

Collection, Standardization and Phytochemical Evaluation of *Trigonella Foenella- Gracum* Against Diabetes Mellitus

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Abstract

The present research work include the Anti-Diabetic, Anti-Oxidant and Anti-Hyperlipidemic activity of *Trigonella foenella- gracum*, focuses on the phytochemical investigation and antidiabetic potential of this same plant. Here in this study the collection and authentication of seeds were done and then finally extraction process of *Trigonella foenum-gracum* seeds plants was completed by maintaining temperature for various solvents. After extraction in various solvents and identification through applying various tests the presence was confirmed. Successive extraction of the plant materials was carried out, followed by detailed phytochemical screening. The extracts were found to contain several bioactive constituents, including tannins, phenolic compounds, carbohydrates, glycosides, terpenoids, proteins, amino acids, and flavonoids. Phenolic compounds and flavonoids, in particular, are widely recognized for their therapeutic value in controlling hyperglycemia and combating oxidative stress. The ethanolic extracts of *T. foenum-graecum* showed a strong presence of these beneficial phytochemicals. The findings of the present investigation demonstrate that the ethanolic extracts of *T. foenum-graecum* exhibit significant antidiabetic effects in streptozotocin (STZ)-induced diabetic rats. Their beneficial activity appears to be associated with improved glucose tolerance, enhanced utilization of glucose, and restoration of depleted liver glycogen stores at both tested doses (200 and 400 mg/kg).

KeyPoints :-STZ, *Trigonella foenum-graecum*, DM, DMSO, Westar rat.

1. Introduction

It is endocrinal metabolic condition marked by elevated blood glucose levels and disturbances in the metabolism of lipids, carbohydrates, and proteins, which in turn heightens the risk of developing cardiovascular complications (Daisy et al., 2009). DM has emerged as a significant global health issue because of its rapidly increasing occurrence. It impacts individuals across all age groups, economic levels, and demographic sectors in almost every part of the world. The highest occurrence of the disease is observed in low- and middle-income countries (Sidahmed et al., 2023). At present, numerous countries are approaching a global diabetes epidemic, which is advancing rapidly across the globe (Selvin and Juraschek, 2020). Poorly managed diabetes often leads to persistent high blood sugar levels (hyperglycemia), which, over time, can cause significant damage to various body systems, affecting blood vessels as well as nerve tissues (Bishu et al., 2019). Persistent hyperglycemia in DM leads to damage, impaired function, and eventual failure of multiple organs and tissues, contributing to onset of microvascular disorders like retinopathy, nephropathy, cardiovascular disorders as well as neuropathy (Harding et al., 2019). A hyperglycemic state, combined with impaired insulin function, hyperlipidemia, and oxidative stress, facilitates the onset of both microvascular and macrovascular disorders. These include conditions like diabetic retinopathy, neuropathy, cardiovascular diseases, foot ulcers, and even limb amputations (Control and Trial, 2005). Early indicators of hyperglycemia may include polydipsia, increased appetite, excessive need to urinate, headaches, blurry sight, fatigue, unintended loss of weight, recurrent vaginal yeast or skin infections, and delayed wound-healing or sores- many of which are also associated with prolonged elevated blood sugar levels (Tegegne et al., 2024). Despite some variations in classification, four primary types of diabetes are widely recognized across the literature: (1) Type 1 DM, resulting from the immune-mediated destruction of pancreatic β -cells; (2) Type 2 DM, characterized by a progressive decline in insulin secretion, often associated with insulin resistance; (3) Gestational DM, which is first identified in the mid to late stages of pregnancy; and (4) Other diabetes types resulting from distinct factors, such as drug/chemical-influenced diabetes as well as monogenic forms, including neonatal diabetes as well as MODY (Tegegne et al., 2024; Leslie et al., 2016).

1.1 Pathophysiology

1.1.1 Type I Diabetes Mellitus

T1DM (Type I diabetes mellitus) is a complex disorder governed by genetic predisposition as well as environmental factors that disrupt immune regulation. It develops due to autoimmune response, in which CD⁴⁺ as well as CD⁸⁺ T-cells, along with macrophages, attack and destruct insulin-secreting beta cells in the pancreas. This autoimmune activity is identified by the presence of specific markers, including: (1) islet cell autoantibodies, (2) insulin autoantibodies, (3) auto-antibodies against GAD65 (glutamic acid decarboxylase) as well as (4) auto-antibodies targeting tyrosine phosphates IA-2 as well as IA-2 β (Toren et al., 2021; Roep et al., 2021).

1.1.2 Type II Diabetes Mellitus

Accounting for more than 90% of total diabetes cases, type 2 diabetes mellitus is the most widespread form and affects individuals across all age groups (Ruze et al., 2023). It is primarily characterized by two key features: reduced insulin secretion due to pancreatic β -cell dysfunction and decreased insulin effectiveness caused by insulin resistance. In response to this resistance, pancreatic β -cells often attempt to compensate by increasing insulin production (Dludla et al., 2023; Hurtado and Vella, 2019). However, when this compensation is insufficient, hyperglycemia ensues. The genetic inheritance pattern of T2DM is generally intricate as well as incompletely understood, with MODY as exception, which follows an autosomal dominant inheritance pattern because of glucokinase gene mutations located on chromosome 7p (Holt, 2004).

2. Materials and methods

2.1 Collection And Authentication Of Plant Material

Seeds of *Trigonella foenum-graecum* were procured from local market and this plant parts were authenticated by a botanist at the Department of Botany of University. The authenticated plants were used for preparation of extracts.

2.2 Extract Preparation:

All the plant materials were washed, dried in the shade at room temperature, and then ground into a fine powder using an electric grinder. The powder was stored in airtight containers. It was also passed through sieve no. 44 before being kept for extraction. Cleaned and powdered seeds of *Trigonella foenum-graecum*, were used for the extraction work. About 500 grams of the powdered material was evenly placed in a Soxhlet apparatus. Extraction was done using different solvents, starting from non-polar to polar, such as petroleum ether, chloroform, acetone, and ethanol. All solvents were purified before use. The extraction was performed through continuous hot percolation for 72 hours. Aqueous extraction was done separately using the cold maceration method (Harborne, 1998).

2.3 Solvent Extraction:

- Plant powder (50 g) extracted with 500 mL 70% ethanol, utilizing a Soxhlet apparatus for 6-8 hours.
- Extracts were filtered through filter paper (Whatman No. 1) as well as concentrated via rotary evaporator at 40°C.
- Concentrated extracts were kept at 4°C until further use, all solvent used at various temperature are shown in table no. 1.

