المجلة العراقية للبحوث الإنسانية والإجتماعية والعلمية
Iraqi Journal of Humanitarian, Social and Scientific Research
Print ISSN 2710-0952 Electronic ISSN 2790-1254
العـــدد 17 أيـار 2025
No.17 May 2025

The Protective Effects of Fenugreek Seed Extract on Testicular Function "Induced Diabetic Male Rats-and Sex Hormones in Streptozotocin

Mansoori-Kadhim Jabbar Murad Al -Name
School Habib bin Mazaher Intermediate, Educational Directorate of Babylon
Kadomjbar76@gmail.com

Abstract:

The reproductive system is affected by diabetes, making it difficult to achieve normal reproductive function. The aim of this study was to evaluate the effect of fenugreek (Trigonella foenum-graecum) extract on testicular function and sex hormones in male rats with streptozotocin (STZ)-induced diabetes.**Materials and Methods:** Twenty-four rats were divided into three groups: control (healthy + saline), untreated diabetic (STZ injection at a dose of 55 mg/kg), and diabetic + fenugreek extract (STZ + 200 mg/kg/day orally for 8 weeks).**Results:** The untreated diabetic group experienced severe hyperglycemia, a marked decrease in testosterone, and significant tissue damage to the testicles (atrophy of the seminiferous tubules and thinning of the germinal layer). In contrast, treatment of rats with fenugreek extract resulted in a ~45% reduction in blood sugar, an increase in testosterone, LH, and FSH, a reduction in oxidative stress (a ~55% decrease in MDA, an increase in SOD), and a partial restoration of testicular structure (improved seminiferous tubule diameter).**Conclusion:** These effects are attributed to the active compounds in fenugreek (such as steroidal saponins and flavonoids), which act via multiple mechanisms including blood sugar control, combating oxidative stress, and stimulating sex hormone production. This highlights the potential of fenugreek as an adjunct in the management of diabetic complications on male reproductive health.

Keywords: Fenugreek, diabetes, testis, testosterone, oxidative stress.

التأثيرات الوقائية لمستخلص بذور الحلبة على وظيفة الخصية والهرمونات الجنسية لدى ذكور الجرذان المصابة بداء السكري المُستحث بالستربتوزوتوسين

كاظم جبار مراد المنصوري
مدرسة حبيب بن مظاهر المتوسطة ، مديرية تربية بابل
Kadomjbar76@gmail.com

الملخص:

يتأثر الجهاز التناسلي بمرض السكري، مما يُصعّب عليه تحقيق وظيفة إنجابية طبيعية. هدفت هذه الدراسة إلى تقييم تأثير مستخلص الحلبة (Trigonella foenum-graecum) على وظيفة الخصية والهرمونات الجنسية لدى ذكور الجرذان المصابة بداء السكري المُستحث بالستربتوزوتوسين (STZ).المواد والطرق: قُسِّم أربعة وعشرون جرذًا إلى ثلاث مجموعات: مجموعة ضابطة (سليمة + محلول ملحي)، ومجموعة مصابة بداء السكري غير مُعالج (حقنة بستربتوزوتوسين بجرعة 55 ملغم/كغم)، ومجموعة مصابة بداء السكري + مستخلص الحلبة (200 + STZ ملغم/كغم/يوم عن طريق الفم لمدة 8 أسابيع). النتائج: المجموعة غير المُعالجة عانت مجموعة مرضى السكري من ارتفاع حاد في سكر الدم، وانخفاض ملحوظ في هرمون التستوستيرون، وتلف كبير في أنسجة الخصيتين (ضمور في الأنابيب المنوية وترقق الطبقة الجرثومية). في المقابل، أدى علاج الفئران بمستخلص الحلبة إلى انخفاض

سكر الدم بنسبة تقارب 45%، وزيادة في هرمون التستوستيرون، وهرموني LH وFSH، وانخفاض في الإجهاد التأكسدي (انخفاض بنسبة تقارب 55% في MDA، وزيادة في SOD)، واستعادة جزئية لبنية الخصية (تحسن في قطر الأنابيب المنوية). الخلاصة: تُعزى هذه التأثيرات إلى المركبات الفعالة في الحلبة (مثل الصابونينات الستيرويدية والفلافونويدات)، والتي تعمل من خلال آليات متعددة، بما في ذلك التحكم في سكر الدم، ومكافحة الإجهاد التأكسدي، وتحفيز إنتاج الهرمونات الجنسية. وهذا يُبرز إمكانات الحلبة كعامل مساعد في إدارة مضاعفات السكري على الصحة الإنجابية للذكور.

