Fenugreek Induces The Sperm Characteristics in Hypothyroid Male Rats

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Abstract

Background: Hypothyroidism, a common hormonal disorder, disrupts the hypothalamic-pituitary-gonadal axis, impacting male reproductive health. Fenugreek (Trigonella foenum-graecum L.), known for its pharmacological properties, has shown potential therapeutic benefits and risks concerning reproductive function. Methods: Male rats were divided into four groups: a negative control, a hypothyroid group, a hypothyroid group treated with fenugreek extract (15 mg/kg/day), and a hypothyroid group treated with L-thyroxine (0.1 mg/kg/day). Thyroid hormone levels and sperm characteristics (motility, viability, morphology) were assessed after 30 days of treatment. Hormonal analysis was performed using ELISA, and semen analysis followed standard protocols. Statistical analysis involved ANOVA with $p < 0.05$. Results: Hypothyroidism led to increased TSH and decreased T3 and T4 levels, alongside impaired sperm parameters. Both fenugreek and L-thyroxine treatments significantly improved thyroid hormone levels and sperm characteristics compared to the hypothyroid group. Conclusion: Hypothyroidism adversely affects male reproductive health, but fenugreek shows promise in ameliorating these effects, potentially due to its antioxidant properties and hormonal modulation. Further research is needed to confirm its clinical application.

Keywords: Hypothyroidism, Fenugreek, Male fertility, Thyroid hormones,Sperm quality.

Significance | This study demonstrated fenugreek's potential to mitigate hypothyroidism-induced male infertility, providing insights into herbal and hormonal therapies' effectiveness.

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1. Introduction

Hypothyroidism, a common hormonal disorder, profoundly affects various physiological processes by disrupting the neuroendocrine axis, including the hypothalamic-pituitary-gonadal axis crucial for testicular function (Kumar et al., 2014; Kaplan, 1984). This condition underscores the intricate relationship between hormonal balance and reproductive health, revealing significant implications for fertility.

Fenugreek (Trigonella foenum-graecum L.), an herbal remedy renowned for its pharmacological properties, has garnered attention in recent years. Belonging to the Fabaceae family, this self-pollinating annual herbaceous plant originates from India and Northern Africa, with India alone contributing 80% of its global production (Gu et al., 2017; Bashtian et al., 2013). Its seeds, known as methi in India, are celebrated for their reported anti-diabetic and cholesterol-lowering effects in both animal models and humans (Rasheed et al., 2015; Zia et al., 2001; Vats et al., 2002). Despite its therapeutic potential, concerns have arisen regarding fenugreek's impact on reproductive health. While generally considered safe with no reported toxicological effects (Al-Habori and Raman, 1998), conflicting data suggests potential risks to fertility and reproductive function. Studies have indicated a sterilizing effect on albino rats and rabbits, noting reduced testis weight and histological changes in seminiferous tubules following fenugreek seed exposure

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(Baby et al., 2016). Moreover, saponins isolated from fenugreek seeds exhibit spermicidal saponins isolated from fenugreek seeds exhibit spermicidal properties in vitro against rat spermatozoa (Mishra et al., 2019), pointing towards mechanisms that could adversely affect reproductive outcomes.

The bioactive compounds in fenugreek seeds, such as sapogenins and diosgenin, serve as precursors for progesterone and possess both anti-gonadotropic and anti-androgenic potential (Raghuram et al., 1994). This dual nature of fenugreek's effects underscores the complexity of its physiological impact, particularly on hormone-sensitive tissues like the testes.

In animal models, particularly male rats, ethanol extracts of fenugreek seeds have demonstrated inhibitory effects on sex organs and fertility, attributed to furostanol glycosides, the major soluble saponins found in fenugreek (Sharma and Jacob, 2002; Madhulika and Verma, 2019). These compounds are known for their cholesterol-complexing properties and their ability to modulate hormonal pathways, highlighting their potential impact on reproductive health.