Table 1 : Showing the temperature of various Solvent used

Sr.No.	Solvent Used	Required Temperature
1	Petroleum Ether	(60-80°C)
2	Chloroform	(55-56°C)
3	Acetone	(55-56°C)
4	Alcohol 90% v/v	(75-78°C)
5	Distilled Water	(40-45°C)

Extraction of plant material using different solvents

• Petroleum Ether Extract of the seeds of *Trigonella foenum-graecum*:

The shade-dried and coarsely powdered seeds of *Trigonella foenum-graecum* were subjected to extraction with petroleum ether (60–80 °C) for 72 hours. Upon completion, the extracts were filtered hot through Whatman filter paper No. 10 to eliminate impurities. The filtrates were concentrated using vacuum distillation until the volume was reduced to one-tenth of the original. The concentrated extracts were then transferred to 100 mL beakers and the remaining solvent was evaporated on a water bath. The residues were placed in a desiccator to remove residual moisture and subsequently stored in airtight glass containers for further experimental use.

- **Chloroform Extract of the seeds of *Trigonella foenum-graecum*:**

The marc remaining after petroleum ether extraction was dried and subsequently subjected to extraction with chloroform (55–56 °C) for up to 72 hours. After the extraction was complete, the solvent was removed by distillation, yielding a dark greenish-yellow residue. This residue was then stored in a desiccator.

- **Acetone Extract of the seeds of *Trigonella foenum-graecum*:**

The marc remaining after chloroform extraction was dried and further extracted with acetone (55–56 °C) for up to 72 hours. Upon completion, the solvent was removed through distillation, resulting in a dark brownish-green residue, which was then stored in a desiccator.

- **Ethanol Extract of the seeds of *Trigonella foenum-graecum*:**

The marc remaining after acetone extraction was dried and subsequently extracted with 95% ethanol (75–78 °C) for 72 hours. After the extraction process, the solvent was removed by distillation, yielding dark greenish-yellow extracts. These extracts were placed in a desiccator to eliminate excess moisture and then stored in airtight glass containers for further use.

- **Aqueous Ether Extract of the seeds of *Trigonella foenum-graecum*:**

The marc remaining after ethanol extraction was dried once more and then subjected to maceration with distilled water in a 2-liter round-bottom flask for 72 hours, with 10 mL of chloroform added daily to prevent fungal growth. After extraction, the mixture was filtered, and the solvent was evaporated to dryness on a water bath. Brown-colored extracts were obtained, which were placed in a desiccator to remove excess moisture and then stored in airtight glass containers for further studies. The percentage yields of all the extracts were subsequently determined.

5.3 Identification Of Phytochemical Active Constituents

5.3.1 Preliminary phytochemical analysis

Extracts obtained (petroleum ether, chloroform, acetone, ethanol, and aqueous) went through preliminary phytochemical screening to detect the presence of bioactive compounds (Kokate et al., 1990; Basset et al., 1985).

5.3.2 Tests for Alkaloids:

a) Dragendorff's Test: About 2 mg of each extract was dissolved in 5 mL of distilled water, followed by the addition of 2 M hydrochloric acid until the solution became acidic. One milliliter of Dragendorff's reagent was then added. Orange/red ppt indicates alkaloid presence.

5.3.3 Tests for Carbohydrates:

a) Anthrone Test: Approximately 2 mg extract was mixed with 10 mL distilled water, shaken thoroughly, filtered. Filtrate was concentrated and treated with 2 mL of Anthrone reagent. The development of a green or blue coloration indicated the presence of reducing sugars in the sample.

5.3.4 Test for Glycosides:

Molisch's Test: Approximately 2 mg of the extract was mixed with 10 mL of distilled water, shaken thoroughly, and filtered. The filtrate was concentrated and treated with 2–3 drops of Molisch's reagent, followed by the careful addition of 2 mL of concentrated sulfuric acid along the side of the test tube to form a distinct layer. The appearance of a reddish-violet ring at the interface indicated the presence of glycosides.

5.3.5 Tests for Proteins and free Amino Acids

plant extract in a small quantity was dissolved in a few millilitres of distilled water, then subjected to these qualitative tests:

1. Million's test: Million's reagent was added to extract solution. Red coloration indicated protein as well as free amino acid presence.

5.3.6 Tests for Fixed oils:

a) Spot Test: A small portion of the extract was pressed between two clean filter papers. The appearance of a translucent oil stain on the paper confirmed the presence of fixed oils.

5.4 Pharmacological Screening

Experimental animals:

The experimental study was conducted using healthy male Wistar albino rats, aged between 8 to 10 weeks and weighing between 180 to 220 grams and they kept under controlled environmental conditions with a temperature of $25 \pm 2^\circ\text{C}$, relative

humidity of 50–60%, and a 12-hour light/dark cycle. The rats were provided with free access to standard laboratory chow and clean drinking water throughout the experimental period.

Induction of Diabetes:

Experimental diabetes was induced in overnight-fasted Wistar albino rats by administering streptozotocin (STZ), a well-known diabetogenic agent. STZ was freshly prepared immediately before use by dissolving it in cold 0.1 M citrate buffer (pH 4.5) to ensure stability and accuracy of dosing. Each rat received a single intraperitoneal injection of STZ at a dose of 50 mg/kg body weight. Following STZ administration, the rats were provided with 5% glucose solution in their drinking water for the next 24 hours to prevent drug-induced hypoglycemia, which may occur due to sudden and rapid insulin release from the damaged pancreatic β -cells. After 72 hours of STZ injection, fasting blood glucose (FBG) levels were determined using a glucometer by collecting a small drop of blood from the tail vein. The rats exhibiting fasting blood glucose levels equal to or greater than 250 mg/dL were considered diabetic and selected for inclusion in the experimental study. These diabetic rats were then stabilized and maintained under standard laboratory conditions before initiating treatment with the respective plant extracts and standard drug.

Experimental groups:

Diabetes induction of experimental animals were randomly allocated into five groups, each consisting of six rats. A separate group of non-diabetic rats was maintained as the normal control group. In total, five groups, each containing six animals, were used for the experimental study. The treatment was continued for a period of 28 consecutive days as per the following grouping plan:

Group I (Normal Control): Non-diabetic rats received 1% DMSO and 1% Tween 80 orally.

Group II (Diabetic Control): Diabetic rats received 1% DMSO and 1% Tween 80 orally.

Group III (Standard): Diabetic rats given metformin (150 mg/kg bw; orally).