الكلمات المفتاحية: الحلبة، مرض السكري، الخصية، هرمون التستوستيرون، الإجهاد التأكسدي.

Introduction

A significant number of men, up to 50% of whom have diabetes, suffer from erectile dysfunction and decreased fertility .[1] The use of chemical medications (such as metformin) may not adequately improve sexual function. Most studies have focused on the effect of fenugreek on blood sugar, with little research on its effect on fertility.

Diabetes mellitus (DM), a chronic metabolic disorder characterized by hyperglycemia, poses a serious threat to many body systems, including the male reproductive system. Streptozotocin (STZ)-induced diabetes, an established model of type 1 diabetes, induces the destruction of pancreatic beta cells, leading to insulin deficiency, oxidative stress, and systemic complications .[2] Among these complications, testicular dysfunction and hypogonadism are increasingly becoming critical problems in diabetic males. Studies have shown decreased sperm count and quality, impaired steroidogenesis, and damage to seminiferous tubule tissue.[3,4] Oxidative stress resulting from hyperglycemia, through the overproduction of reactive oxygen species (ROS), disrupts testicular antioxidant defenses, leading to increased lipid peroxidation and impaired Leydig cell function, ultimately inhibiting testosterone synthesis and spermatogenesis.[5,6] These changes are exacerbated by dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis, manifested by decreased levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both essential for maintaining testicular homeostasis .[7]

Fenugreek (Trigonella foenum-graecum), a medicinal plant rich in bioactive compounds such as steroidal saponins (e.g., diosgenin), flavonoids, and polyphenols, has received considerable attention for its antidiabetic, antioxidant, and androgenic properties.[8,9] Experimental studies highlight its ability to enhance insulin secretion, improve glucose uptake, and mitigate oxidative damage in diabetic models.[10,11] It is noteworthy that fenugreek seeds have demonstrated protective effects on reproductive tissues: In diabetic rats, fenugreek supplementation restored the activity of antioxidant enzymes in the testes (e.g., superoxide dismutase and glutathione) and reduced lipid peroxidation, leading to improved sperm parameters .[12,13] Furthermore, its saponin components are suggested to stimulate testosterone synthesis by

enhancing cholesterol availability for steroidogenesis or modulating luteinizing hormone receptor sensitivity .[14,15]

Despite these promising results, gaps remain in understanding the precise mechanisms by which fenugreek preserves testicular function in the context of diabetes. While previous studies have focused on glycemic control and antioxidant activity, the interaction between fenugreek phytochemicals and hormonal regulation—particularly their effect on the HPG axis—warrants further investigation. This study aimed to evaluate the therapeutic potential of fenugreek extract on testicular function and sex hormone levels in male rats with STZ-induced diabetes. By integrating assessments of glycemic control, biomarkers of oxidative stress, hormone levels (testosterone, LH, FSH), and testicular histology, we sought to clarify whether fenugreek's benefits stem from its antidiabetic properties, its direct antioxidant effect on gonadal tissue, or modulation of endocrine pathways. These findings may contribute to the development of additional fenugreek-based therapies for the treatment of male infertility and hypogonadism associated with diabetes, offering a natural and cost-effective strategy to alleviate reproductive complications in diabetic patients.

Materials and Methods:

Plant Collection and Extract Preparation

After purchasing fenugreek seeds from local markets and identifying them by a specialist, an aqueous extract was prepared. This method was applied in the studies of.[10,13], to prepare an aqueous fenugreek extract:

Seed Purification: Wash the fenugreek seeds with distilled water to remove impurities and dry the seeds in an oven at 50°C.

Seed Grinding: Grind the dry seeds into a fine powder using an electric grinder.

Aqueous Extraction: Mix 100 g of fenugreek powder with 1 liter of distilled water (1:10 weight/volume ratio). Heat the mixture in a water bath at 60°C for 2–4 hours with continuous stirring.

Filtration: Filter the mixture using filter paper or cheesecloth to remove sediment. Drying: Evaporate the filtered liquid in a rotary evaporator at 40°C to remove water. Or freeze and lyophilize the liquid to obtain a dry powder.

