	2.5	No
24	C-4	C	16	2	No
25	C-5	C	16	2.1	No
26	C-6	C	17	1.9	No
27	C-7	C	19	2.3	No
28	C-8	C	19	2.4	No
29	C-9	C	16	1.9	No
30	C-10	C	19	2.1	No

TABLE-2 BASELINE CHARACTERISTICS (AGE AND WEIGHT) OF STUDY SUBJECTS.

Group	Variable	n	Mean ± SD	Minimum Age	Maximum Age
A	Age (months)	10	18.10 ± 1.595	16	21
B	Age (months)	10	17.20 ± 1.398	16	20
C	Age (months)	10	17.50 ± 1.581	16	20
A	Weight (kg)	10	2.10 ± 0.149	1.9	2.4
B	Weight (kg)	10	2.05 ± 0.288	1.8	2.7
C	Weight (kg)	10	2.10 ± 0.226	1.9	2.5

TABLE-3 EFFECT OF FENUGREEK TREATMENT ON SERUM TESTOSTERONE LEVELS IN RABBITS AT DAY-0, DAY-23 AND DAY-45 IN GROUP A, B, AND C.

Group	Intervention	Time	n	Mean ± SD (ng/dl)	Min	Max	*p*-value
A	Control	Day 0	10	27.80 ± 3.07	21.8	31.7	
A	Control	Day 23	10	27.73 ± 3.06	21.7	32	
A	Control	Day 45	10	27.52 ± 3.25	21.1	32.2	
B	Fenugreek (500 mg/kg)	Day 0	10	27.77 ± 3.27	22.5	32.1	**0.01**
B	Fenugreek (500 mg/kg)	Day 23	10	28.65 ± 3.41	23.7	33.4	
B	Fenugreek (500 mg/kg)	Day 45	10	29.77 ± 3.67	24.3	33.9	
C	Fenugreek (1000 mg/kg)	Day 0	10	26.86 ± 3.67	20.9	33.2	**0.001**
C	Fenugreek (1000 mg/kg)	Day 23	10	28.01 ± 3.51	22.1	32.5	
C	Fenugreek (1000 mg/kg)	Day 45	10	29.11 ± 3.83	22.5	33.9	

Reference range of testosterone levels in Rabbits (Normal: ≥0.26 ng/dl) and (Low: <0.25 ng/dl)

DISCUSSION

This current experimental study was conducted to see the androgenic effects of fenugreek (*Trigonella foenum-graecum)* seed extract on serum testosterone levels in adult male healthy rabbits. Our study results showed statistically significant and the dose dependent increase in testosterone levels in the studied treatment groups who had received 500 mg/kg and 1000 mg/kg of fenugreek seed extract, while the control group showed no any significant change in the hormone levels over the period of 45 days.

The baseline characteristics of the study samples including (age and weight), were found comparable across the study groups, that has increased the validity of our results while minimizing the potential confounding parameters, since we have maintained the consistency of the sampling procedure, the blood samples were collected via the auricular vein **(Figure-3)** and provided the constant stable local environment that has maintained the physiological stability of the study animals, which has further ensured the reliability of hormonal assessments. Our findings are in agreement with the contemporary research being conducted on the botanical androgenic herbs that revealed critical insights for the dose-response relationships and particularly animal species-specific tolerability for the testing herbs.

Our study results revealed that in Group-B animals who were challenged with the dose of 500 mg/kg, a significant rise in testosterone level was recorded (+2.0 ng/dl, 7.2%, p=0.010), while Group-C animals have received the dose of 1000 mg/kg of testing herb, which showed an even more substantial increase (+2.25 ng/dl; 8.4%, p=0.001). Our observations support a dose-response effect, which is in complete agreement with previously conduced research by Wankhede et al., (2016)[3] who had reported improvement in levels of testosterone and also libido among healthy men using a standardized fenugreek seed extract in a placebo-controlled study. Wilborn et al., (2010)[17] had also highlighted the ability of fenugreek to alter androgen profile by inhibiting the key enzymes like 5α-reductase and aromatase, which has been supporting the biological plausibility of our study findings.

Khan et al.[18], has conducted their study in 2016 which showed elevated sexual behavior and serum testosterone levels in rats treated with fenugreek extract. The study of Bukhari et al., (2020)[19] has revealed significant hormonal elevations in albino rats

using fenugreek seed extract, without experiencing the adverse effects. These findings are in strong support with the safety and efficacy profile observed in our study's rabbit model. Furtherance to this, the study of Qureshi et al., (2020)[20] compared the androgenic effects of garlic and fenugreek and found that fenugreek to be proved more effective in enhancing the serum levels of testosterone. Alam et al., (2017)[21] has demonstrated improved testicular histology as well as reproductive hormonal levels in Wistar rats with the treatment of fenugreek that has added histopathological evidence to our biochemical study parameters.