Group IV: Diabetic rats given ethanolic extract of *Trigonella foenum-graecum* (200 mg/kg bw; orally).

Group V: Diabetic rats given ethanolic extract of *Trigonella foenum-graecum* at (400 mg/kg bw; orally).

All treatments were given once a day by oral gavage for the full 28-day experimental period.

Sample collection

Before initiating the experiment, the fasting blood glucose (FBG) levels of all rats were recorded. Blood samples were collected weekly through tail vein puncture throughout the study period. During the 28 days of continuous drug administration, blood glucose levels were monitored on days 0, 7, 14, 21, and 28. On the 28th day, following an overnight fast, blood samples were obtained from the retro-orbital plexus under mild ether anesthesia. Collected blood was transferred into clean, dry test tubes and allowed to clot at room temperature for 30 minutes. The samples were then centrifuged at 2000 rpm for 10 minutes, and the clear serum obtained was used for the estimation of various biochemical parameters. For glucose and insulin analysis, blood was collected in tubes containing a mixture of potassium oxalate and sodium fluoride (1:3). Liver tissues were carefully excised, blotted, weighed, and stored at -80°C for the determination of antioxidant enzyme activities.

Biochemical analysis

Blood glucose levels were determined using the glucose oxidase-peroxidase (GOD-POD) method. Serum insulin concentrations were analyzed using a microplate ELISA Kit (Diasorin, Saluggia, Italy). Liver glycogen content was determined following standard procedure (Reitman and Frankel, 1957). Total protein content was estimated using the method of Lowry et al. (1951). Serum urea and creatinine levels were determined using the diacetyl monoxime method (Wybenga et al., 1971) and Jaffe's method (Slot, 1965), respectively. Liver homogenates were prepared in ice-cold 10% potassium chloride solution and used for the assessment of antioxidant enzyme activities- superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as described by Rotruck et al. (1973).

Biochemical parameter investigated in liver tissue

a) Estimation of Plasma Thiobarbituric Acid Reactive Substances (TBARS)

Lipid peroxidation levels in liver tissue were determined using the thiobarbituric acid (TBA) reaction as described by Ohkawa et al. (1979).

Reagents:

1. Trichloroacetic acid (TCA) – 15% solution

2. Hydrochloric acid (HCl) – 0.25 N
3. Thiobarbituric acid (TBA) – 0.38% prepared in hot distilled water
4. TCA–TBA–HCl reagent – freshly prepared by mixing reagents 1, 2, and 3 in the ratio of 1:1:1
5. Stock standard (4.8 mM) – prepared by diluting 0.079 ml of 1,1,3,3-tetramethoxypropane to 100 ml
6. Working standard – obtained by diluting the stock solution to achieve a concentration of 4.8 nM/ml

Procedure

Liver tissue homogenates were made using Tris–HCl buffer (pH 7.5). To 1 ml of the homogenate, 2 ml of freshly prepared TCA–TBA–HCl reagent was added and mixed well. The mixture was heated in a boiling water bath for 15 minutes to develop the color. After cooling, the samples were centrifuged for 10 minutes, and the absorbance of the clear supernatant was read at 535 nm using a reagent blank. Standard solutions (2–10 nM) were processed in the same way to create the calibration curve. The results were reported as nmol of TBARS per 100 g of wet tissue. Here is a simple and clear paraphrased version: Liver tissue homogenates were made using Tris–HCl buffer (pH 7.5). To 1 ml of the homogenate, 2 ml of freshly prepared TCA–TBA–HCl reagent was added and mixed well. The mixture was heated in a boiling water bath for 15 minutes to develop the color. After cooling, the samples were centrifuged for 10 minutes, and the absorbance of the clear supernatant was read at 535 nm using a reagent blank. Standard solutions (2–10 nM) were processed in the same way to create the calibration curve. The results were reported as nmol of TBARS per 100 g of wet tissue.

b) Measurement of Lipid Peroxidation

Lipid peroxidation levels in liver tissue were determined following the method of Ohkawa et al. (1979).

Reagents

1. Sodium dodecyl sulfate (SDS) – 4% solution
2. Acetic acid – 20% solution
3. Hydrochloric acid (HCl) – 1.27 M (pH 3.5)
4. Thiobarbituric acid (TBA) – 0.8% (pH 7.4)
5. Tetraethoxypropane – used as a standard

Procedure

The extent of lipid peroxidation, indicated by the formation of thiobarbituric acid reactive substances (TBARS) such as malondialdehyde (MDA), was measured in liver homogenates. To 1 ml of the sample, 0.2 ml of 4% (w/v) SDS, 1.5 ml of 20% acetic acid (adjusted to pH 3.5 with 1.27 M HCl), and 1.5 ml of 0.8% TBA (pH 7.4) were added. The reaction mixture was then heated in a water bath at 85°C for one hour. After cooling, the samples were centrifuged at 1200 g for 10 minutes, and the absorbance of the resulting pink-colored supernatant was measured at 532 nm against a reagent blank. Tetraethoxypropane was used to prepare the standard calibration curve. Results were expressed as millimoles of MDA formed per 100 g of wet tissue.

Result sand Discussion

6.1 Overview

This work provides a comprehensive presentation of the experimental findings obtained from the study of *Trigonella foenum-graecum* (Fenugreek). The chapter is structured to systematically describe the outcomes of the extraction procedures, preliminary phytochemical investigations, and pharmacological evaluations conducted on these plant extracts. Initially, the extraction process for this plant was assessed, and the percentage yield of the respective extracts was calculated to determine the efficiency of the extraction methods employed. Following this, a detailed phytochemical screening was carried out to identify the presence of various bioactive constituents, including alkaloids, flavonoids, glycosides, saponins, tannins, phenols, and other secondary metabolites, which are known to contribute to the therapeutic properties of the plants. Subsequently, the pharmacological activities of the extracts were evaluated, focusing on their anti-diabetic, anti-hyperlipidemic, and antioxidant potential. The anti-diabetic activity was assessed through relevant *in vivo* models, measuring parameters such as blood glucose levels, glucose tolerance, and insulin modulation. The anti-hyperlipidemic effect was determined by evaluating lipid profile parameters, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels. Additionally, the antioxidant potential of the extracts was examined using standard assays to measure their ability to scavenge free radicals and reduce oxidative stress markers.

All experimental data were carefully analyzed using appropriate statistical methods to evaluate the significance of the observed effects in treated groups compared to control groups. This statistical evaluation helped in confirming the reliability and reproducibility of the results, thereby providing a solid foundation for interpreting the pharmacological efficacy of the

studied plant extracts. The findings are presented in a structured format, including tables, figures, and descriptive analyses, allowing for a clear understanding of the therapeutic potential and biochemical characteristics of *T. foenum-graecum*.