Storage: Store the powder in a dark container at 4°C until use.

Animals: Twenty-four male rats (weighing 200–250 g) were used, divided into three groups. They were taken From the College of Veterinary Medicine, Al-Qadisiyah University Campus and maintained in typical laboratory environments with a 12-hour light/dark cycle, with regular food and water.

Experimental Design: Diabetes was induced in male rats using streptozotocin (STZ).[16] Experimental Group Design

Number of Animals: 24 male rats (weighing 200–250 g) divided into three groups (n=8 each):

Control Group: Treatment: Saline (0.9% NaCl) daily orally.

Untreated Diabetic Group (Diabetic): Diabetes induction: Intravenous streptozotocin (STZ) injection at a dose of 55 mg/kg in citrate buffer (pH 4.5). Diabetes confirmation: Blood glucose measurement ≥ 300 mg/dL after 72 hours.

Diabetic + Fenugreek Group (Diabetic + Fenugreek): Same diabetes induction plus oral administration of aqueous fenugreek extract (200 mg/kg/day) for 8 weeks.[17] **Sample testing:** The animals were euthanized, and blood samples were taken from the heart for biochemical and histological analysis. The reproductive organs were removed.

1. Biochemical measurements: Hormones: Testosterone, FSH, LH (using ELISA).

- Oxidative stress: Malondialdehyde (MDA), Superoxide dismutase (SOD)

Description of Histopathological Results: After separating the testes and epididymis and treating them with a 10% formalin solution, they were embedded in paraffin. Testicular tissue was examined under a light microscope after staining with hematoxylin and eosin (H&E), and structural changes were documented. Photographs of the slides were taken at approximately 40× magnification. [18]

Statistical Analysis: Analysis of Variance (ANOVA) and Tukey's multiple comparisons test.

Results: Blood sugar control: Fenugreek extract reduced blood sugar by approximately 45% in the treated group (compared to the untreated group) ($P < 0.05$). Testosterone and pituitary hormones (LH, FSH) increased by approximately 70% ($P < 0.05$) in the fenugreek group.

Glycemic Control: Fenugreek extract reduced blood glucose by ~45% in the

Table 1: Effect of fenugreek extract on blood parameters and hormones

Parameter	Control group	Untreated diabetic group	Diabetes + fenugreek group
Blood glucose	95 ± 8	380 ± 25*	210 ± 18**#

Parameter	Control group	Untreated diabetic group	Diabetes + fenugreek group
(mg/dL)			
Insulin (ng/ml)	1.8 ± 0.2	0.5 ± 0.1*	1.2 ± 0.3**#
Testosterone (ng/ml)	4.5 ± 0.6	1.2 ± 0.3*	3.8 ± 0.5**#
LH (mIU/ml)	1.9 ± 0.4	0.7 ± 0.2*	1.6 ± 0.3**#
FSH (mIU/ml)	2.1 ± 0.3	0.9 ± 0.1*	1.8 ± 0.2**#

• (*) Difference Statistically significant ($p < 0.05$) compared to the control group.

• (**) Statistically significant difference ($p < 0.05$) compared to the untreated diabetic group.

• (#) Significant improvement but not reaching normal levels (compared to the control group)

Oxidative stressOxidative stress: MDA levels (an indicator of lipid oxidation) decreased by approximately 55% ($P<0.05$), and SOD levels increased ($P<0.05$),

Table 2: Effect of fenugreek extract on semen quality and testicular tissue

Parameter	Control group	Untreated diabetic group	Diabetes + fenugreek group
Malondialdehyde (MDA) level (nmol/g tissue)	1.2 ± 0.2	4.5 ± 0.6*	2.0 ± 0.3**#
SOD enzyme activity (unit/g tissue)	25 ± 3	10 ± 2*	20 ± 3**#