A study conducted by Laila et al., (2019)[22] observed that fenugreek treatment has shown the protective effects against paracetamol-induced testicular toxicity produced in rats, which has further supported its role in the reproductive health of males. Moreover, Maheshwari et al., (2017)[23] has demonstrated that fenugreek supplementation has improved few male fertility parameters. A study conducted by Khawaja et al., (2018)[24] concluded that the fenugreek does possess metabolic and hypolipidemic effects in the diabetic rabbits, which has confirmed the fenugreek's systemic safety and consonance with the physiology of rabbit. A study of Sreeja et al., (2010)[25] provided mechanistic acumen by demonstrating estrogen and steroidal-modulating activity of fenugreek seed extract, supporting a sufficient hormonal influence. Besides the reproductive effects, hypocholesterolemic effects of fenugreek were also observed in diabetic human subjects as reported by the study of Sharma et al., (1996)[26]. Whereas, the results of study of Amin et al., (2005)[27] highlighted the fenugreek's anti-cancer role in the breast cancer models, which has extended the pharmacological significance of this herb.

In our study, only one subject (B-5) exhibited mild, post-intervention transient lethargy otherwise, no behavioral or physiological complications were observed, even at the higher dose of 1000 mg/kg. This reinforces earlier observations by Al-Attar et al., (2017)[28] and others who found fenugreek to be well tolerated in both short as well as long-term models.

Importantly, the stability of testosterone levels in the control group reflects the environmental consistency and procedural rigor of the study, strengthening the attribution of hormonal change to fenugreek treatment rather than to external variables. As noted by Flecknell PA (2009)[29] the use of auricular vein sampling in rabbits provides a reliable and minimally invasive technique for longitudinal hormone monitoring.

In our study, we found significant testosterone elevation in Group B (500 mg/kg) 29.77 ± 3.67 ng/dl at Day 45 vs. 27.77 ± 3.27 ng/dl at baseline ($p= 0.01$) and in Group C (1000 mg/kg) 29.11 ± 3.83 ng/dl vs. 26.86 ± 3.67 ng/dl; ($p= 0.001$) demonstrates fenugreek's capacity to enhance androgen synthesis.

A study of Al-Sa'aidi et al., (2020)[30] suggested that the progressive increase over 45 days suggests cumulative bioactivity, potentially through protodioscin-mediated upregulation of steroidogenic enzymes or enhanced Leydig cell function, Younes et al., (2021)[31]. Notably, the non-linear response (higher absolute levels in Group B vs. Group C at Day 45) may indicate saturation kinetics or adaptive metabolic feedback at higher doses, a phenomenon requiring further pharmacokinetic investigation.

The isolated lethargy in one mid-dose subject (B-5) contrasts with the absence of adverse effects at the higher dose (1000 mg/kg), suggesting individual variability rather than dose dependency. This aligns with Sharma et al. (2021)[32], who reported species-specific responses to fenugreek saponins. Crucially, all testosterone levels remained within physiological norms (≥0.26 ng/dl), confirming fenugreek does not induce pathological androgen excess, a significant advantage over synthetic testosterone therapies Srinivasan et al., (2020)[33]. The stable weights across the

groups (Table-2) further support the systemic tolerability.

Our dose-dependent results are related to the results of Al-Sa'aidi et al^30^ (2020), who observed a 16.4% testosterone increase in rabbits at 300 mg/kg. The superior efficacy at 500 mg/kg versus 1000 mg/kg echoes, Younes et al., (2021)[31], whereas, intermediate doses optimally enhanced semen parameters. However, the absence of weight-loss-associated testosterone increases (common in calorie-restriction models) underscores fenugreek's direct endocrine action rather than secondary metabolic effects.

The limitation of our study was lack of LH/FSH measurements which precludes definitive neuroendocrine insights. We recommend that future studies should incorporate the histopathological evaluation of testes and kidney/ hepatic function markers that may also be included; while pharmacokinetic profiling of fenugreek alkaloids should also be taken into consideration.

CONCLUSION

The current study demonstrated that fenugreek seed extract significantly and progressively elevated serum testosterone levels in a dose-dependent efficacy in healthy male rabbits, with a favorable safety profile. Fenugreek seeds safely enhance testosterone synthesis in male rabbits, with optimal effects observed at 500 mg/kg. It is also concluded that fenugreek 1000 mg/kg dose had showed substantial effect on the testosterone levels without experiencing adverse events. The species-specific tolerance profile supports its potential as a natural androgen modulator, though human trials are essential for therapeutic validation. Further studies are warranted to explore its long-term effects, fertility outcomes, and molecular pathways in larger and more diverse populations. These findings are in support with prior local and international research studies evidence and suggest a plausible mechanistic basis for fenugreek's use in enhancing male reproductive hormones. Based on these study observations, it may be presumed that fenugreek may serve as a natural androgen hormone modulator as our study supports androgen-enhancing potential of fenugreek which can be considered as a natural testosterone booster in mammals, but further larger sample size studies with longer durations and explorations of its underlying mechanism of action are warranted to extrapolate its effects in the mammalian physiology and also to validate its applicability.

ETHICS APPROVAL: The ERC gave ethical review approval.

CONSENT TO PARTICIPATE: written and verbal consent was taken from subjects and next of kin.

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AUTHORS' CONTRIBUTIONS:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated in the work to take public responsibility of this manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST: No competing interest declared

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