6.2 Percentage Yield Of Extracts

Successive solvent extraction of each plant yielded extracts of varying colors, textures, and quantities depending on the solvent polarity. The results are summarized in **Table 6.1**.

Solvent Extraction: Observation

Both the aqueous and ethanolic extracts of *Trigonella foenum-graecum*, exhibited the highest percentage yields among the different solvents tested. This observation suggests that these solvents are particularly effective in extracting a larger proportion of the plant material. The higher extractive yields in water and ethanol can be attributed to the greater solubility of polar bioactive constituents, including phenolic compounds, glycosides, and flavonoids, in polar solvents. These phytochemicals are known for their hydrophilic nature, which allows them to dissolve more readily in aqueous and alcoholic media compared to non-polar solvents. Consequently, the results indicate that aqueous and ethanolic extraction methods are more efficient for isolating the biologically active constituents responsible for the medicinal properties of these plants. Also, the higher yields observed in aqueous and ethanolic extracts can be attributed to the superior solubility of polar bioactive compounds, such as carbohydrates, glycosides, and alkaloids, in these solvents. Polar solvents are more effective at breaking down plant cell walls and solubilizing hydrophilic constituents, resulting in a greater recovery of biologically active compounds. In contrast, non-polar solvents yielded lower extractive values, likely due to their limited ability to dissolve these polar phytochemicals. These findings indicate that water and ethanol are the most suitable solvents for extracting the primary pharmacologically active constituents from these plants. The data also provide a basis for selecting solvents in subsequent phytochemical and pharmacological evaluations, ensuring optimal isolation of compounds responsible for anti-diabetic, anti-hyperlipidemic, and antioxidant activities.

Table 6.1 Extractive values of *Trigonella foenum-graecum* seeds

Plant Name and Part Used	Solvent Used	Color of Extract	Yield (% w/w)
<i>Trigonella foenum-graecum</i> (Seeds)	Petroleum ether	Yellowish brown	4.8
	Chloroform	Greenish yellow	3.2
	Acetone	Dark brown	2.9
	Ethanol	Brownish green	6.4
	Aqueous	Dark brown	7.8

6.3 Phytochemical Screening

Preliminary phytochemical screening of *Trigonella foenum-graecum*, extracts have bioactive constituents, including alkaloids, flavonoids, saponins, glycosides, tannins, and phenolic compounds. The qualitative composition and relative abundance of these phytochemicals varied based on extraction solvent used. Polar solvents such as water and ethanol were found to extract higher concentrations of hydrophilic compounds like carbohydrates and glycosides, , whereas non-polar solvents preferentially extracted lipophilic constituents. These variations highlight the influence of solvent polarity on the solubility and extractability of specific phytochemical classes. The results of this screening provide insight into the chemical profile of each plant and form the basis for understanding their pharmacological activities, as different bioactive compounds contribute to anti-diabetic, anti-hyperlipidemic, and antioxidant effects. The results are summarized in Table 6.2.

Observation

The preliminary phytochemical screening demonstrated that all three plants- *Trigonella foenum-graecum* contained substantial amounts of alkaloids, flavonoids, and phenolic compounds. These classes of bioactive constituents are widely recognized for their therapeutic potential, particularly in exerting antidiabetic and antioxidant effects through mechanisms such as enhancement of insulin sensitivity, modulation of carbohydrate metabolism, and scavenging of free radicals. In addition, *T. foenum-graecum* exhibited a notably high content of saponins, which have been reported to contribute to lipid-lowering, hypoglycemic, and cardioprotective activities.

Table 6.2: Preliminary phytochemical screening of various extracts of *Trigonella foenum-graecum* seeds

Phytochemical Class	Test Method	Petroleum Ether	Chloroform	Acetone	Ethanol	Aqueous
Alkaloids	Mayer's Test	-	+	+	+	+
	Dragendorff's Test	-	+	+	+	+
Flavonoids	Shinoda Test	-	+	+	+	+
	Alkaline Reagent Test	-	+	+	+	+
Saponins	Froth Test	-	-	+	+	+
Glycosides	Keller-Killiani Test	-	+	+	+	+
	Borntrager's Test	-	+	+	+	+
Tannins	Ferric Chloride Test	-	+	+	+	+
	Gelatin Test	-	+	+	+	+
Phenols	Ferric Chloride Test	-	+	+	+	+
	Lead Acetate Test	-	+	+	+	+
Terpenoids	Salkowski Test	+	+	+	+	+
Steroids	Liebermann-Burchard Test	+	+	+	+	+

6.4 Pharmacological Studies

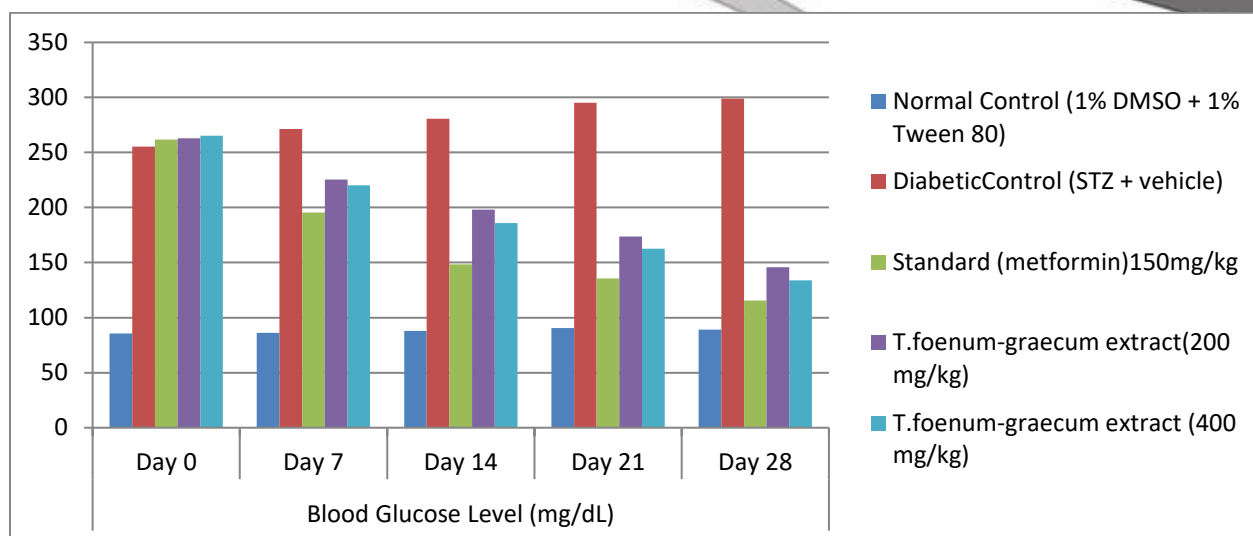
• Screening of Antidiabetic Activity

6.4.1 Hypoglycemic Study in Normal Fasted Rats (Acute) :

In normal fasted rats, a single oral administration of ethanolic extracts of *T. foenum-graecum* at doses of 200 mg/kg and 400 mg/kg did not produce any significant reduction in blood glucose levels up to the 3rd hour of observation. Similarly, the standard drug metformin did not exhibit any marked hypoglycemic effect at the 3rd hour, and glucose levels remained within the normal range. These findings are summarized in **Table 6.4.1**.

