Fenugreek oil improves metabolic parameters and mitigates reproductive dysfunctions in type-2 diabetic male Wistar rats.

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Abstract

Aim: The study investigated the impact of Fenugreek oil (FGO) on blood glucose levels and reproductive dysfunctions in male Wistar rats with type 2 diabetes mellitus (T2DM).
Methods: Animals were divided into 5 groups (n=5). Group 1 (Control) received water (0.2 mL/day), Group 2 (T2DM) while Group 3, 4 and 5 with T2DM were treated with FGO (1 mL/Kg), FGO (0.5 mL/Kg) and metformin (100mg/Kg). T2DM was induced via administration of fructose *ad libitum* for two weeks, followed by intraperitoneal streptozotocin injection (40 mg/Kg). Administration of FGO was oral for 4 weeks after confirmation of T2DM. Fasting blood glucose levels were monitored weekly, and after the last treatment, the rats were sacrificed to analyze sperm parameters, insulin, hypothalamic-pituitary-testicular hormones, and oxidative stress biomarkers. Cytoarchitecture of the testes and pancreas were also analyzed.
Results: The T2DM group showed increased blood glucose and homeostatic model assessment for insulin resistance (HOMA-IR) while homeostatic model assessment for beta-cell function (HOMA-β) decreased significantly. Following FGO treatment, improvements were observed in blood glucose levels and insulin sensitivity. Sperm motility, viability, and count were enhanced, and the occurrence of abnormal sperm morphology decreased. Insulin and reproductive hormones that were significantly decreased in T2DM also improved following FGO administration. Testicular malondialdehyde was significantly reduced with an improved antioxidants status following administration of FGO. Histological analysis revealed damage in the testes and pancreas of the diabetic group, which was improved post-treatment.
Conclusion: Fenugreek oil effectively mitigates reproductive dysfunctions and improves metabolic parameters related to type 2 diabetes in male Wistar rats.

L'huile de fenugrec améliore les paramètres métaboliques et atténue les dysfonctionnements reproductifs chez les rats Wistar mâles diabétiques de type 2 .

Abstrait
Objectif : L'étude a examiné l'impact de l'huile de fenugrec (FGO) sur les niveaux de glucose sanguin et les dysfonctionnements reproductifs chez les rats Wistar mâles atteints de diabète sucré de type 2 (DT2).
Méthodes : Les animaux ont été divisés en 5 groupes (n = 5). Le groupe 1 (témoin) a reçu de l'eau (0,2 mL/jour), le groupe 2 (DT2) tandis que les groupes 3, 4 et 5 atteints de DT2 ont été traités avec du FGO (1 mL/kg), du FGO (0,5 mL/kg) et de la metformine (100 mg/kg). Le DT2 a été induit par l'administration de fructose *ad libitum* pendant deux semaines, suivie d'une injection intrapéritonéale de streptozotocine (40 mg/kg). L'administration de FGO a été orale pendant 4 semaines après confirmation du DT2 . Les glycémies à jeun ont été surveillées chaque semaine et, après le dernier traitement, les rats ont été sacrifiés pour analyser les paramètres du sperme, l'insuline, les hormones hypothalamo-hypophyso-testiculaires et les biomarqueurs du stress oxydatif. La cytoarchitecture des testicules et du pancréas a également été analysée.
Résultats : Le groupe DT2 a montré une augmentation de la glycémie et une évaluation du modèle homéostatique pour la résistance à l'insuline (HOMA-IR) tandis que l'évaluation du modèle homéostatique pour la fonction des cellules bêta (HOMA-β) a diminué significativement. Après le traitement par FGO, des améliorations ont été observées dans les niveaux de glycémie et la sensibilité à l'insuline. La motilité, la viabilité et le nombre de spermatozoïdes ont été améliorés, et l'apparition de morphologie anormale des spermatozoïdes a diminué. L'insuline et les hormones reproductives qui étaient significativement diminuées dans le DT2 se sont également améliorées après l'administration de FGO . Le malondialdéhyde testiculaire a été significativement réduit avec un statut antioxydant amélioré après l'administration de FGO. L'analyse histologique a révélé des lésions dans les testicules et le pancréas du groupe diabétique, qui se sont améliorées après le traitement.
Conclusion : L'huile de fenugrec atténue efficacement les dysfonctionnements reproductifs et améliore les paramètres métaboliques liés au diabète de type 2 chez les rats mâles Wistar
Mots clés : diabète sucré, huile de fenugrec, dysfonctionnements de la reproduction.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized expressed by hyperglycemia, abnormal metabolism of carbohydrates, lipids, and proteins, and an elevated risk of consequences from vascular disease (1). The underlying cause of DM is connected to either a complete loss of insulin, a relative deficiency, or resistance to the hormone. DM is broadly classified into primary and secondary types. Primary DM is type 1 and type 2 diabetes, while secondary DM arises from other medical conditions, including gestational diabetes, which occurs during pregnancy (2). Specifically, type 2 diabetes mellitus (T2DM) is marked by disruptions in the metabolism of lipids, carbohydrates, and proteins, and results from defective insulin secretion, insulin resistance, or a combination of these factors (2). If left unmanaged, this metabolic disorder can progressively lead to macrovascular and neuropathic life-threatening complications. The various complications linked with DM include retinopathy, nephropathy, neuropathy, renal failure, cardiovascular dysfunction, and infertility (3).

Infertility is a worldwide concern, affecting approximately 10% to 15% of couples attempting to conceive, with male factor infertility contributing to nearly half of these cases (4). Hyperglycemia causes disturbance in the male reproductive system due to the induction of oxidative stress. Oxidative stress disrupts the delicate balance between reactive oxygen specie (ROS) production and antioxidant defense mechanisms, leading to oxidative damage to sperm cells and impairing their viability and function (5).

