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# Anti-Lipidemic and Hepatoprotective Effects of Fenugreek Supplementation on STZ-Induces Type 2 Diabetes: An Animal Study

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**Abstract** | Despite significant efforts to manage diabetes mellitus (DM), its prevalence and related complications continue to increase. There are already well-established antidiabetic drugs in the pharmaceutical sector like Dapagliflozin (DAPA). However, medicinal plant remedies, e.g., Fenugreek (Trigonella foenum-graecum), are efficiently utilized for DM treatment with low side effects. Accordingly, the study aimed to compare the effects of DAPA and Fenugreek seeds powder (FSP) or extract (FSE) on hyperglycemia, dyslipidemia, and hepatic alterations of streptozotocin (STZ)-provoked DM rats. Here, DM was triggered by administrating 10% fructose (Fr) for two weeks, then intraperitoneally injected with a single STZ dosage (40 mg/kg). The study was carried out using 50 male albino rats allocated into 5 groups (n = 10/group): Control, diabetic non-treated, and diabetic groups treated by DAPA (1 mg/Kg b.wt), FSE (250 mg/kg, b.wt) and FSP (100mg/kg, b.wt). All doses were daily administered by stomach tube for 8 weeks. Serum biochemical analyses of insulin, glucose, lipid profile, and liver function tests, besides histopathological analyses for the liver and pancreas, were performed. The FSE or FSP and DAPA treatment ameliorates the measured parameters in diabetic rats compared to diabetic rats: Significantly (P≤0.05) elevated body weight and insulin levels, reduced glucose, and improved lipid profile (lowered total cholesterol, LDL, and triacylglycerides while increased HDL). The enhancement in liver function was recorded by decreased liver enzymes (ALT and AST) and increased both albumin and total protein, and proved by the histopathological examination of hepatic tissue compared to diabetic rats. It was obvious that the results of FSE or FSP were better than that of DAPA. It was concluded that FSE and FSP significantly (P≤0.05) improved dyslipidemia and hyperglycemia in STZ-provoked diabetic rats, offering a safe herbal medication for DM treatment. However, Further investigations are needed using different methods of extractions of fenugreek, different doses and durations, different comparisons with other antidiabetic drugs having different mechanism of action than DAPA.

**Keywords**: Fenugreek, Dapagliflozin, Liver function, Diabetes mellitus, Streptozotocin

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## INTRODUCTION

The rising global prevalence of diabetes mellitus (DM) has made it one of the most significant health concerns to the contemporary human population. Diabetes is a heterogeneous syndrome characterized by defined hyperglycemia which is classified as type 1 diabetes (T1DM), type 2 diabetes (T2DM), specific types of diabetes and gestational diabetes mellitus (Singh *et al*., 2022).

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic disorders worldwide, accounts for over 90–95% of all diabetes and its development is primarily caused by a combination of two main factors: Defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin. Typically, T2DM is defined by hyperglycemia, a continued elevated BGLs appears in a chronic and heterogeneous manner as fat, carbohydrate, and protein metabolic malfunctions (Roglic, 2016).

The liver is one of the main organ that control metabolic homeostasis by participating in metabolite detoxification, protein synthesis, production of required biochemical for digestion, hormone formation, and storage of glycogen (Doroudian *et al*., 2024). The liver maintains blood glucose levels (BGLs) by storing glucose as glycogen and then releasing it when its levels fall below the normal range, engaging in gluconeogenesis, a process in which glucose is formed from non-carbohydrates.

Inflammation, endoplasmic reticulum stress (ERS), oxidative stress and ectopic lipid deposition especially in liver are involved in progression of T2DM by impairing insulin sensitivity and/or β cell dysfunction, causing insulin resistance (IR) reciprocal with metabolic disorders (Lu *et al*., 2024). T2DM is just one component of metabolic dysfunction syndrome (MDS), and is often accompanied by other components of MDS, such as pre-obesity/obesity, metabolic dysfunction associated steatotic liver disease (MASLD), dyslipidemia (Lu *et al*., 2024).

IR of T2DM accompanied with an increased delivery of NEFAs from adipose tissue to the liver and pancreas, leading to intracellular fat deposition in hepatic tissue (Lu *et al*., 2024). This accumulation of large lipid droplets in hepatocytes, referred to macrovascular hepatocellular steatosis leads to a metabolic syndrome called non-alcoholic fatty liver disease (NAFLD) (Bi *et al*., 2024).

Type 2 diabetes is a major risk factor for non-alcoholic fatty liver disease (NAFLD) and/or nonalcoholic steatohepatitis (NASH), as the prevalence of NAFLD is as high as 40% to 50% among diabetic patients (Williamson *et al*., 2011; Shimizu *et al*., 2019). Approximately 20 to 30% of patients with NAFLD also have nonalcoholic steatohepatitis (NASH), which can cause liver fibrosis and progresses to cirrhosis with a risk of hepatocellular carcinoma in 10 to 20% of patients (Shimizu *et al*., 2019).

Currently, NAFLD remains one of the most frequent liver diseases, affecting up to 25% of the general adult population (Pimpin *et al*., 2018). The co-existence of NAFLD and type 2 diabetes significantly increases the prospect of developing nonalcoholic steatohepatitis (NASH) and cirrhosis compared to the presence of NAFLD without persistent hyperglycemia (Calzadilla and Adams, 2016).

Dapagliflozin (DAPA) is an oral antidiabetic medication that impedes the sodium-glucose cotransporter 2 (SGLT2), decreasing glucose reabsorption in the kidneys and increasing its excretion. This approach aids in reducing plasma glucose levels without inducing weight gain (Sayed *et al*., 2020). SGLT2 inhibitors offer benefits for individuals with diminished pancreatic function or insulin resistance (IR). Research has revealed that DAPA effectively decrease oxidative stress, lipid metabolism, fibrosis, and inflammation, besides improving IR (Kojima *et al*., 2015; Terami *et al*., 2014).