Table 6.4.1: Effect of Ethanolic solvent extracts of *Trigonella foenum-graecum* on blood glucose levels (mg/dL) of STZ-induced diabetic rats after single dose treatment.

Groups	Treatment	Blood Glucose Level (mg/dL)				
		Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal Control (1% DMSO + 1% Tween 80)	85.52 ± 4.21	86.26 ± 3.81	88.01 ± 4.11	90.65 ± 4.35	89.17 ± 3.95
II	Diabetic Control (STZ + vehicle)	255.31 ± 6.84	271.41 ± 8.93 [#]	280.72 ± 7.24 [#]	295.12 ± 9.33 [#]	298.85 ± 8.46 [#]
III	Standard (metformin) 150mg/kg	261.85 ± 6.56	195.63 ± 5.44 [*]	148.34 ± 4.85 [*]	135.73 ± 3.92 ^{**}	115.54 ± 3.24 ^{***}
IV	<i>T. foenum-graecum</i> extract (200 mg/kg)	262.96 ± 7.42	225.55 ± 6.38 [*]	198.16 ± 5.83 [*]	173.79 ± 4.92 ^{**}	145.64 ± 4.13 ^{***}
V	<i>T. foenum-graecum</i> extract (400 mg/kg)	265.11 ± 6.92	220.32 ± 5.77 [*]	185.92 ± 4.62 [*]	162.54 ± 4.45 ^{**}	133.73 ± 3.77 ^{**}



Biochemical Parameters

6.4.2 Effect on Serum Lipid Profile

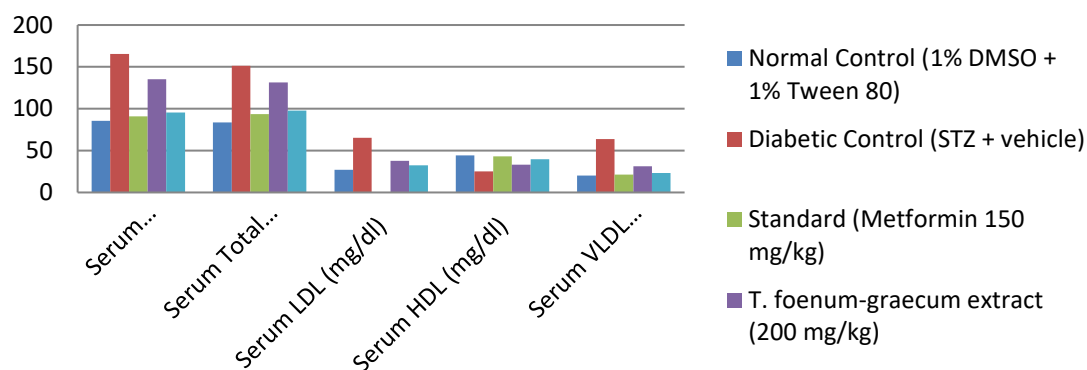
In the STZ-induced diabetic group, a marked alteration in lipid metabolism was observed, as evidenced by a significant rise in total triglycerides (85.42 ± 3.21 to 165.25 ± 6.42 mg/dL), cholesterol (83.56 ± 4.27 to 151.47 ± 5.81 mg/dL), LDL (27.13 ± 2.06 to 65.38 ± 4.95 mg/dL), and VLDL (20.28 ± 0.64 to 63.65 ± 1.28 mg/dL), along with a notable decrease in HDL levels (44.36 ± 2.31 to 25.17 ± 1.43 mg/dL) compared to the normal control group. Treatment with *Trigonella foenum-graecum* at 200 mg/kg and 400 mg/kg for 28 days significantly ameliorated these alterations in a dose-dependent manner.

The extracts produced a marked reduction in total cholesterol, triglycerides, LDL, and VLDL, while HDL levels increased significantly, suggesting an overall improvement in the lipid profile. These findings are summarized in **Table 6.4.2**.

Table 6.4.2: Effect of Ethanolic solvent extracts of *Trigonella foenum-graecum* on serum lipid profile.

Group	Treatment	Serum Triglycerides (mg/dl)	Serum Total Cholesterol (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)	Serum VLDL (mg/dl)
I	Normal Control (1% DMSO + 1% Tween 80)	85.42 ± 3.21	83.56 ± 4.27	27.13 ± 2.06	44.36 ± 2.31	20.28 ± 0.64
II	Diabetic Control (STZ + vehicle)	$165.25 \pm 6.42^{\#}$	$151.47 \pm 5.81^{\#}$	$65.38 \pm 4.95^{\#}$	$25.17 \pm 1.43^{\#}$	$63.65 \pm 1.28^{\#}$
III	Standard (Metformin 150 mg/kg)	90.72 ± 3.54	$93.64 \pm 4.03^{**}$	28.46 ± 2.44	43.18 ± 2.10	21.34 ± 0.72
IV	T. foenum-graecum extract (200 mg/kg)	$135.13 \pm 5.22^*$	$131.42 \pm 5.35^*$	$37.84 \pm 3.68^{**}$	$33.24 \pm 1.96^*$	$31.43 \pm 1.04^{**}$
V	T. foenum-graecum extract (400 mg/kg)	$95.58 \pm 4.63^*$	$97.75 \pm 4.11^*$	$32.42 \pm 2.95^{**}$	$39.69 \pm 2.05^*$	$23.31 \pm 0.88^{***}$