Studies have demonstrated that antioxidant therapies can mitigate diabetic complications by preventing cellular components from oxidative damage (6,7). In terms of conventional pharmacological interventions for hyperglycemia, several classes of drugs have been established, including biguanides (which lower the production of hepatic glucose); peroxisome proliferator-activated receptor-γ (PPARγ) agonists (build-up insulin action); sulfonylureas (stimulate pancreatic islet to release insulin) and α-glucosidase inhibitors (delay absorption of glucose in the gastrointestinal tract) (8). In modern years, there has been growing interest in the use of herbal medicines as hypoglycemic agents, owing to their rich source of compounds that can be utilized in drug synthesis. Herbal drugs are often characterized by their safety, low toxicity, minimal side effects, and affordability, making them an attractive alternative for disease treatment (9).

Trigonellafoenum-graecum (fenugreek seed) has demonstrated promising hypoglycemic, anti-hypertensive, and hypolipidemic properties in both animal and human studies (10). The bioactive compounds within fenugreek seeds, including diosgenin, trigonelline, galactomannans, fiber, protein, and bitter principles, contribute to its therapeutic potential, particularly in managing hyperglycemia and hypercholesterolemia (11). Notably, fenugreek oil causes a decrease in blood glucose levels by blocking alpha-amylase activity in a dose-dependent manner, thus reducing the breakdown and absorption of starch (12). Additionally, the fenugreek hypoglycemic effect is linked to the inhibition of glucose uptake, with studies showing that fenugreek oil impairs sodium-dependent glucose transport in the intestines (13).

MATERIALS AND METHODS

Experimental Animals

For the investigation, a total of twenty-five (25) male Wistar rats weighing about 120g– 150g were employed. The animals were acquired and grown under a continuous 12-hour light-dark cycle in the Animal House Section of the College of Health Sciences, Osun State University Osogbo. Before the experiment started, they were given two weeks to acclimatized and were provided conventional pelletized feeds and clean drinking water *ad libitum*. This study was performed in alignment with the College of Health Sciences animal rights policy at Osun State University in Osogbo, under reference number UNIOSUNHREC 2024/011B

Induction of Diabetes Mellitus

This was done following method of Mohammed *et al*. (14). To induce insulin resistance, the animals were administered a 10% fructose solution for a two-week period *ad libitum*. Subsequently, a single injection of streptozotocin (STZ) at 40 mg/Kg mixed in citrate buffer (pH 4.5) was administered intraperitoneally to overnight-fasted rats to induce pancreatic beta-cell dysfunction. Fasting blood samples were taken from the tail vein to measure fasting blood glucose levels using a portable glucometer (Accu-Chek Active) and test strips. Rats with blood glucose levels exceeding 200 mg/dL were identified as diabetic (1).

Experimental design

The rats were split into five groups, with five (5) per group as follows:

Group 1 (non-diabetic control group)

Group 2 (untreated Type 2 Diabetes)
Group 3 (Type 2 diabetic + 1ml/Kg of Fenugreek oil)
Group 4 (Type 2 diabetic +0.5ml/Kg of Fenugreek oil)
Group 5 (Type 2 diabetic + 100mg/kg of Metformin)
The route of administration of fenugreek was oral for 28 days.

Animal Sacrifice and Sample Collection

The animals were anesthetized using sodium thiopentone (50 mg/kg i.p.) and then incised from the abdominal region to the thoracic region. The caudal epididymis was used to examine various sperm parameters, while the testes and pancreas were preserved in Bouin's fluid and 10% formalin for histological analysis. The blood was collected via cardiac puncture, and drawn into the plain bottle to obtain serum.

Sperm Analysis

Sperm analysis was performed as described by Adedokun *et al*. (15). Spermatozoa obtained from the caudal epididymis were examined under an Olympus research microscope (Olympus, Japan) with a 40x objective lens. Progressive motility was evaluated promptly and expressed as a percentage rounded to the closest whole number. Sperm viability percentage (live sperm) was assessed using the eosin-nigrosin staining technique, as recommended by the World Health Organization. The non-motile (dead) sperm took up the stain, leaving the active (live) sperm cells unstained. Sperm morphology and count were equally evaluated.

Hormonal Assay

The blood samples were collected via cardiac puncture and then centrifuged at 3000 rpm for 10 minutes to isolate the serum samples. The resulting serum was subsequently used to measure levels of gonadotrophin releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and insulin using the enzyme-linked immunosorbent assay (ELISA) kits (Calbiotech, USA) as described by Adedokun *et al*. (16)

Assessment of Oxidative Stress Markers

Malondialdehyde (MDA) concentration and superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) activities according to the procedure described by Adedokun *et al*. (16).

Histological Studies

This procedure was carried out as outlined by Akpantah et al. (17). The testes and pancreas were initially fixed in 10% formalin for 24 hours, followed by dehydration in 70% alcohol. Subsequently, the tissues were passed through 90% alcohol and chloroform for 30 minutes each before being embedded in molten paraffin wax at 57°C in an oven for 20 minutes each. Serial portions were cut to a thickness of 5 microns using a rotary microtome. Tissue slides were prepared and deparaffinized by passing through two changes of absolute alcohol, followed by 70% alcohol and water for 5 minutes. The slides were subsequently stained using hematoxylin.