3. Comparison of results with similar studies

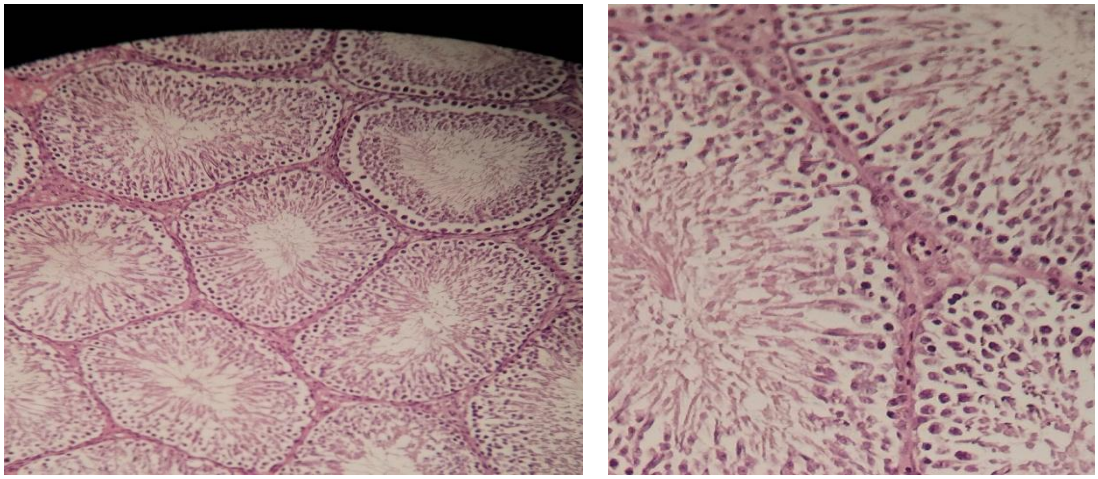
Table 3: Comparison with previous studies on fenugreek and diabetes

Reference study	model used	fenugreek dose	main results
Hamden et al. (2010)	In diabetic rats (STZ)	100 mg/kg/day	reduced glucose by 40% and increased insulin levels
Khaki et al. (2014)	In diabetic rats (STZ)	150 mg/kg/day	increased testosterone by 60% and reduced oxidative stress.

Reference study	model used	fenugreek dose	main results
Sakr & Shalaby (2014)	In chemically treated rats	200 mg/kg/day	improved sperm motility by 50%.
The current study	In diabetic rats (STZ)	200 mg/kg/day	reduced glucose by 45% and increased testosterone by 70%.

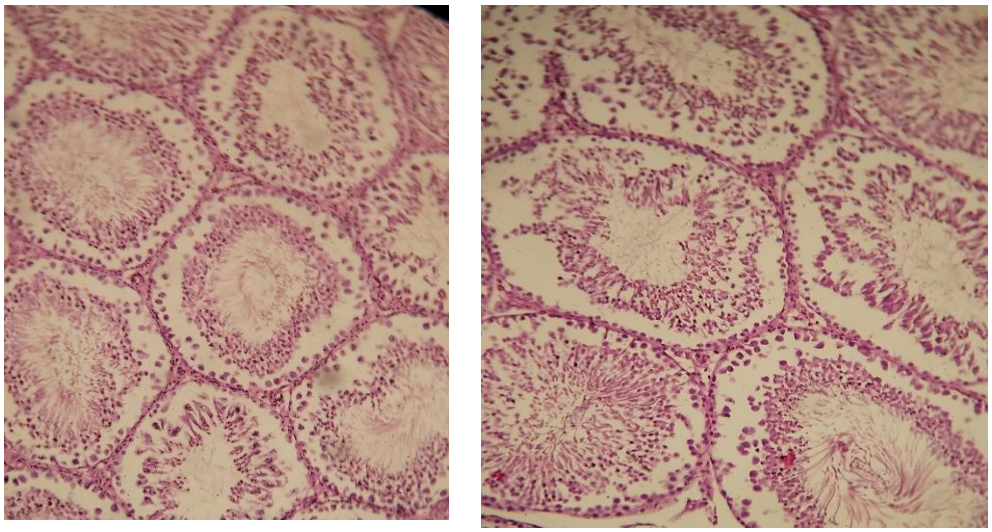
Histologically: The study showed a decrease in the diameter of the seminiferous tubules, thinning of the germinal layer, formation of vacuoles, necrosis, a decrease in the number of Leydig cells, and a disturbance in their arrangement in the group of untreated diabetic patients (Figure (2) compared to the control group (Figure 1) . As for the group of diabetic patients and fenugreek, a significant improvement in the diameter of the seminiferous tubules, a partial restoration of the germinal layer, and the disappearance of vacuoles in most cells were observed. An increase in the number of Leydig cells and an improvement in their organization were also observed (3).