Effect on Serum Lipid Profile

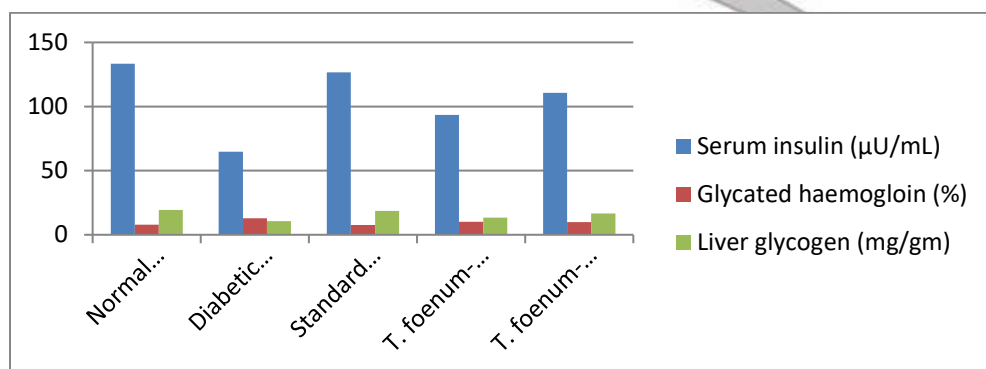


6.4.3 Effect on serum insulin, liver glycogen, and glycated hemoglobin

HbA1c (glycated hemoglobin) serves as a reliable biomarker for assessing the long-term glycemic control in diabetes mellitus. It reflects the extent of non-enzymatic glycation of hemoglobin due to prolonged exposure to elevated blood glucose levels, thereby correlating with the chronic complications of diabetes. In the present study, the STZ-induced diabetic rats exhibited a significant reduction in serum insulin levels (from 133.46 ± 1.65 to 64.78 ± 2.89 $\mu\text{U/mL}$) and liver glycogen content (from 19.43 ± 1.56 to 10.74 ± 0.34 mg/g tissue), accompanied by a marked increase in HbA1c (from 7.92 ± 0.23 to 12.85 ± 0.40 %). Following 28 days of oral treatment with *Trigonella foenum-graecum*, (200 mg/kg and 400 mg/kg), there was a significant improvement in biochemical parameters. Serum insulin levels increased to 93.37 ± 2.58 , 98.42 ± 1.68 , 102.05 ± 1.56 (200 mg/kg) and 110.65 ± 1.34 , 119.34 ± 1.23 , 120.89 ± 1.09 $\mu\text{U/mL}$ (400 mg/kg), while liver glycogen levels rose to 13.29 ± 0.18 , 14.74 ± 0.22 , 15.45 ± 0.12 (200 mg/kg) and 16.58 ± 0.36 , 17.84 ± 0.20 , 18.92 ± 0.85 mg/g tissue (400 mg/kg). Simultaneously, a significant reduction in HbA1c was observed, decreasing to 10.18 ± 0.57 , 10.84 ± 0.61 , 9.63 ± 0.21 % (200 mg/kg) and 9.79 ± 0.23 , 8.09 ± 0.34 , 8.57 ± 0.72 % (400 mg/kg), respectively, when compared with diabetic control rats. These results are presented in **Table 6.7**

Table 6.4.3: Effect of different solvent extracts of *Trigonella foenum-graecum* on serum insulin, glycated haemoglobin and liver glycogen.

Group	Treatment	Serum insulin ($\mu\text{U/mL}$)	Glycated haemoglobin (%)	Liver glycogen (mg/gm)
I	Normal Control (1% DMSO + 1% Tween 80)	133.46 ± 1.65	7.92 ± 0.23	19.43 ± 1.56
II	Diabetic Control (STZ + vehicle)	$64.78 \pm 2.89^{\#}$	$12.85 \pm 0.40^{\#}$	$10.74 \pm 0.34^{\#}$
III	Standard (Metformin 150 mg/kg)	$126.61 \pm 2.08^*$	$7.67 \pm 0.33^{**}$	$18.58 \pm 0.67^*$
IV	<i>T. foenum-graecum</i> extract (200 mg/kg)	$93.37 \pm 2.58^*$	$10.18 \pm 0.57^{**}$	$13.29 \pm 0.18^{***}$
V	<i>T. foenum-graecum</i> extract (400 mg/kg)	$110.65 \pm 1.34^*$	$9.79 \pm 0.23^{**}$	$16.58 \pm 0.36^*$



6.4.4 Effect on in vivo antioxidant parameters:

The antioxidant enzymes SOD, CAT, and GPx play a critical role in cellular defense against oxidative stress. SOD converts superoxide radicals into hydrogen peroxide, which is further decomposed by CAT and GPx to non-toxic products, preventing oxidative injury. In diabetes, excessive glucose levels may glycate and inactivate these enzymes, enhancing oxidative stress and promoting lipid peroxidation. In STZ-induced diabetic rats, there was a significant reduction in liver SOD (8.46 ± 0.32 to 3.24 ± 0.18 U/mg protein), CAT (62.48 ± 2.14 to 38.63 ± 1.36 nM H₂O₂ decomposed/min/gm), and GPx (6.27 ± 0.25 to 2.14 ± 0.12 U/mg protein) compared with normal rats. Treatment with Trigonella foenum-graecum (200 and 400 mg/kg), as well as metformin, significantly restored these antioxidant enzyme levels toward normal, reflecting enhanced free radical scavenging activity and activation of the antioxidant defence system. Conversely, diabetic rats showed an increase in lipid peroxidation markers, TBARS (1.24 ± 0.06 to 3.56 ± 0.18 nmoles/g protein) and LPO (1.86 ± 0.08 to 4.92 ± 0.22 nmoles/g protein), which were significantly reduced after extract and standard treatment, confirming their antioxidant and cytoprotective effects. The results are presented in Table 6.4.4