Statistical Analysis

The Guassian distribution test was done using Anderson-Darling test and Shapiro-Wilk normality test. All the data were normally distributed with p value < 0.05 considered statistically significant.The data were presented as means ± standard error of the mean (SEM) and analysed using one-way analysis of variance (ANOVA). All statistical comparisons and post-hoc tests (Tukey test) were conducted using GraphPad Prism 5. A significance level of $P < 0.05$ was considered statistically significant

RESULTS

Effects of Fenugreek Oil on Blood Glucose Levels in Type 2 Diabetic Male Wistar Rats

A significant increase in blood glucose levels was observed in the T2DM, T2DM + FGO 1 mL/Kg, T2DM + FGO 0.5 mL/Kg, and T2DM + MET 100 mg/Kg groups compared to the non-diabetic control group. However, a significant reduction in blood glucose levels was noted in the T2DM + FGO 1 mL/Kg, T2DM + FGO 0.5 mL/Kg, and T2DM + MET 100 mg/Kg groups when compared to the untreated diabetic group (T2DM).

Effects of Fenugreek Oil on Serum Insulin, HOMA-IR, and HOMA-Beta in Type 2 Diabetic Male Wistar Rats.

Serum insulin and HOMA-beta (homeostatic model assessment for beta cells) significantly reduced, while HOMA-IR (homeostatic model assessment for insulin resistance) notable increase in T2DM, T2DM + 0.5mL/kg and T2DM + 100mg/Kg groups when compared with the control group. However serum insulin and HOMA-beta notably increase while HOMA-IR significantly reduced in T2DM + 1mL/kg and T2DM +100mg/kg groups in

comparison with T2DM group, indicating improved insulin sensitivity and pancreatic beta-cell function.

Effects of Fenugreek Oil on Sperm Parameters in Type 2 Diabetic Male Wister Rats:

Sperm motility significantly decreases ($p<0.05$) in T2DM group in comparison with non-diabetic control group. However, sperm motility in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg) and T2DM + MET (100 mg/ Kg) groups significantly increased when compared with T2DM group.

When analyzed, the sperm viability level significantly decreases ($p<0.05$) in the T2DM group in comparison with the non-diabetic control group. However, a notable increase ($p<0.05$) in sperm viability was observed in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg), and T2DM + MET (100 Mg/Kg) when compared with the untreated diabetic group (T2DM).

Normal sperm morphology significantly decreased ($p<0,05$) in T2DM when compared with non-diabetic control group. Whereas, sperm morphology in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg), and T2DM + MET (100 mg/Kg) groups significantly increased in comparison with T2DM group.

Sperm count significantly decrease ($p<0.05$) in T2DM, T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg), and T2DM + MET (100 mg/Kg) groups when compared with the control. However, sperm count significantly increased in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg), and T2DM + MET (100 mg/Kg) groups when compared with T2DM group.

The effect of Fenugreek Oil on testicular oxidative stress biomarker (serum) in type II diabetic male Wistar rats

The testicular MDA in T2DM, T2DM + FGO 1 mL/Kg, T2DM + FGO 0.5 mL/Kg, and T2DM + MET 100 mg/Kg groups notably increased in comparison with non-diabetic control. However, there was a notable reduction in MDA level in T2DM + FGO 1 mL/Kg, T2DM + FGO 0.5 mL/Kg, and T2DM + MET 100 mg/Kg groups when compared with the untreated diabetic group.

The level of GSH, SOD, and CAT activities notably decreased in the untreated diabetic group in comparison with the non-diabetic control. However, a notable increase in GSH, SOD, and CAT were noted in T2DM + FGO 1mL/Kg, T2DM + FGO 0.5mL/Kg, and T2DM + MET 100mg/Kg groups when compared with the T2DM group.

Effect of Fenugreek oil on Serum GnRH in Type 2 diabetic male Wistar rats

There was a notable decrease ($p<0.05$) in GnRH in T2DM in comparison with the non-diabetic control while there was no relevant difference in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg) and T2DM + MET (100 mg/Kg) when compared with control. However, GnRH levels in T2DM + FGO (1mL/Kg), T2DM + FGO (0.5mL/Kg), and T2DM + MET (100 mg/Kg) treated groups significantly increased when compared with the untreated diabetic group.

Effect of Fenugreek oil on Serum FSH in Type 2 diabetic male Wistar rats

There was a notable decrease ($p<0.05$) in FSH in T2DM in comparison with non-diabetic control while there was no relevant difference in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg) and T2DM + MET (100 mg/Kg) when compared with control. However, FSH levels in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg), and T2DM + MET (100 mg/Kg) treated groups significantly increased when compared with the untreated diabetic group.

Effect of Fenugreek oil on Serum LH in Type 2 diabetic male Wistar rats

There was no relevant decrease ($p<0.05$) in LH in T2DM, T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg), and T2DM + MET (100 mg/Kg) when compared with non-diabetic control. However, LH level in T2DM + FGO (1 mL/Kg) treated group notably increased in comparison with the untreated diabetic group, and T2DM + FGO (0.5 mL/Kg) and T2DM + MET (100 mg/Kg) treated groups had no relevant difference when compared with untreated diabetic group.

Effect of Fenugreek Oil on Serum Testosterone in Type 2 Diabetic Male Wistar Rats

There was a notable decrease ($p<0.05$) in testosterone in T2DM in comparison with non-diabetic control while there was no relevant difference in T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg) and T2DM + MET (100 mg/Kg) when compared with non-diabetic control. Thus, testosterone levels in T2DM + FGO (1 mL/Kg) treated group notably increased in comparison with the untreated diabetic group, and T2DM + FGO (0.5 mL/Kg) and T2DM + MET (100 mg/Kg) treated groups had no relevant difference when compared with untreated diabetic group.

