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RESEARCH ARTICLE

Fenugreek (*Trigonella foenum-graecum*) Sprout Extract for Diabetes Management in Streptozotocin Induced Rat Model

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Abstract

Diabetes mellitus is a widespread metabolic disorder marked by persistent high blood sugar due to inadequate insulin secretion or action. While conventional antidiabetic drugs are available, their side effects have led to the exploration of alternative treatments. Fenugreek (*Trigonella foenum-graecum*) is noted for its medicinal properties, particularly in managing diabetes. In this study, fenugreek seeds were sprouted, and methanolic extracts were prepared. Diabetes was induced in Long-Evans rats through streptozotocin (STZ) injection, and diabetic rats were treated with fenugreek sprout extract at a dosage of 400 mg/kg body weight per day for 28 days. Blood glucose levels (BGL) were regularly monitored, and pancreatic tissues were examined histologically to assess the protection of β-cells. The fenugreek sprout extract demonstrated significant antidiabetic effects, as the treated rats exhibited a notable reduction in blood glucose levels (BGL) compared to diabetic control groups (both positive and negative). Histopathological examination revealed that the pancreatic β-cells of fenugreek-treated rats were protected, exhibiting improved morphology and reduced immune cell infiltration. The study aimed to investigate the antidiabetic potential of methanolic extracts of fenugreek sprouts in STZ-induced diabetic Long-Evans rats. The results suggest that fenugreek sprouts have antidiabetic properties, offering a natural alternative for diabetes management.

Keywords: Antidiabetic, Diabetes, Fenugreek sprouts, streptozotocin, pancreatic β-cells.

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Introduction

Diabetes mellitus (DM) is a long-term metabolic disorder marked by elevated blood sugar levels, resulting from impaired insulin secretion, action, or both. The worldwide incidence of diabetes has been consistently increasing, leading to serious health concerns. According to the World Health Organization (WHO), diabetes impacts millions of individuals globally and is a leading cause of illness and death. It also contributes to various complications, including cardiovascular diseases, neuropathy, kidney damage (nephropathy), and eye problems (retinopathy) (Roglic 2016). Antidiabetic drugs, including metformin and sulfonylureas, are widely use for the management of diabetes. However, these medications are often associated with various side effects and limitations, such as gastrointestinal disturbances and the risk of hypoglycemia (Inzucchi et al. 2015, Maruthur et al. 2016). Consequently, awareness is growing in health sectors, including physicians, general people and patients for the exploration of plant based (leaves or plant extracts) therapies for diabetes management. Among the various medicinal plants, fenugreek sprout (*Trigonella foenum-graecum*) is very familiar for its potential antidiabetic properties. Fenugreek is a plant belonging to the family of Fabaceae, extensively cultivated in India, North Africa, and the Mediterranean region. The seeds and leaves of fenugreek are used both as a spice and a medicinal herb. Fenugreek seeds, leaves, and sprouts exhibit antidiabetic properties that are attributed to a variety of bioactive compounds, including saponins, alkaloids, flavonoids, and dietary fiber (Sharma et al. 1990, Neelakantan et al. 2014).

The antidiabetic activity of fenugreek sprout extract is primarily attributed to several pharmacological mechanisms: Insulinotropic effect: Compounds like 4-hydroxyisoleucine stimulate insulin secretion from pancreatic β-cells, Improved insulin sensitivity: Polyphenols and flavonoids enhance peripheral glucose uptake and insulin receptor sensitivity, Enzyme inhibition: Fenugreek inhibits digestive enzymes like α-amylase and α-glucosidase, delaying carbohydrate absorption, Antioxidant activity: The extract scavenges free radicals and reduces oxidative stress in pancreatic tissues, protecting β-cells from STZ-induced damage., Hypolipidemic effects: Fenugreek lowers serum triglycerides and cholesterol levels, contributing to better glycemic and lipid profile control. Anti-inflammatory properties: Bioactive components suppress inflammation in pancreatic and hepatic tissues, supporting metabolic function. Streptozotocin (STZ) is widely used in animal models to induce Type 1 diabetes by selectively destroying pancreatic β-cells through DNA alkylation and oxidative stress. This results in hypoinsulinemia, hyperglycemia, and various secondary complications, including disturbances in lipid metabolism, hepatotoxicity, and nephrotoxicity. In this study, we aim to evaluate the antidiabetic potential of methanolic fenugreek sprout extract in rats with STZ-induced diabetes, focusing on its effects on blood glucose levels, pancreatic β-cell function, and associated biochemical parameters. By elucidating the therapeutic potential of fenugreek sprouts, this study seeks to contribute to the growing body of evidence supporting the use of natural products in diabetes management.