Thyroid dysfunction is also well-documented to affect male reproductive physiology, with thyroid hormones (T3 and T4) playing crucial roles in metabolic regulation and spermatogenesis (Maia et al., 1990; Kundu et al., 2006). Imbalances in thyroid hormone levels, whether hypo- or hyperthyroidism, can disrupt spermatogenesis and sperm quality, leading to infertility issues (Liu et al., 1999, Somaya et al. 2024, Vinay et al. 2023, Srivalsa et al. 2021, A.S. Abdul Majid et al. 2019).

The intersection of fenugreek and thyroid function in male reproductive health is an emerging area of interest. Studies exploring the effects of fenugreek seeds on sperm characteristics in male rats with induced hypothyroidism provide valuable insights into potential therapeutic or adverse effects. Understanding these interactions is critical for evaluating fenugreek's safety and efficacy in clinical and dietary contexts, given its widespread use as a natural remedy for various health conditions.

While fenugreek offers promising therapeutic benefits, particularly in metabolic disorders, its potential impact on male reproductive health warrants careful consideration. Further research is essential to elucidate the underlying mechanisms of fenugreek's effects on hormone-sensitive tissues and to establish comprehensive guidelines for its safe use in clinical practice. Clarifying these interactions will not only enhance our understanding of fenugreek's broader pharmacological effects but also inform recommendations for its judicious use in therapeutic settings.

2. Materials and Methods

The comprehensive experimental setup aimed to evaluate the effects of fenugreek seed extract and L-thyroxine on male reproductive parameters in a hypothyroid rat model, with appropriate controls to assess the impact of each treatment regimen. The methodology ensured rigorous scientific standards and ethical considerations throughout the study were maintained.

2.1Ethical Approval:

All procedures conducted in this study received approval from The Scientific Committee of the College of Veterinary Medicine, University of Kufa, ensuring compliance with ethical standards for animal welfare.

2.2 Animal Groups and Experimental Design:

Male rats weighing between 200-250 g were assigned to four experimental groups, each comprising 10 rats. Group 1 (Negative Control - C-negative) received oral saline daily for 30 days. Group 2 (Positive Control - HYPO positive) also received oral saline for 30 days and underwent induction into a hypothyroid state. Group 3 (Fenugreek - HYPO+FN) received daily oral gavage of fenugreek (15 mg/kg body weight) for 30 days, following the protocol by Stark and Madar (1993). Group 4 (L-T4 - HYPO+ Thyroxin) received daily intraperitoneal injections of L-thyroxine (0.1 mg/kg/day) for 30 days, as per the method described by Swaroop et al. (2015). This experimental design allowed for the systematic evaluation of fenugreek seed extract and L-thyroxine on male reproductive parameters within a hypothyroid rat model, ensuring controlled conditions for accurate assessment of treatment effects.

2.3 Preparation of Ethanol Extracts of Fenugreek Seeds:

Fenugreek seeds were sourced from local markets in Najaf province. A total of 100 g of powdered fenugreek seeds underwent extraction using a Soxhlet apparatus with 70% ethanol. The extraction process continued until complete extraction was achieved. The ethanol solvent was subsequently evaporated under reduced pressure using a rotary evaporator until complete dryness. The resulting ethanol extract was stored at 4°C for further use (Singh et al., 2020).

2.4 Hormonal Analysis:

Serum levels of thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) were measured using the enzyme-linked immunosorbent assay (ELISA) method with Elabscience kits.

2.5 Semen Analysis:

After blood collection, semen was retrieved from the left caudal epididymis for evaluation of several parameters. These included general motility, progressive motility, viability, and morphology. Semen obtained from the right caudal epididymis was homogenized in Tris buffer (pH = 7.4) to achieve a concentration of 15% (g/ml), following the method described by Lim et al. (2002). This standardized approach ensured thorough assessment of sperm characteristics, providing essential data for analyzing the impact of experimental treatments on male reproductive health within the study's framework.