The effect of Fenugreek Oil on testicular oxidative stress biomarker (serum) in type 2 diabetic male Wistar rats

The testicular MDA in T2DM, T2DM + FGO 1 mL/Kg, T2DM + FGO 0.5 mL/Kg, and T2DM + MET 100 mg/Kg groups notably increased in comparison with non-diabetic control. Thus, there was a notable reduction in MDA, T2DM + FGO 1 mL/Kg, T2DM + FGO 0.5 mL/Kg, and T2DM + MET 100 mg/Kg when compared with the untreated diabetic group.

The GSH, SOD, and CAT activities notably decreased in the untreated diabetic group in comparison with the non-diabetic control. However, a notable increase in GSH, SOD, and CAT antioxidant activities were noted in T2DM + FGO 1 mL/Kg, T2DM + FGO 0.5 mL/Kg, and T2DM + MET 100 mg/Kg groups when compared with the T2DM group.

Effect of Fenugreek oil on Histology of Testis in type 2 diabetic male Wistar rats.

Photomicrograph of the normal testicular section showing several normal seminiferous tubules showing lumen containing spermatozoa (white arrow), normal and completely developed germinal cells in non-diabetic control, T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg) and T2DM + METFORMIN (100mg/Kg). The interstitial spaces contained normal Leydig cells (slender arrow). In contrast, some seminiferous tubules in the T2DM group showed maturation arrest (black arrow), indicating disrupted spermatogenesis.

Effect of Fenugreek oil on Histology of Pancreas in type 2 diabetic male Wistar rats.

Photomicrograph of the pancreatic section showing several normal pancreatic parenchyma, including acinar and zymogenic cells, normal interlobular connective tissue (blue arrow), normal islets of Langerhans (white arrow), in non-diabetic control, T2DM + FGO (1 mL/Kg), T2DM + FGO (0.5 mL/Kg) and T2DM + MET (100mg/Kg). In contrast, T2DM showed degeneration and infiltration of islets of langerhans by inflammatory cells (stroked white arrow), highlights acinar and zymogenic cells with inter-acinar spaces infiltrated by inflammatory cells (red slender arrow).

DISCUSSION

Studies have shown that DM, as an endocrine disorder, currently affects over 400 million people worldwide, accounting for 9.1% of the global population (18). Its prevalence is increasing rapidly, with estimates suggesting that by 2040, 642 million individuals will be impacted by diabetes (18). Type 2 diabetes mellitus (T2DM) is known to contribute to male sexual dysfunction, primarily through autonomic neuropathy and endothelial dysfunction (19). In addition, T2DM induces oxidative stress in nearly all tissues, including the male reproductive organs, which is a key factor in the reproductive impairments, observed in diabetic individuals (20). However, Fenugreek has demonstrated antioxidant and hypoglycemic properties, potentially improving male reproductive health (21). This study investigates the impacts of fenugreek oil on blood glucose levels and reproductive dysfunction in male Wistar rats with T2DM.

The significant elevation in blood glucose levels and reduction in insulin secretion noted in this study align with previous research demonstrating the administration of fructose *ad libitum* for two weeks, followed by streptozotocin can induce insulin resistance and cause hyperglycemia (14). This increase in blood glucose was however alleviated following administration of fenugreek oil. This confirmed earlier reports of the hypoglycemic properties of fenugreek oil (22). Fenugreek oil has been shown to lower hyperglycemia through various mechanisms, including delayed gastric emptying, reduced glucose absorption rate, inhibition of α-amylase activity, and decreased hepatic glucose-6-phosphatase activity, thereby suppressing hepatic gluconeogenesis (23).

The increase in insulin production observed in fenugreek-treated rats after induction of type-2 diabetes shows that fenugreek oil can regenerate the pancreatic beta cell. This is in line with earlier studies that insulin secretion increases due to the regeneration of pancreatic tissue (24&25). An increase in insulin sensitivity also observed in fenugreek-treated rats is an indication that fenugreek oil might have increased the sensitivity of the tissue to insulin. This is also in concordance with earlier report that fenugreek improves insulin function at the cellular level, leading to improved insulin sensitivity (22). Photomicrograph of the pancreas equally corroborated the result observed on insulin level in this study by showing poor architecture in type 2 diabetic treated rats while better cytoarchitecture was observed following administration of fenugreek oil.

The significant decrease in sperm parameters observed in the diabetic group indicates the detrimental impact of type 2 diabetes mellitus (T2DM) on male fertility. This finding aligns with Gandhi et al. (19), who reported that

T2DM contributes to male sexual dysfunction through autonomic neuropathy and endothelial dysfunction. However, the reduction in sperm parameters was mitigated following the administration of fenugreek oil, suggesting its protective effect on male reproductive health. The histological distortion of the testes in the diabetic group further highlights the negative impact of T2DM on testicular cytoarchitecture, which was restored following fenugreek oil treatment. This restoration corroborates the improvements in sperm parameters and supports previous research by Korejo et al. (26), who noted significant pathological degenerative changes in epididymal tissues and secretory dysfunction in diabetic rats.

Furthermore, a significant reduction in serum GnRH, FSH, LH, and testosterone levels noted in the diabetic group in this research corresponds with the results of Annie et al., (27), who reported that diabetic rats exhibited decreased concentrations of serum insulin, testosterone, FSH, and LH. This decrease in HPT hormones was corrected following the administration of Fenugreek oil.