Materials and Methods

Sample collection:

Fenugreek (*T. foenum-graecum*) was sourced from local retail shops in the Sirajganj district of Bangladesh between September-December 2023. This study was carried out in the Microbiology Lab. of the Dept. of Botany at Jahangirnagar University, Savar, Dhaka, and at Khwaja Yunus Ali University, Sirajganj, Bangladesh.

Preparation of Fenugreek sprout extract

Germination process of Fenugreek seeds

Fenugreek seeds were collected and brought into the laboratory. The seeds were cleaned thoroughly to remove debris, dust, or other particles. The cleaned fenugreek seeds were soaked in distilled water for 12 h at 25°C. After 12 h, the seeds were drained and spread on a moist muslin cloth on a tray. The seeds were placed in a dark condition at 30°C for 10 days. To keep moist, we added 5 drops of distilled water to the cloths during germination period. After 10 days, the germinated fenugreek sprouts were harvested. Next, the sprouts were washed thoroughly using distilled water to remove any remaining seed coats.

Preparation of methanolic extracts

Harvested fenugreek sprouts were air-dried at 25°C in a paper on a table in the laboratory for 48 hrs. Next, the dried sprouts were ground into a fine powder in a grinder. A measured amount of fenugreek sprout powder was mixed with 70% ethanol in a ratio of 1:10 (w/v). The sprout mixtures were kept in a shaking incubator at 25°C for 24 hours to ensure thorough extraction of bioactive compounds. After 24 h of incubation, the mixture was filtered through a sterile muslin cloth, and next through Whatman No. 1 (11 µm) filter paper to obtain a clear methanolic extract. The concentrated extracts were freeze-dried to obtain a powder form of methanolic extract. Then, the extract powders were preserved in airtight containers at -20°C for further analysis.

Animal model

Long-Evans rats were chosen for this study because of their well-documented characteristics and consistent response to streptozotocin, which various researchers have identified as an appropriate model for diabetes investigation. We selected ten-week-old rats weighing between 150-180 grams to ensure they were mature, which is crucial for reliable responses to diabetes induction and subsequent treatments. By maintaining a standard age and weight for the animals, we aimed to support the development of Type 1 diabetes in our experimental subjects. The rats were kept in normal cages with a 12-hour light/dark cycle at a controlled temperature of 22±2°C and humidity of 55±10%. Throughout the experiment, we kept a close eye on the rats, attending to and recording any indications of discomfort, disease, or suffering. In order to ensure a swift and painless death, the rats were humanely put to sleep at the conclusion of the study by inhaling CO_2 and then having their cervical dislocation (Shibly et al. 2018).

Induction of diabetes

Preparation of STZ solution: Streptozotocin was dissolved in a cold citrate buffer (0.1 M, pH 4.5) to maintain its stability and effectiveness. A single intraperitoneal injection of STZ at a dose of 50 mg/kg was administered to the rats (Kulkarni et al. 2012).

Post-injection care

Following the STZ injection, the rats were provided a 5% glucose solution in their drinking water for the first 24 h to prevent hypoglycemia, which is a very common side effect of STZ administration. The rats were regularly monitored for signs of distress or adverse reaction to the STZ injection.