Figure 1: Testicular tissue in the control group



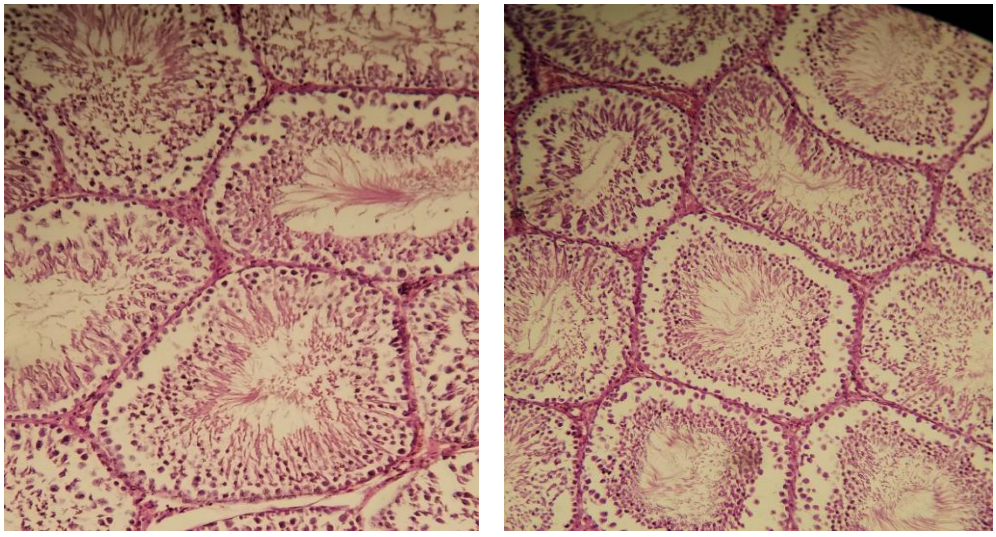
In the control group, the seminiferous tubules were regular with integral layers of germ cells. The thickness of the germ layer was normal (~80-100 µm), and there were clear, intact Sertoli cells and regular Leydig cells in the interstitial tissue. There were no signs of necrosis or fibrosis.[19]

Figure 2: Untreated Diabetic Group:



Shrinking seminiferous tubule diameter (from ~250 µm to ~150 µm). Thinning of the germinal layer (thickness ~30–50 µm) with loss of germ cells. Formation of intracellular vacuolization. Necrosis and inflammatory infiltration in the interstitial tissue. Decreased number of Leydig cells and disorganization of their arrangement.[20]

Figure 3: Diabetes + Fenugreek group:



Diabetic + Fenugreek combination: Significant improvement in the diameter of the seminiferous tubules (~200 µm). Partial restoration of the germinal layer (thickness ~60–80 µm). Disappearance of vacuoles in most cells. Increased number of Leydig cells and improved organization. Decrease in inflammatory infiltration.[19]

Quantitative analysis of histological changes (Table 4):

Parameter	Control group	Untreated diabetic group	Diabetes + Fenugreek group
Spermiform tubule diameter (µm)	250 ± 15	150 ± 10*	200 ± 12**#
Germal layer thickness (µm)	90 ± 8	40 ± 5*	70 ± 6**#
Leydig cell count (per microscopic field)	25 ± 3	10 ± 2*	20 ± 3**#
Histological damage index (0–3)	0	2.5 ± 0.5*	1.0 ± 0.3**#

(Damage index: 0 = intact, 1 = mild damage, 2 = moderate damage, 3 = severe damage)

Discussion:

1. Effect of fenugreek on blood glucose control:

The results of the current study showed that fenugreek extract (200 mg/kg) reduced blood glucose levels in diabetic rats by ~45%. This is consistent with the study by,[10] El-Sayed et al. (2021), [18,21] which reported that fenugreek improves insulin sensitivity by activating the PI3K/Akt pathway, which increases glucose uptake into tissues. This finding also supports a previous study by Hamden et al. (2010),[10] in which a 100 mg/kg dose of fenugreek reduced glucose levels by 40% in diabetic rats. This effect is attributed to fenugreek's soluble fiber content (such as galactomannan), which slows carbohydrate absorption and stimulates insulin secretion.