The testicular oxidative stress biomarkers evaluated in this study include MDA, CAT, GSH, and SOD. A notable increase in MDA levels as well as decrease in cellular antioxidant activities observed in the diabetic group suggests heightened lipid peroxidation. Previous study has shown that MDA blood level increases with a marked reduction in the functionalities of antioxidant enzymes in diabetic conditions (28). Oxidative stress has been recognized as a significant factor in reproductive dysfunction and impairments in individuals with T2DM (20). However, the MDA level significantly decreases following the administration of Fenugreek oil. This is in line with earlier report which stated that MDA level decreases following administration of Fenugreek oil (29). Importantly, the administration of fenugreek oil restored the levels of CAT, SOD, and GSH, aligning with findings by Rahigude et al., (30) that fenugreek oil enhances antioxidant enzyme activity in the testes. Fenugreek's well-documented antioxidant and hypoglycemic properties (21) contribute to its protective effects on male reproduction. Therefore, this shows that Fenugreek oil is potent in decreasing lipid peroxidation and increasing antioxidant enzyme levels.

CONCLUSION

Type-2 diabetes causes male reproductive dysfunction by a reduction in sperm indices, a decrease in hypothalamic pituitary testicular hormones, and induction of testicular oxidative stress as well as distortion of testicular cytoarchitecture. However, fenugreek oil attenuates the glucose level as well as reproductive dysfunctions induced by type 2 diabetes in male Wistar rats. Therefore, fenugreek oil may serve as a promising therapeutic agent for addressing type 2 diabetes and its related reproductive complications in males.

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Authors' Contribution

KIA: Conceptualization, Methodology, Writing- Original draft preparation;
***ITJ**.:* Methodology, collection of data, original draft preparation;
***JTA**.:* Methodology, collection of data, original draft preparation;
OOO: Investigation, Writing- Reviewing and Editing;
AOA: Visualization, Software analysis;
OSO: Reviewing and Editing;
TGA: Conceptualization, Software analysis.

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Table 1: The Effects of Fenugreek Oil on insulin, HOMA-IR Serum and HOMA-Beta in Type 2 Diabetic Male Wistar Rats.

	CONTROL	T2DM	T2DM + 1mL/Kg FGO	T2DM+0.5mL/ Kg FGO	T2DM +MET(100mg/Kg)
SERUM INSULIN (MIu/mL)	4.96 ± 0.37	2.16 ± 0.25$^{\alpha}$	4.58 ± 0.63$^{\beta}$	4.01 ± 0.52$^{\alpha\beta}$	3.84 ± 0.41$^{\alpha\beta}$
HOMA-IR	1.06 ± 0.05	2.12 ± 0.67$^{\gamma}$	1.14 ± 0.16$^{\delta}$	1.35 ± 0.50	1.15 ± 0.36$^{\delta}$
HOMA-β	86.73± 22.59	2.32 ± 0.50$^{\alpha}$	44.81 ±12.17$^{\alpha\beta}$	21.17 ± 9.02$^{\alpha\beta}$	35.95 ±11.27$^{\alpha\beta}$

α - Illustrates a notable decrease compared to the non-diabetic control group
β - Illustrates a notable increase compared to untreated Type 2 DM
γ- Illustrates a notable increase compared to non-diabetic control group
δ- Illustrates a notable decrease compared to untreated Type 2DM

Table 2: Effects of Fenugreek Oil on Sperm Parameters in Type 2 Diabetic Male Wistar Rats.

	CONTROL	T2DM	T2DM+FGO (1 mL/Kg)	T2DM+FGO (0.5 mL/Kg)	T2DM+MET (100 mg /Kg)
MOTILITY (%)	81.20 ± 1.07	60.40 ± 0.98$^{\alpha}$	77.60 ± 1.12$^{\beta}$	77.60 ± 1.12$^{\beta}$	81.00 ± 1.00$^{\beta}$
VIABILITY(%)	89.00 ± 1.00	75.80 ± 1.24$^{\alpha}$	87.60 ± 1.12$^{\beta}$	85.80 ± 1.20$^{\beta}$	87.00 ± 1.09$^{\beta}$
MORPHOLOGY(%)	83.20 ± 1.02	68.60 ± 1.12$^{\beta\sigma}$	83.60 ± 1.21$^{\alpha}$	75.00 ± 1.14$^{\alpha}$	81.20 ± 1.24$^{\alpha}$
COUNT (10^6/mL)	234.8 ± 1.20	158.60 ± 1.33$^{\alpha}$	222.80 ± 1.20$^{\alpha\beta}$	221.00 ± 1.30$^{\alpha\beta}$	220.20 ± 1.16$^{\alpha\beta}$

Values were expressed as Mean ± SEM
α - Illustrates a notable decrease ($p<0.05$) when compared with non-diabetic control group
β- Illustrates a notable increase ($p<0.05$) when compared with untreated diabetic group
T2DM- implies type II diabetic mellitus
FGO- implies Fenugreek Oil
MET- implies Metformin

Table 3: The Effect of Fenugreek Oil on Testicular Oxidative Stress Biomarker (Homogenate) in Type 2 Diabetic Male Wistar Rats

	CONTROL	T2DM	T2DM+FGO (1 mL/Kg)	T2DM+FGO (0.5 mL/Kg)	T2DM+MET (100 mg/Kg)
MDA (μ/mg)	0.60 ± 0.04	0.77 ± 0.03$^{\delta\beta}$	0.60 ± 0.03$^{\alpha\sigma}$	0.64 ± 0.02$^{\delta\beta}$	0.64 ± 0.04$^{\delta\beta}$
GSH (μ/mg)	0.05 ± 0.006	0.02 ± 0.002$^{\alpha\sigma}$	0.06 + 0.002$^{\delta\beta}$	0.04 ± 0.007$^{\alpha\sigma}$	0.03 ± 0.002$^{\alpha\sigma}$
SOD (μ/mg)	0.14 ± 0.02	0.02 ± 0.002$^{\alpha\sigma}$	0.15 ± 0.05$^{\beta\delta}$	0.14 ± 0.005$^{\alpha}$	0.14 ± 0.004$^{\alpha}$
CAT (μ/mg)	13.99 ± 0.54	10.65 ± 0.83$^{\alpha\delta}$	20.65 ± 1.64$^{\delta\beta}$	16.16 ± 1.97$^{\delta\beta}$	14.91 ± 3.52$^{\delta\beta}$