Confirmation of diabetic status (BGL)

After 72 hours of STZ injection, blood glucose levels were measured to successfully confirm the induction of diabetes. In summary, blood samples were taken from the rats' tail veins using standard procedures to minimize stress and ensure precise measurement (Chevassus et al. 2009). Blood glucose levels were measured using a calibrated glucometer (BioHermes Limpid Blood glucose monitoring Meter, China). The measurements were taken in a fasted state (after eight hours fast) to ensure consistency, and reliability. In fasting stage, blood glucose levels in rats >7.2 mmol/dl were considered as diabetic in the study (Stedman 2002). Diabetic rats were randomly assigned to different experimental groups for subsequent treatments with fenugreek sprout extract, ensuring equal distribution based on their initial blood glucose levels.

Experimental design

Group-I (negative control): Healthy, non-diabetic rats served as the control group (n = 5). The rats received diet and water throughout the study period. Group-II (positive control): The diabetic control group (n = 5) consisted of streptozotocin-induced diabetic rats that were not treated. During the course of the trial, they were given a regular diet and water. Streptozotocin (STZ)-induced diabetic rats in Group-III (treatment 1) were given glibenclamide, a typical medication, at a dosage of 5 mg/10 ml WFI/kg of body weight as the standard control group (n = 5). During the course of the trial, they were given a regular diet and water. Group-IV (treatment 2: fenugreek sprout extract): Five rats with diabetes were given a lab meal containing 400 mg/kg body weight/day of fenugreek sprout extract of methanol (Abedin et al. 2024).

Dosages and administration schedule of Fenugreek sprout extract

Dosages selection

Dosages of fenugreek sprout extract for this experiment (Brown et al. 2009) are to determine the effective doses that demonstrate significant antidiabetic effects without causing toxicity. The dose range was 400 mg/kg per day administered orally.

Administration protocol

The selected doses of fenugreek sprout extracts were dissolved in distilled water. Administration was done once daily in the morning via oral gavage, ensuring consistent delivery of extract to the rats.

Duration of treatment

The period of treatments was set for 4 weeks and the expected onset of observable effects on blood glucose levels and pancreatic histology. Rats were treated daily throughout the study period, and body weight, food intake, and water consumption were monitored regularly.

Biochemical analysis

Blood glucose level monitoring

Fasting blood glucose levels were measured daily throughout the study. Blood samples were obtained from the rats' tail veins following a 12-hour overnight fast. A calibrated glucometer, specifically designed for small animals, was used to determine glucose levels. Measurements were taken at baseline (prior to STZ induction), after STZ induction to verify the onset of diabetes, and at regular intervals during the treatment period, such as on a weekly basis.

Measurement of serum glucose levels

Blood samples were taken by heart puncture while the animals were sedated at the end of the research (six weeks into the treatment). The serum was separated by centrifuging the blood at 3000 rpm for 15 minutes at 4°C while the blood was allowed to coagulate. After that, the serum samples were kept for subsequent examination at -20°C.

Measurement of liver function tests (LFT) and lipid profiles

The blood serum of the experimental rats was analyzed with Automated Biochemistry analyzer (Dimension EXL with LM Siemens Healthcare, USA) for estimation of LFTs namely serum creatinine, Serum glutamic pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), and lipid profiles namely Cholesterol (Total), Cholesterol (HDL) and Triglycerides (Tg).

Histopathological examination of pancreatic tissues

Hematoxylin and eosin-stained sections were examined under a light microscope at 40x magnification to identify histopathological features, such as inflammation, necrosis, or neoplasia, providing valuable insights into the disease processes.

Data analysis

Changes in blood glucose levels were recorded and analyzed statistically to assess the effectiveness of fenugreek sprout extract in lowering hyperglycemia in diabetic rats. Results were presented as mean ± standard error for each experimental group. Data were statistically analyzed to compare differences between experimental groups and control groups.