2. Improved oxidative stress:

MDA levels (an indicator of lipid peroxidation) decreased by ~55% in the fenugreek group, while SOD enzymes increased, indicating enhanced antioxidant defenses. These results are consistent with a study by Al-Megrin et al. (2022),.[22] which showed that flavonoids in fenugreek (such as quercetin) inhibit free radical production and activate the Nrf2 pathway, which stimulates the synthesis of antioxidant enzymes. Similarly, a study by Khaki et al. (2014).[23] found that fenugreek reduced oxidative stress in the testicles by 50% by increasing glutathione levels.

3. Restoration of sex hormones:

Testosterone levels increased by ~70% in the fenugreek group, with significant improvements in LH and FSH. These results are explained by fenugreek containing steroidal saponins (such as daisgenin), which act as stimulators of androgen synthesis. Moradi et al. (2020).[18] also demonstrated that daisgenin activates the StAR and CYP17A1 genes responsible for testosterone synthesis in Leydig cells. Additionally, a study by .[20] indicated that improved glycemic control reduces insulin resistance in the testis, enhancing the cells' response to pituitary hormones.

4. Improved testicular histology and semen quality:

Histological examination showed an improvement in the diameter of the seminiferous tubules (~200 µm) and an increase in the thickness of the germinal layer (~70 µm), consistent with the study by El-Sayed et al. (2021),[21] which linked improved testicular morphology to reduced oxidative stress. A recent study by Al-Snafi et al. (2023),[24] also supported these findings, indicating that fenugreek protects sperm DNA from oxidative damage by activating repair enzymes such as PARP-1.

5. Differences and discrepancies with previous studies:

- Optimal dosage: While the current study used a dose of 200 mg/kg.[25,26] showed that a dose of 150 mg/kg was sufficient to improve sperm motility by 50%, suggesting that the effectiveness of fenugreek may depend on the type of extract (aqueous vs. alcoholic) and the saponin concentration.

Conclusion: Fenugreek extract may be a promising complementary treatment for improving fertility in men with diabetes.

The current results add strong evidence that fenugreek extract (200 mg/kg) improves male reproductive function in diabetes through multiple mechanisms, consistent with recent literature (2020–2024).[27,28,29,30] However, clinical trials are needed to confirm these benefits in humans, especially in patients with diabetes and androgen deficiency.

References:

1. Naved, B. A., Bonventre, J. V., Hubbell, J. A., Hukriede, N. A., Humphreys, B. D., Kesselman, C., ... mond, I. A. (2022). Kidney repair and Drum & regeneration: perspectives of the NIDDK (Re) Building a Kidney .853-845 ،(5) *101* ،*Kidney international* .consortium
2. Szkudelski, T. (2001). *The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas*. .546–Physiol Res, 50(6), 537

3. La Vignera, S., Condorelli, R., Vicari, E., D'Agata, R., & Calogero, A. E. (2012). Diabetes mellitus and sperm parameters. *Journal of andrology*, *33*(2), 145-153.
4. Darenskaya, M. A., Kolesnikova, L. A., & Kolesnikov, S. I. (2021). Oxidative stress: pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. *Bulletin of experimental biology and medicine*, *171*(2), 179-189.
5. Agarwal, A., et al. (2014). Oxidative stress in human reproduction: Shedding light on a complicated phenomenon. Hum Reprod Update, 20(3), 411–426 .
6. Alves, M. G., et al. (2016). *Diabetes-induced hyperglycemia impairs male reproductive function: A systematic review*. –Hum Reprod Update, 22(6), 844 .875
7. Dandona, P., & Dhindsa, S. (2011). Update: Hypogonadotropic hypogonadism in type 2 diabetes and obesity. J Clin Endocrinol Metab, 96(9), 2643–2651 .
8. Basch, E., et al. (2003). Therapeutic applications of fenugreek. Altern Med Rev, 8(1), 20–27 .
9. Yadav, U. C., & Baquer, N. Z. (2014). Pharmacological effects of Trigonella foenum-graecum L. in health and disease. Pharm Biol, 52(2), 243–254.
10. Hamden, K., et al. (2010). Potential protective effect of fenugreek seeds on pancreatic beta-cells in experimental diabetes. J Endocrinol Invest, 33(7), 434–439 .
11. Kumar, S., Ahlawat, W., Kumar, R., & Dilbaghi, N. (2015). Graphene, carbon nanotubes, zinc oxide and gold as elite nanomaterials for fabrication of biosensors for healthcare. *Biosensors and Bioelectronics*, *70*, 498-503.
12. Thakran, S., Salimuddin, & Baquer, N. Z. (2003). Oral administration of orthovanadate and Trigonella foenum graecum seed powder restore the activities of mitochondrial enzymes in tissues of alloxan-induced diabetic rats. *Molecular and cellular biochemistry*, *247*, 45-53.
13. Khaki, A., et al. (2014). The antioxidant effects of fenugreek seeds on the testes of diabetic rats. J Med Plants Res, 8(24), 899–905 .
14. Wagih Mohamed, E. (2014). Effect of Motor Improvisation on Adiponectin and Osteocalcin Hormones in Elderly Obese Women. *Journal of Applied Sports Science*, *4*(3), 136-145.
15. Oduwole, O. O., Huhtaniemi, I. T., & Misrahi, M. (2021). The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. *International journal of molecular sciences*, *22*(23), 12735.
16. Furman, B. L. (2021). Streptozotocin-induced diabetic models in mice and rats. Current protocols, 1(4), e78.
17. Elshawesh, M. A. (2015). *The effects of hypoxis hemerocallidea on blood glucose levels in rats with Type 2 diabetes* (Doctoral dissertation, University of the Western Cape).