Values were expressed as Mean ± SEM
α – Illustrates a notable decrease ($p<0.05$) when compared with non-diabetic control group
β- Illustrates a notable increase ($p<0.05$) when compared with non-diabetic control group
σ- Illustrates a notable decrease ($p<0.05$) when compared with untreated diabetic group
δ- Illustrates a notable increase ($p<0.05$) when compare with untreated diabetic group
T2DM- implies type II diabetic mellitus
FGO- implies Fenugreek Oil
MET- implies Metformin

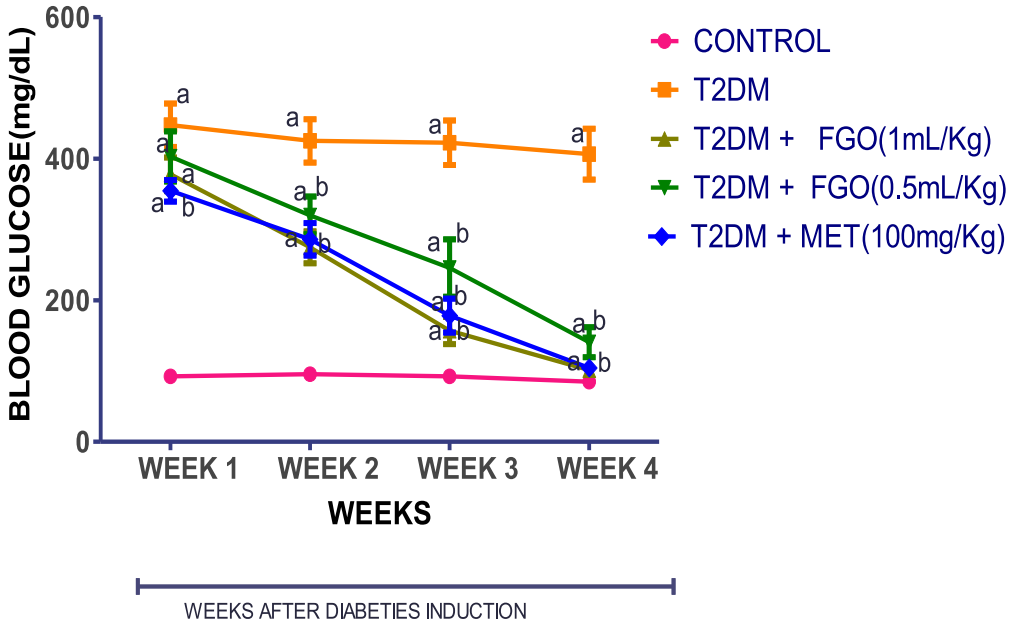

Fig. 1: Blood glucose levels in Type 2 Diabetic Male Wistar Rats in 4 weeks of administration of Fenugreek oil (FGO).

a - significant increase compares to non-diabetic control group
b - significant decrease compares to untreated Type 2 diabetes mellitus group

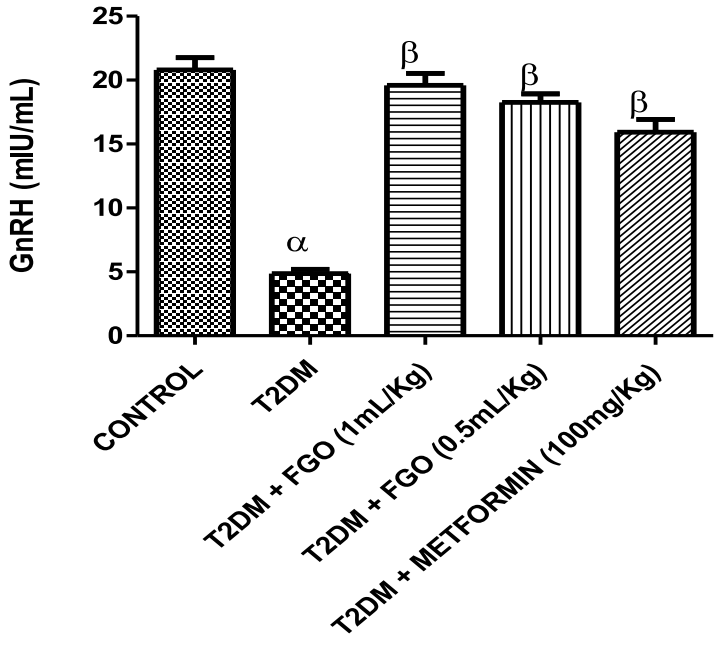

Fig 2: Effect of Fenugreek oil on Serum GnRH in Type 2 Diabetic male Wistar rats

α Illustrates a notable decrease ($p<0.05$) compared with non-diabetic control group
β Illustrates a notable increase ($p<0.05$) compared with untreated diabetic group