Results

Blood glucose levels

The effects of fenugreek sprout extracts on blood glucose levels were evaluated in rats over a 28-day period. As shown in Table 1 and 2, significant changes were observed in both body weight and serum glucose levels across all experimental groups.

Table 1: Effects of methanolic extracts of Fenugreek *sprouts* on body weight and serum glucose levels in experimental diabetic rats induced by streptozotocin (STZ).

Groups		Group - I		Group - II		Group - III		Group - IV	
Weight and sugar levels		W	SL	W	SL	W	SL	W	SL
Before injection with STZ		115	5.7	140	6.3	110	6.0	123.3	6.1
Treated	7th	128	6.0	161	10.3	133	10.5	149	10.7
	14th	142	6.1	193	11.3	155	7.3	181	7.3
	21st	165	5.8	205	10.0	162	6.4	192.3	6.5
	28th	171	5.5	205	10.9	163	6.6	195.5	5.7
	Average	144	5.9	181	10.6	145	7.0	189.6	6.5

W = Weight in gm, S = Sugar level (mmol/dL). The healthy normal value of sugar is 7.2 mmol/dL (Gupta et al. 2001). Data are the mean value ($p<0.05$) of three rats in each group.

Table 2: The effect of sprout extracts on blood glucose levels.

Groups		Group - I	Group - II	Group - III	Group - IV
Before injection with STZ (Baseline)		5.7±0.26	6.3±0.13	6.0±0.31	4.8±0.19
Treated	7th day	6.0 ±0.15	10.3 ±0.17	10.5±0.20	10.7±0.31
	14th day	6.1 ±0.32	11.3±0.09	7.3±0.19	7.3±0.27
	21st day	5.8 ±0.28	10.0±0.30	6.4±0.26	6.5±0.31
	28th day	5.5 ±0.24	10.9 ±0.50	6.6±0.29	5.7±0.20
	Average	5.9 ±0.04	10.6±0.38	7.0±0.19	6.5±0.87

Each value is the mean ± standard error.

Table 3: Effects of blood glucose levels in experimental STZ-induced diabetic long-evans rats treated with Fenugreek sprout extracts and Glibenclamide.

Groups		Group - I	Group - II	Group - III	Group - IV
Before injecting with STZ (Baseline)		5.7	6.3	6.0	4.8
After being injected with STZ (on 7th days)		6.0 (100%)	10.3 (100%)	10.5 (100%)	10.7 (100%)
Treatment	14th day	6.1 (101.7%)	11.3 (109.7%)	7.3 (69.5%)	7.3 (68.2%)
	21st day	5.8 (96.7%)	10.0 (97.1%)	6.4 (60.9%)	6.5 (60.7%)
	28th day	5.5 (91.7%)	10.9 (105.8%)	6.6 (62.8%)	5.7 (53.3%)
	Average	5.9 (98.3%)	10.6 (102.9%)	7.0 (66.7%)	6.5 (61.7%)

Lipid profile

The effect of fenugreek sprout extracts on the lipid profile was evaluated, focusing on total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and triglycerides (Tg). The lipid profile results, presented in Figure 1, revealed that fenugreek sprout treatment helped maintain lipid levels within the normal range for rats.