2.6 Statistical Analysis:

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using ANOVA (Analysis of Variance) to

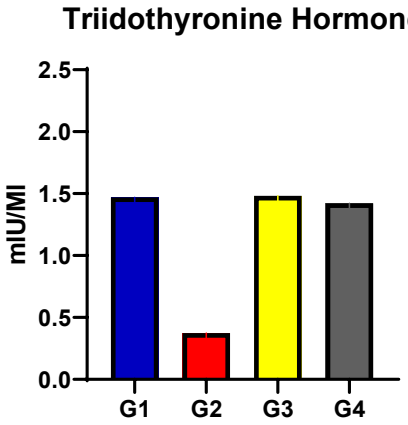

Figure 2. Thyorid hormanes (T3) level in the experimental groups.

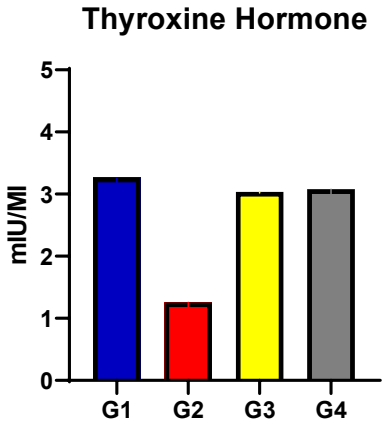

Figure 3. Thyorid hormanes (T4) level in the experimental groups.

Table 1. Analysis of sperm characteristics

Parameters / **Groups**	**Motility**		**Morphology (%)**	**Viability (%)**
	General Motility (%)	**Progressive Motility (%)**		
Control (Normal saline)	87.80 ± 2.5 a	78.00 ± 1.00 a	90.00 ± 1.0a	90.48 ± 1.9a
Hypothyroidism	36.00 ± 3.3 b	35.92 ± 8.5 b	52.80 ± 1.9 c	41.80 ± 3.8 b
HYPO+FN	85.80 ± 2.1 a	74.80 ± 2.16 a	89.50 ± 1.8 a	88.00 ± 2.6 a
HYPO+Thyroxin	85.40 ± 3.0 a	73.40 ± 3.5 a	85.72 ±3.2b	87.70 ± 21.0a

Each letter represents a statistically significant difference between the groups at the ($p \leq 0.05$) level.

compare differences among groups, with a significance level set at $p < 0.05$ (Xavier et al., 2019).

3. Results

3.1 Thyroid Gland Hormonal Tests Results

The analysis of thyroid hormone levels, as presented in Figures 1, 2, and 3, indicated varied responses to the experimental treatments. In the HYPO (positive control) group, there was a significant increase in thyroid-stimulating hormone (TSH) levels, alongside significant decreases in both triiodothyronine (T3) and thyroxine (T4) levels. In contrast, treatments with HYPO+Thyroxin resulted in significant decreases in TSH levels compared to the HYPO group, as well as significant increases in both T3 and T4 levels. Similarly, the HYPO+FN treatment also led to significant decreases in TSH levels and significant increases in T3 and T4 levels compared to the HYPO group. These results suggested that both Thyroxin and fenugreek (FN) treatments effectively modulated thyroid hormone levels, counteracting the effects of hypothyroidism.

3.2 Sperm Characteristics Analysis Results

The analysis of sperm characteristics, summarized in Table 1, included general motility, progressive motility, viability, and morphology from the left testis of the experimental groups. In the HYPO (positive control) group, there were significant decreases in all sperm parameters compared to the negative control group. Conversely, both the HYPO+Thyroxin and HYPO+FN treatments resulted in improvements across all sperm parameters compared to the HYPO group. These findings indicated that both Thyroxin and FN supplementation had beneficial effects on sperm quality under hypothyroid conditions, improving motility, viability, and morphology.

4.Discussion:

The present study underscores the complex interplay between thyroid hormones and male reproductive function, particularly under conditions of hypothyroidism. Our findings reveal significant impairments in sperm viability, motility, and morphology in hypothyroid male rats, underscoring the critical role of thyroid hormones in spermatogenesis and sperm quality.