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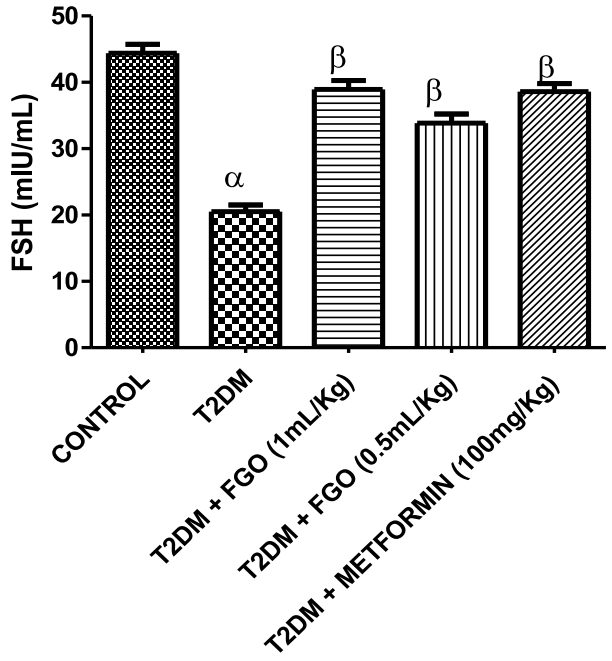

Fig 3: Effect of Fenugreek oil in Serum FSH in Type 2 diabetic male Wistar rats

α Illustrates anotable decrease ($p<0.05$) compared with non-diabetic control group
β Illustrates anotable increase ($p<0.05$) compared with untreated diabetic group

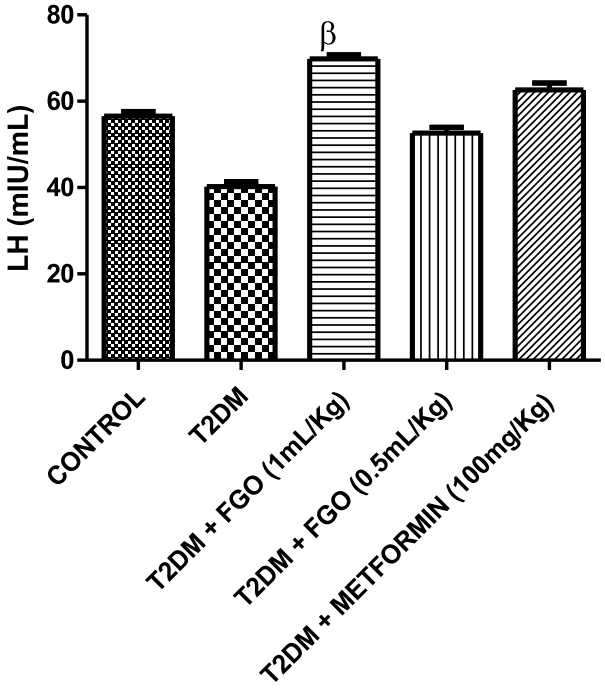

Fig 4 Effect of Fenugreek oil on Serum LH in Type 2 diabetic male Wistar rats

α Illustrates anotabledecrease ($p<0.05$) compared with non-diabetic control group
β Illustrates anotable increase ($p<0.05$) compared with untreated diabetic group

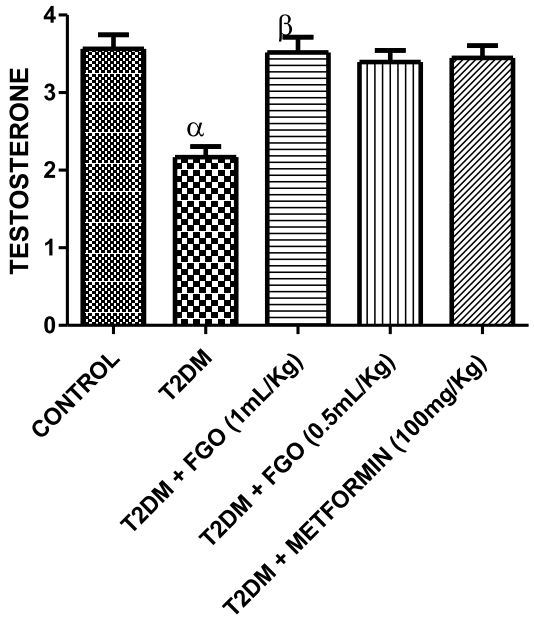

Fig 5 Effect of Fenugreek oil on Serum Testosterone in Type 2 diabetic male Wistar rats

α Illustrates anotable decrease ($p<0.05$) compared with non-diabetic control group
β Illustrates anotable increase ($p<0.05$) compared with untreated diabetic group