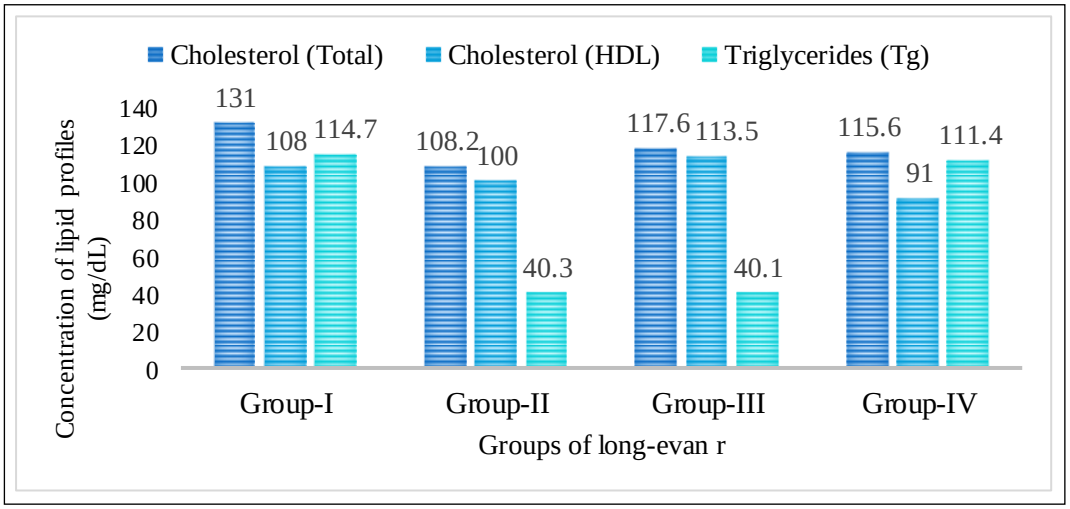

Fig. 1: The impact of Fenugreek sprouts (*Trigonella foenum-graecum*) extracts and Glibenclamide treatment.

Liver function tests

Liver function tests (LFTs) were undertaken to investigate the hepatic effects of fenugreek sprout administration. The results, given in Table 4, demonstrated that creatinine levels were normal in all groups, indicating that renal function was intact.

Table 4: Impact of Glibenclamide treatment and Fenugreek sprout (*Trigonella foenum-graecum*) extracts at four-week intervals on liver function tests (Serum Creatinine, SGPT, SGOT, and SALP) in STZiDLERs.

Groups	Creatinine (mg/dl)	SGPT (U/L)	SGOT (U/L)	S.ALP (U/L)
Group-I	0.95 ±0.05	37.0 ±4.2	33.0 ±3.1	290.0 ±12.4
Group-II	0.52 ±0.03	22.0 ±2.7	19.0 ±1.5	209.0 ±8.9
Group-III	0.43 ±0.02	25.0 ±3.5	26.0 ±2.2	189.0 ±7.8
Group-IV	0.93 ±0.06	290.0 ±15	33.0 ±2.9	284.0 ±11.7

Note: normal values for creatinine (0.4-1.4 mg/dL), SGPT (7–45 U/L), SGOT (8–40 U/L), and S. ALP (40–130 U/L).

Histopathological analysis

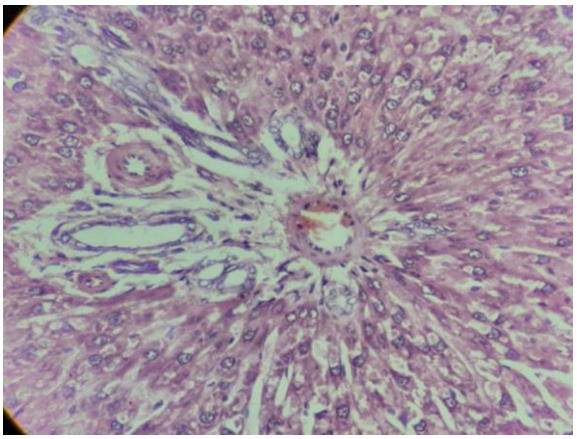

Fig. 2: Group-I (negative control): Normal hepatocytes are intact structure and supportive connective tissue.