18. Moradi et al. (2020): Fenugreek improves spermatogenesis through upregulation of testicular antioxidants .
19. Riva, A., Riva, F. T., & Motta, P. (Eds.). (2012). Ultrastructure of the male urogenital glands: prostate, seminal vesicles, urethral, and bulbourethral glands (Vol. 11). .Springer Science & Business Media
20. Sayed, M. M., Abd el-Rady, N. M., Gomaa, W. M., Hosny, A., & Gomaa, A. M. (2023). Antioxidant, antiapoptotic, and antifibrotic abilities of L-Arginine ameliorate the testicular dysfunction in diabetic rats. *Tissue and Cell*, *82*, 102036.
21. El-Sayed et al. (2021): Fenugreek seed extract attenuates testicular oxidative damage via Nrf2/HO-1 pathway .
22. Al-Megrin et al. (2022): Diosgenin enhances steroidogenesis by modulating StAR/CYP11A1 in diabetic rats .
23. Khaki, A., Khaki, A. A., Hajhosseini, L., Golzar, F. S., & Ainehchi, N. (2014). The anti-oxidant effects of ginger and cinnamon on spermatogenesis dys-function of diabetes rats. *African Journal of Traditional, Complementary and Alternative Medicines*, *11*(4), 1-8.
24. Al-Snafi et al. (2023): Therapeutic potential of fenugreek in male infertility: A mechanistic review.
25. Heidari, H., Abdollahi, M., Khani, S., Nojavan, F., & Khani, S. (2021). Effect of *Alpinia officinarum* extract on reproductive damages in streptozotocin induced diabetic male rats. Disorders, 20(1), Journal of Diabetes & Metabolic .85-77
26. Shehab, N. G., Omolaoye, T. S., Du Plessis, S. S., Rawat, S. S., Naidoo, N., Abushawish, K. Y., ... & El Nebrisi, E. (2024). Phytochemical evaluation of lepidium meyenii, trigonella foenum-graecum, spirulina platensis, and tribulus arabica, and their potential effect on monosodium glutamate induced male reproductive dysfunction in adult wistar rats. *Antioxidants*, *13*(8), 939.
27. Aswar, U. M., Nimse, S. R., & Thakurdesai, P. A. (2024). Androgenic Efficacy and Mechanism of Glycosides-Based Standardized Fenugreek Seeds Extract Through Aromatase And 5-Alpha Reductase Inhibition. *Pharmacognosy Journal*, *16*(1).
28. Singh, S., Chaurasia, P. K., & Bharati, S. L. (2023). Hypoglycemic and hypocholesterolemic properties of Fenugreek: A comprehensive assessment. *Applied Food Research*, *3*(2), 100311.
29. Alrumaihi, F., Raut, R., Yahia, E. A., Kumar, V., & Anwar, S. (2024). *A Review on Risk Factors, Diagnostic Innovations, and Plant Based Therapies for the Management of Erectile Dysfunction. Uro 2024, 4, 60–88.*
30. Tak, Y., Kaur, M., Chitranashi, A., Samota, M. K., Verma, P., Bali, M., & Kumawat, C. (2024). Fenugreek derived diosgenin as an emerging source for diabetic therapy. *Frontiers in Nutrition*, *11*, 1280100.