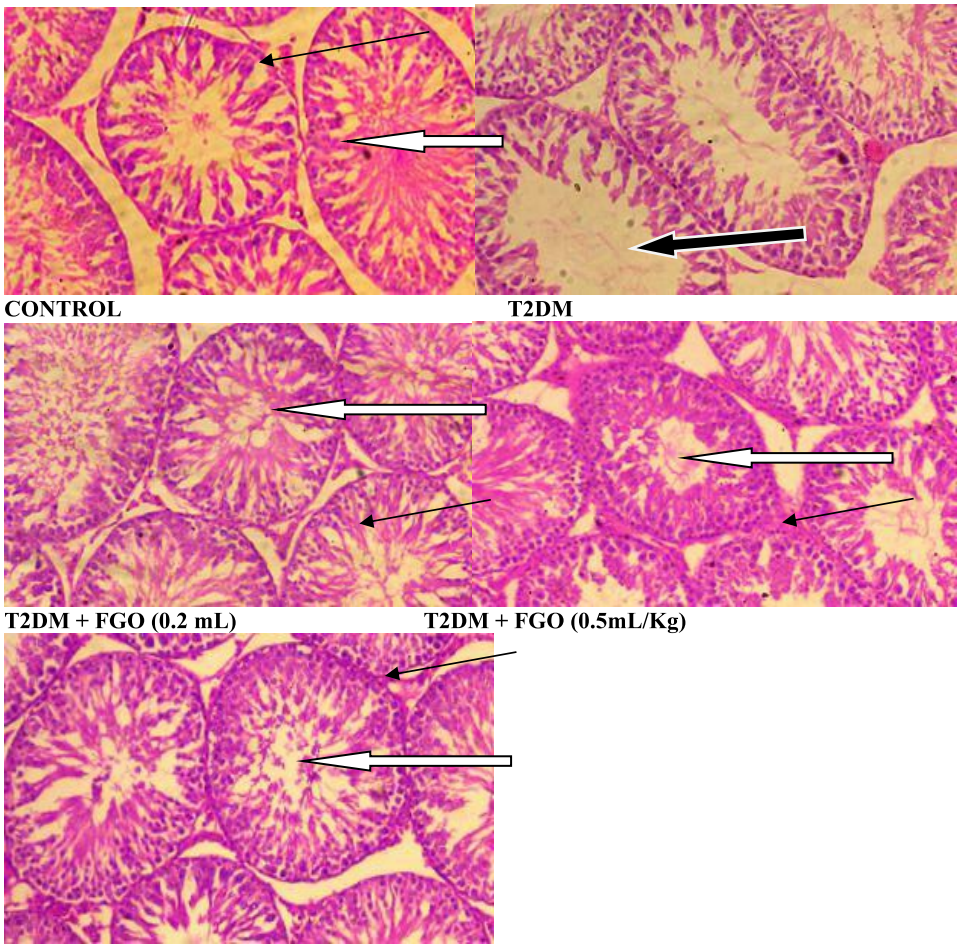

Figure 6: Photomicrograph of testicular section stained by Hematoxylin and Eosin (X400).
KEYS
White arrow indicates normal lumen with spermatozoa
Black arrow indicates maturation arrest
Slender arrow indicates normal Leydic cells

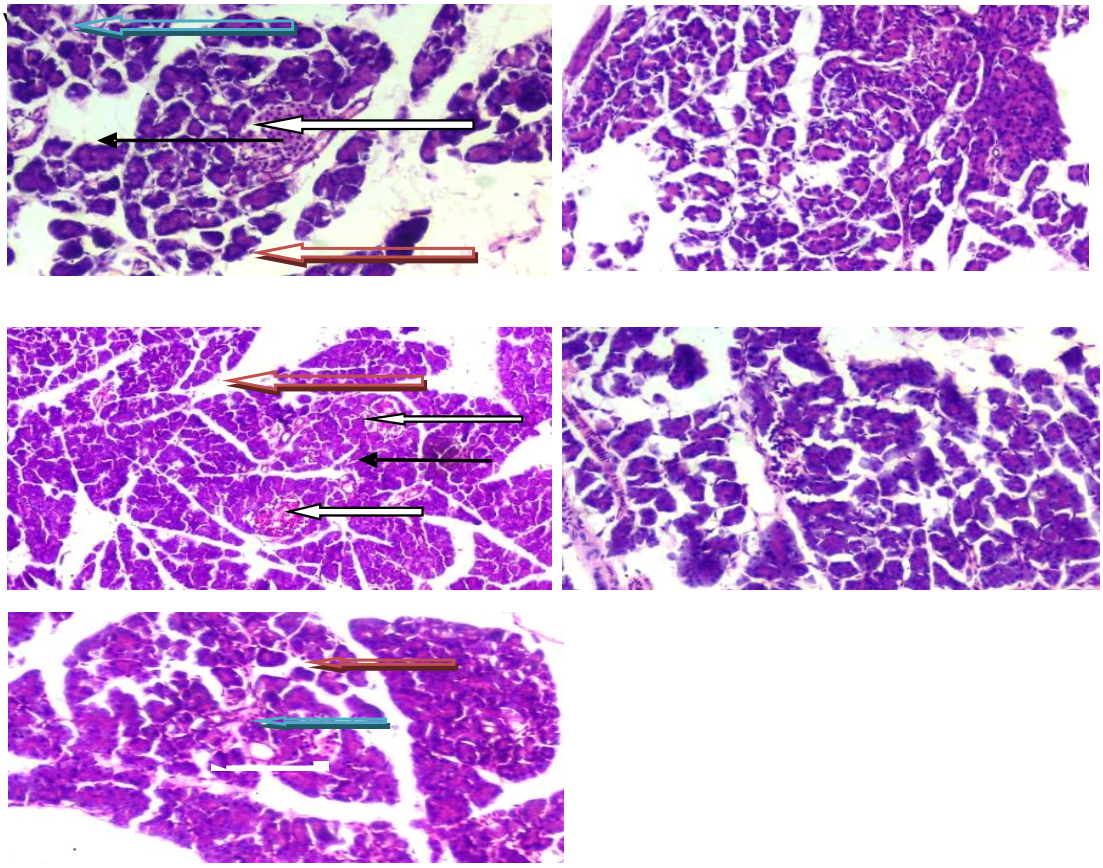

Figure7 Photomicrograph of pancreatic section stained by Hematoxylin (X400) E

KEY
Slender arrow- Normal pancreatic parenchyma, including acinar and zymogenic cells.
Blue arrow- Represents normal interlobular connective tissue.
Red arrow- Normal septa.
White arrow- Denotes the normal islets of Langerhans, comprising round to oval endocrine cells.
Red slender arrow- Highlights acinar and zymogenic cells with interspaces infiltrate by inflammatory cells.
Stroked white arrow- Degenerated and infiltrated of islets of langerhans by inflammat
White slender arrow- Acinar and zymogenic cells shows a mild infiltrating inflamma