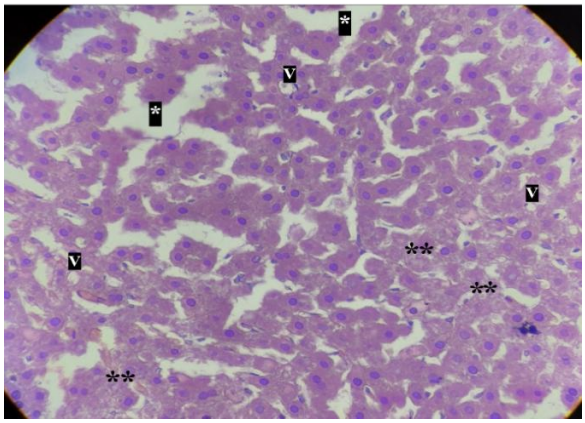

Fig. 3: Group-II (Positive Control): Microscopic view of liver slide at 40X for histopathological analysis of Group-II (Diabetic Control) in Stzidlers.

The dilatation of sinusoidal regions (*), vacuolization of hepatocytes (V), and degeneration of hepatocytes (**) (Fig. 4).

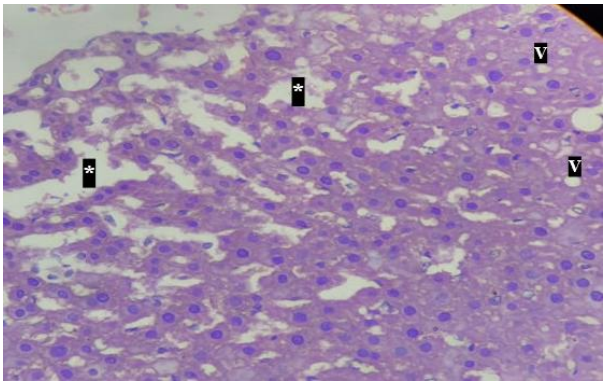

Fig. 4: Group-III (Treatment 1-Glibenclamide): Microscopic view of liver slide at 40X for histopathological analysis of Group-III (Standard).

The Fig. 5 shows the dilatation of sinusoidal regions (*), and vacuolization of hepatocytes(V).

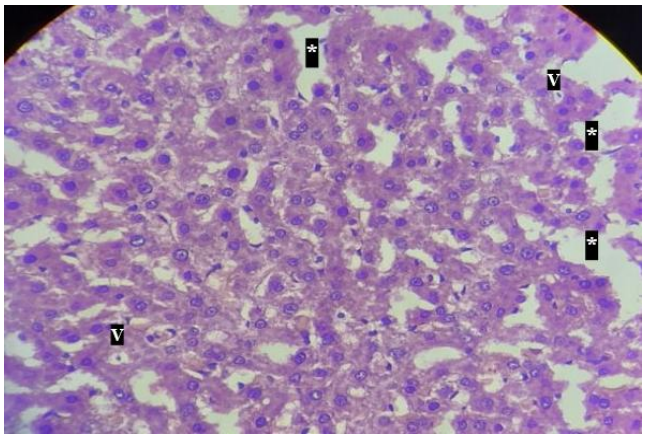

Fig. 5: Group-V (Treatment 2–Fenugreek sprout extract): Microscopic view of liver slide at 40X for histopathological analysis of Group-IV (Experimental-Fenugreek sprouts).