Hypothyroidism is recognized for its adverse effects on male fertility through multiple mechanisms. The deficiency in thyroid hormones often results in decreased sperm count, impaired motility, and altered sperm morphology, all of which are commonly observed consequences of this condition (SPSS, 2010). These changes can substantially compromise fertility outcomes, highlighting the importance of effectively managing thyroid disorders to mitigate their impact on reproductive health.

Beyond traditional measures of sperm quality, recent research has expanded our understanding of the broader influence of thyroid hormones on male reproductive physiology. Studies suggest that hypothyroidism may disrupt epididymal function, leading to histological and endocrinological changes that adversely affect sperm maturation and quality (Biswas et al., 1994; Buzzard et al., 2003). Specifically, impaired epididymal epithelial function under hypothyroid conditions may contribute to increased cytoplasmic droplets on spermatozoa, further compromising fertility potential.

The therapeutic potential of fenugreek (Trigonella foenum-graecum) in mitigating these effects is of particular interest. Fenugreek, a traditional herbal remedy, has shown promise in enhancing male fertility through various mechanisms (Maheshwari et al., 2017). Our study corroborates previous findings indicating that fenugreek supplementation can improve sperm motility and reduce abnormalities in male rats with hypothyroidism (Kiss et al., 2019).

The observed benefits of fenugreek on sperm parameters may be attributed to several factors. First, fenugreek contains bioactive compounds with antioxidant properties, which can counteract oxidative stress induced by hypothyroidism and protect sperm from oxidative damage (Shamim et al., 2016). Oxidative stress is known to impair sperm function and viability, making antioxidant supplementation a promising strategy to preserve male fertility in thyroid disorders.

Moreover, fenugreek may exert regulatory effects on hormone levels, including thyroid hormones, although the precise mechanisms require further elucidation (Sakr et al., 2012). By modulating thyroid hormone levels or enhancing their utilization, fenugreek could indirectly improve sperm characteristics and overall reproductive outcomes in hypothyroid conditions.

Our findings underscore the potential therapeutic role of fenugreek in managing male infertility associated with thyroid dysfunction. However, translating these results to clinical practice requires rigorous clinical trials to establish safety, efficacy, and optimal dosage regimens. Future research should focus on unraveling the molecular mechanisms underlying fenugreek's effects on male reproductive health, including its interactions with thyroid hormones and its long-term impact on fertility.

This study contributes to a deeper understanding of how thyroid hormones influence male reproductive physiology and highlights fenugreek as a promising adjunct therapy for improving sperm quality in hypothyroidism. Addressing thyroid-related male infertility through integrated approaches, including pharmacological interventions and lifestyle modifications, holds potential for enhancing reproductive health outcomes in affected individuals.

5. Conclusion

In conclusion, our study emphasizes the significant impact of hypothyroidism on male reproductive health, revealing impairments in sperm viability, motility, and morphology. Fenugreek (Trigonella foenum-graecum) emerges as a potential therapeutic agent, demonstrating improvements in sperm parameters in hypothyroid male rats. Fenugreek's antioxidant properties and possible regulatory effects on hormone levels contribute to these benefits. However, translating these findings into clinical practice requires further research to ensure safety and efficacy. Integrated approaches, combining pharmacological interventions and lifestyle modifications, offer promise in managing thyroid-related male infertility and enhancing reproductive health outcomes.

Author contributions

F.Z.H.A.Q. conceptualized the study, developed the methodology, wrote the original draft, reviewed and edited the manuscript, and administered the project. B.A.M.A.M. curated the data, conducted the formal analysis, developed the software, visualized the data, and reviewed and edited the manuscript. M.A.F.A. validated the findings, provided resources, supervised the project, and reviewed and edited the manuscript. Z.M.K.H. conducted the investigation, wrote the original draft, and participated in the reviewing and editing process. K.T.S.A. acquired funding, supervised the project, and reviewed and edited the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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