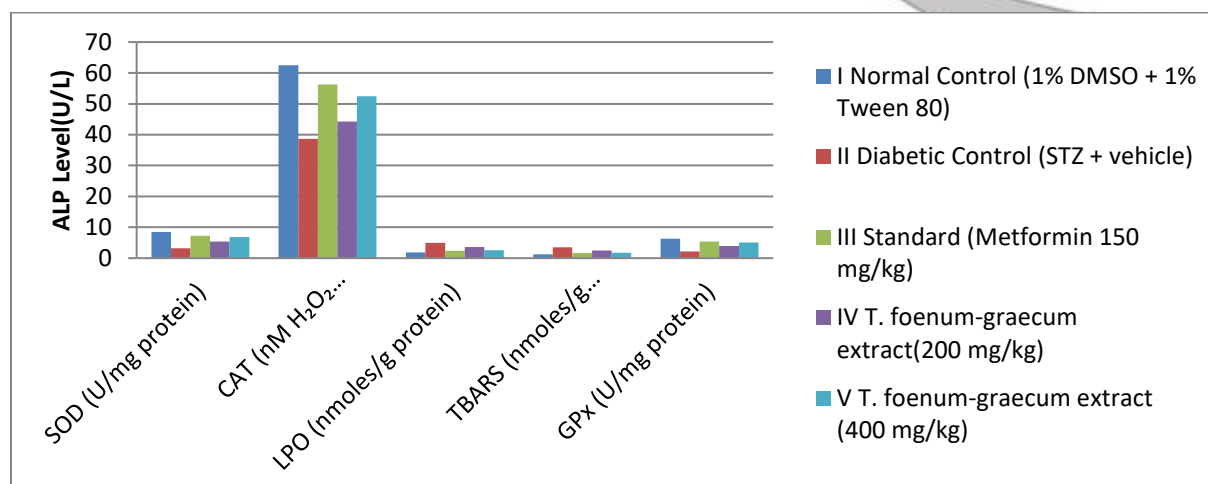
Table 6.4.4: Effect of Ethanolic solvent extracts of Trigonella foenum-graecum on antioxidant marker levels.

Group	Treatment	SOD (U/mg protein)	CAT (nM H ₂ O ₂ decomposed /min/gm)	LPO (nmoles/g protein)	TBARS (nmoles/g protein)	GPx (U/mg protein)
I	Normal Control (1% DMSO + 1% Tween 80)	8.46 ± 0.32	62.48 ± 2.14	1.86 ± 0.08	1.24 ± 0.06	6.27 ± 0.25
II	Diabetic Control (STZ + vehicle)	3.24 ± 0.18 [#]	38.63 ± 1.36 [#]	4.92 ± 0.22 [#]	3.56 ± 0.18 [#]	2.14 ± 0.12 [#]
III	Standard (Metformin 150 mg/kg)	7.28 ± 0.28 [*]	56.31 ± 2.03 [*]	2.34 ± 0.10 ^{**}	1.62 ± 0.07 ^{**}	5.41 ± 0.21 [*]
IV	T. foenum-graecum extract (200 mg/kg)	5.36 ± 0.21 [*]	44.25 ± 1.85 [*]	3.67 ± 0.16 [*]	2.47 ± 0.11 ^{**}	3.92 ± 0.17 ^{***}
V	T. foenum-graecum extract (400 mg/kg)	6.83 ± 0.27 [*]	52.46 ± 1.94 [*]	2.58 ± 0.12 [*]	1.78 ± 0.08 ^{**}	5.02 ± 0.20 [*]

6.4.5 Effect on hepatic parameters

SGOT, SGPT, and ALP are reliable biochemical indicators of liver function. In the STZ-induced diabetic rats, marked hepatic damage was evident, as reflected by significant increases in SGOT, SGPT, and ALP levels. These elevations can be attributed to the leakage of hepatic enzymes from damaged liver cells into the bloodstream, indicating STZ-induced hepatotoxicity. Following 28 days of oral administration of Trigonella foenum-graecum, (200 and 400 mg/kg), there was a significant reduction in SGOT, SGPT and ALP enzyme levels- indicating a hepatoprotective effect of the extracts against diabetic liver injury. Additionally, a decline in total protein levels was observed in diabetic rats, suggesting altered protein metabolism due to enhanced proteolysis and reduced synthesis. Treatment with the extracts restored total protein levels towards normal, demonstrating their ability to improve hepatic function. The findings are summarized in Figure 6.1.

Figure 6.1: Effect of ethanolic solvent extracts of *Trigonella foenum-graecum*, on hepatic parameters.



6.4.6 Effect on renal parameters:

Serum urea and creatinine levels serve as key markers of renal function. The diabetic control group showed a notable increase in urea and creatinine levels compared with normal rats, indicating impaired renal function. After 28 days of treatment with *Trigonella foenum-graecum* (200 and 400 mg/kg), there was a significant decrease in serum urea and creatinine compared to the diabetic control group. The standard drug, metformin, also produced similar improvements. The outcomes are presented in ss

Discussions

Diabetes mellitus is a long-standing, lifestyle-related, and complex metabolic disorder for which no complete cure is currently available. Its development involves multiple organs, and the underlying mechanisms are still not fully understood. Persistent high blood glucose is known to contribute significantly to functional damage in different organs, ultimately leading to complications such as cardiomyopathy, nephropathy, retinopathy, and neuropathy, depending on the system affected. Also, diabetes mellitus is characterized by persistent hyperglycemia, impaired insulin secretion or action, oxidative stress, and dyslipidemia, often leading to multiple organ complications. Medicinal plants have historically served as an important source of bioactive compounds capable of targeting multiple pathways involved in diabetes and its associated metabolic abnormalities. Diabetes is also commonly associated with conditions like hyperlipidemia, oxidative stress, and inflammation, all of which promote the formation of reactive free radicals that trigger inflammatory pathways (Del Prato, 2009).

Over the past 20–30 years, modern therapeutics has introduced around ten classes of synthetic antidiabetic drugs targeting different organs, including the intestine, pancreas, skeletal muscle, liver, and kidney (Chaudhary et al., 2017). These agents, being chemically pure compounds, have been extensively evaluated through in silico, in vitro, and in vivo studies to establish their mechanisms, pharmacokinetics, pharmacodynamics, and safety profiles (Safavi et al., 2013; Bharathi et al., 2014; Bibi and Sakata, 2016). Although several synthetic drugs effectively reduce blood glucose and HbA1c within a specific timeframe, none are completely free from side effects. Moreover, a notable proportion of patients continue to experience diabetes-related complications despite achieving normal blood glucose levels with these medications, highlighting gaps that remain in our understanding and management of the disease (Chaudhury et al., 2017; Palanisamy et al., 2018).

Despite significant progress in modern allopathic medicine and the development of several effective synthetic antidiabetic agents, identifying a single drug or formulation capable of addressing the wide spectrum of complications linked to diabetes remains a major challenge. This gap has encouraged researchers from traditional medical systems to continue exploring holistic remedies that can offer therapeutic benefits without undesirable side effects. In India, nearly 400 medicinal plants and herbs with antidiabetic potential have been used for centuries to manage hyperglycemia and its associated complications, particularly in individuals with type II diabetes mellitus (Modak et al., 2007). Many of these remedies are incorporated into polyherbal formulations, which combine multiple botanicals. Such combinations are believed to generate a diverse range of bioactive metabolites capable of targeting several molecular pathways simultaneously (Mandlik et al., 2008; Patel et al., 2009). While some herbs and their combinations have shown promising results and demonstrated good safety

profiles for type II diabetes, only a limited number have undergone rigorous scientific validation to confirm their efficacy and mechanisms of action. The present study evaluated the anti-diabetic, anti-hyperlipidemic, and antioxidant activities of three selected medicinal plants *Trigonella foenum-graecum*, their therapeutic potential in streptozotocin-induced diabetic rats.