Discussion

Long-Evans rats with streptozotocin-induced diabetes were used to test the Fenugreek (*Trigonella foenum-graecum)* sprouts' antidiabetic properties. Their effects on liver function tests, lipid profiles, and blood glucose levels were measured as part of this assessment. The findings showed that in rats with STZ-induced diabetes, the fenugreek sprout extract dramatically reduced blood glucose levels. Rats in the fenugreek sprout-treated group experienced a reduction in blood glucose levels from 10.7 ± 0.31 mmol/L to 5.7 ± 0.20 mmol/L after 28 days of treatment, which was an average of 61.7% lower than the baseline post-STZ value. Compared to Treatment 1, which included glibenclamide and showed a reduction of 66.7%, this decline was more substantial (Table 2 and 3). The administration of fenugreek sprout methanolic extracts to STZ-induced diabetic rats over 28 days significantly reduced blood glucose levels in treatment groups compared to diabetic controls. Group III and IV, treated with different doses of fenugreek sprout extract, showed significant glycemic control with an average reduction to 7.0 mmol/L and 6.5 mmol/L, respectively, by the 28th day. This hypoglycemic effect supports previous research indicating that fenugreek contains bioactive compounds like 4-hydroxyisoleucine, trigonelline, and fiber, which enhance insulin sensitivity and delay glucose absorption from the intestine (Abedin et al. 2024, Srinivasan 2006, Gupta et al. 2001). Fenugreek sprout extract treatment demonstrated positive modulation of lipid parameters in diabetic rats: Total cholesterol (TC) and triglyceride (TG) levels were lowered, and HDL-C was maintained within normal ranges, indicative of improved lipid metabolism. These changes align with studies indicating that fenugreek's saponins and dietary fiber reduce cholesterol absorption and enhance excretion, while also improving lipid peroxidation and antioxidant defense (Singh et al. 2023)

Fenugreek sprouts were found to reduce triglyceride levels significantly and maintain total cholesterol and HDL-C levels. These lipid-lowering effects align with findings from previous studies that have reported the lipid-modulating potential of fenugreek in diabetic conditions (Zia et al. 2001). Normal serum creatinine, indicating no renal toxicity from fenugreek treatment. Lowered SGPT and SGOT levels in Group III suggest hepatic protective effects of fenugreek, while a spike in SGPT in Group IV (290 U/L) may indicate a dose-dependent toxicity risk. Fenugreek's antioxidant flavonoids and polyphenols have been previously reported to protect hepatic tissue against oxidative stress in diabetic models (Kaviarasan et al. 2008). However, excessive doses may exert stress on hepatocytes. However, the fenugreek sprout-treated group (Gr-IV) exhibited significantly elevated levels of SGPT and SALP compared to the normal and diabetic control groups. These suggest liver stress or injury associated with fenugreek sprout treatment. Elevated liver enzymes may indicate hepatotoxicity, a finding that necessitates further

investigation (Kaviarasan et al. 2008). The results found from this study highlight fenugreek sprouts effects that can be an effective natural therapy for the management of diabetes. The significant reduction of blood glucose levels and the favourable effects on lipid profiles suggest that fenugreek sprouts can serve as a complementary approach to conventional antidiabetic therapy (Naicker 2017). These findings are in line with previous studies that have demonstrated the antidiabetic and lipid-lowering effects of fenugreek in both human and animal models. The presence of bioactive compounds such as diosgenin, trigonelline, and 4-hydroxyisoleucine in fenugreek is believed to contribute to these beneficial effects. Fenugreek sprouts showed an antidiabetic activity, particularly in reducing blood glucose level and improving lipid profiles.

Conclusion

This study indicates that methanolic extracts of fenugreek sprouts have the potential to lower glucose levels in diabetic rats while preserving normal lipid profiles and liver function. The results reveal significant anti-diabetic and anti-lipidemic activities of these extracts in both normal and diabetic rats. Future investigations are warranted to identify the specific chemical constituents in fenugreek sprouts that contribute to these effects. The combination of fenugreek sprouts and streptozotocin resulted in a reduction in blood glucose levels, while SGOT, SGPT, and SALP remaining unchanged.

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Author's contribution: MZA designed the experiment, supervised the study, and corrected the manuscript. AAA, RH, LJ, MMRK and RYS conducted experiments, collected data and its statistical analysis and wrote the draft of the manuscript. All authors have read and approved the final manuscript.

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Ethical considerations and approval: The research proposal was approved by the ethical review boards of Jahangirnagar University [Reference: BBEC, JU/M2022/11(1)] and Khwaja Yunus Ali University (Ref. No. KYAU/IRB/08-2021), ensuring adherence to institutional and national regulations.

Data availability: All data generated in the study are reported in the article, and unprocessed data will be available to the corresponding author upon request.

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