The extraction procedure produced the maximum yield of bioactive constituents in the ethanolic and aqueous extracts, indicating that most of the phytochemicals present in the plant are predominantly polar in nature. Preliminary phytochemical screening confirmed the presence of substantial quantities of flavonoids, phenolics, saponins, alkaloids, and glycosides—classes of compounds widely recognized for their therapeutic relevance in managing diabetes and its associated metabolic disturbances. Flavonoids and phenolic compounds, in particular, are well-established antioxidants that neutralize free radicals and reduce oxidative stress, a key factor contributing to β -cell dysfunction and insulin resistance. These compounds have also been linked to enhanced insulin secretion, improved peripheral glucose uptake, and overall better glycemic control. Saponins and alkaloids, on the other hand, are known for their roles in regulating lipid metabolism, reducing cholesterol levels, and providing protective effects to pancreatic β -cells against oxidative and inflammatory damage. Glycosides may also support glucose homeostasis through their influence on carbohydrate digestion and absorption. The abundance of these bioactive constituents in the polar extracts strongly aligns with the pharmacological benefits observed during experimental evaluation, suggesting that their synergistic actions contribute significantly to the antidiabetic potential of the plant.

The antidiabetic assessment revealed that all three plant extracts produced a significant reduction in fasting blood glucose levels in streptozotocin-induced diabetic rats. Among them, *Momordica charantia* demonstrated the strongest antihyperglycemic response, showing results that were nearly comparable to those of the standard drug metformin. This potent activity is supported by its rich phytochemical profile, compounds such as charantin, polypeptide-p, and vicine are reported to exhibit insulin-like properties, enhance peripheral glucose utilization, and improve overall glycemic control. Similarly, *Trigonella foenum-graecum* contains 4-hydroxyisoleucine and saponins, which are known to stimulate insulin secretion and promote β -cell responsiveness. *Syzygium cumini* contributes through constituents like jamboline and various flavonoids that can decrease intestinal glucose absorption and enhance insulin sensitivity. These outcomes corroborate earlier experimental and clinical findings demonstrating substantial glucose-lowering effects of these medicinal plants. Beyond glucose regulation, the extracts also showed notable improvements in the lipid profiles of diabetic rats. Diabetes-induced dyslipidemia, characterized by elevated total cholesterol, triglycerides, and LDL levels, along with reduced HDL, is a major contributor to cardiovascular complications. Treatment with the plant extracts significantly reversed these abnormalities. Both *T. foenum-graecum* were particularly effective in lowering total cholesterol, triglycerides, and LDL while simultaneously raising HDL, indicating a favourable impact on lipid homeostasis. These improvements may be attributed to the ability of flavonoids and saponins to inhibit hepatic cholesterol synthesis, promote the excretion of bile acids, and prevent oxidative modification of LDL particles. Overall, the combined antihyperglycemic and antihyperlipidemic activities of this plant extracts highlight their therapeutic potential and provide a scientific basis for their longstanding application in traditional medicinal systems.

Oxidative stress plays a central role in the onset and progression of β -cell impairment and various secondary complications associated with diabetes. In the present study, diabetic rats showed a marked decline in the activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), accompanied by a significant rise in malondialdehyde (MDA), a biochemical marker of lipid peroxidation. These alterations reflect a weakened endogenous antioxidant defence system and increased oxidative damage to cellular components. Administration of the plant extracts effectively countered these changes by enhancing the activities of SOD, CAT, and GPx and simultaneously reducing MDA levels. This restoration of antioxidant status strongly indicates that the extracts possess robust free radical-neutralizing properties. Such effects are largely attributed to the presence of flavonoids, phenolic compounds, and other antioxidant phytochemicals, which can directly scavenge reactive oxygen species, chelate pro-oxidant metal ions, stabilize cell membranes, and prevent oxidative degradation of lipids, proteins, and DNA. By re-establishing the balance between oxidants and antioxidants, the extracts likely helped preserve pancreatic β -cell integrity and function, contributing to improved insulin secretion and better glycemic control. This suggests a synergistic relationship between the antioxidative and antihyperglycemic actions of the plants, both of which play a crucial role in mitigating the metabolic disturbances observed in diabetes.

Conclusion

The present thesis, titled “Evaluation of Anti-Diabetic, Anti-Oxidant and Anti-Hyperlipidemic Activity of Selected Medicinal Plant,” focuses on the phytochemical investigation and antidiabetic potential of three traditionally used Indian medicinal plants: the seeds of *Trigonella foenum-graecum*. This plants have long been employed by local communities and tribal groups across India for managing diabetes and related ailments. After proper botanical authentication, all three species were selected for systematic evaluation of their antidiabetic properties.

STZ administration produced a clear diabetic state in rats, characterized by marked hyperglycemia, excessive thirst (polydipsia), increased food intake (polyphagia), loss of body weight, dyslipidemia, and elevated oxidative stress. Treatment with ethanolic extracts of *T. foenum-graecum* for 28 days effectively countered these abnormalities. The extracts significantly reduced fasting blood glucose levels toward normal values, normalized lipid parameters, improved body weight, and stabilized food and water intake. They also rejuvenated the antioxidant defence system, indicating a strong ability to reduce oxidative stress. These results suggest that the extracts not only possess antihyperglycemic activity but also confer antioxidant protection and may help preserve pancreatic β -cell function.

Based on these outcomes, it can be inferred that the ethanolic extracts of *T. foenum-graecum* hold considerable promise for the management of diabetes mellitus. Their utility is particularly relevant in countries like India, where conventional antidiabetic medications may not be easily accessible or affordable for a large portion of the population. The traditional use of these plants, combined with the scientific evidence from this study, underscores their potential value as cost-effective therapeutic options. Future investigations should focus on isolating and characterizing the specific bioactive constituents responsible for the observed antidiabetic effects. Additionally, detailed mechanistic studies and well-designed clinical trials are necessary to establish their efficacy and safety in humans. Such research could contribute greatly to developing novel plant-based therapies for diabetes and other disorders associated with oxidative stress.

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