Although pharmaceutical medications are commonly used to treat DM, medicinal plant-based remedies are also beneficial in managing DM. Fenugreek (FG; Trigonella foenum-graecum) is widely consumed as a nutritional staple in many countries. Recent research conducted on both humans and animals has demonstrated that extracts derived from Fenugreek seed (FSE) have qualities that can help in managing DM, reducing total cholesterol (TC) levels, and acting as antioxidants. Studies have identified the pharmacological properties of FG in connection to DM by investigating its effects on peripheral glucose uptake and insulin secretion (Alu'datt *et al*., 2024).

However, no studies evaluated the clinical and metabolic effects of DAPA versus fenugreek. Hence, fenugreek has no known side effects; therefore, it is generally safe to use in patients with diabetes or lipid disorders. Fenugreek can also be used in combination with other herbal medications or as a complementary treatment along with other medications and lifestyle modifications in patients with diabetes.

This research aimed to compare the impact of Fenugreek and DAPA on hyperglycemia and dyslipidemia, liver function of streptozotocin (STZ)-triggered diabetic rats. So, can fenugreek prevent hepatic changes and what are the best therapeutic approaches in T2DM that have NAFLD, and the new desirable possible therapeutic options.

## MATERIALS AND METHODS

### Plant collection

The FG seeds (Ismailia local markets, Egypt) were rinsed with distilled water 2–3 times, dried at 40 ±5°C for 12 h, and crushed with a mechanical blender to get a fine powder.

### Plant extraction

The FSP was extracted with 95% ethanol (PERFECT, India), repeating the extraction three times. Each mixture was filtered by filter paper and evaporated in a rotary evaporator. The obtained FSE ethanol was maintained at – 20°C until subsequent usage (Lim *et al*., 2013). Prior to administration, FSE and FSP were dissolved in distilled water.

### Experimental design and feeding trial

The study included 50 male albino rats (160 ±10g) that were purchased from Pharmaceutical Pharco Company, Cairo, Egypt. The experiment was conducted at the Faculty of Veterinary Medicine Animal House and Faculty of Science and Chemistry Department, Suez Canal University, Ismailia, Egypt. Rats were housed at 20°C, 55%–60% humidity, and 12 h light/dark cycle. After a one-week accommodation, Rats were assigned into five groups (n = 10/group): Control, diabetic non-treated, and DAPA-, FSE-, and FSP-diabetic-treated groups. Through the experiment, the Control Group accessed freely to a balanced diet and water. In the other groups, T2DM was induced by providing the rats unrestricted access to a 10% solution of Fructose (Fr; Loba Chemie, India) dissolved in water for the initial two weeks. Then, rats were fasted for a whole night, succeeded by intraperitoneal injection with STZ (a single dosage of 40 mg/kg b.w dissolved in 0.1 M citrate buffer; pH 4.5; Sigma-Aldrich, USA) (Wilson and Islam, 2012). During the initial 24 h of DM, rats were administered a glucose solution (10% w/v; Loba Chemie, India) to prevent acute hypoglycemia. DM was verified 72h after injecting STZ by assessing BGLs, with non-fasting BGLs > 300 mg/dl indicating DM incidence (Srinivasan *et al*., 2005).

Group III comprised DAPA-treated diabetic rats (1mg/Kg b.wt; AstraZeneca, United Kingdom) (Oraby *et al*., 2019b); group IV treated with FSE (250 mg/kg b.w) Hl *et al*. (2010); and group V treated with FSP (100 mg/kg b.w) (Baset *et al*., 2020). The treatments were given orally daily by stomach tube for two months, recording the bodyweight weekly to adjust the doses of the given treatments. Moreover, BGLs were checked weekly to ensure DM occurrence and persistence.

### Sampling

After 12-h fasting, rats were anesthetized with ether, and the collected blood samples from the retro-orbital venous plexus were placed into a plain centrifuge tube in an inclined position at room temperature for 20 min and then placed in the refrigerator to facilitate clot retraction. The samples underwent centrifugation at 3000 rpm for 10 min, carefully collecting the clear serum samples and storing them in Eppendorf tubes at –20°C. Thereafter, serum samples were used for the biochemical analyses: insulin, glucose, lipid profile, and liver function [alanine transaminase (ALT) and aspartate transaminase (AST)], total protein (TP), and albumin (ALB)]. Rats were euthanized to collect the liver and pancreas for histopathological examination.

### Serum biochemical parameters

Serum glucose was assessed using glucose commercial kits (46861S, CliniChem) (Trinder, 1969), serum insulin level using rat insulin ELISA kits (CSB-E05070r, CUSABIO). serum TC level using TC commercial kits (303113050, ELITech Diagnostic, France, (Rivellese *et al*., 1994), serum triglycerides (TG) level using TG commercial kits (304710050, ELITech Diagnostic, France) (Dufour *et al*., 2000), serum high-density lipoprotein (HDL) level using HDL commercial kits (0599, Stanbio Laboratory, USA) (Dufour *et al*., 2000). All of them were determined by the enzymatic calorimetric method. Low-density lipoprotein (LDL) calculation was performed by Friedewald *et al*. (1972) formula explained by Davidson and Rosenson (2009): LDL = TC – (TG/5 + HDL). Both AST and ALT were determined using readymade kits provided by Randox (SC 2643) (Schmidt and Schmidt, 1963), total protein (TP) level was ascertained by the Biuret method using commercial kits (41951S, CliniChem company) (Gornall *et al*., 1949; Weichselbaum, 1946), and serum ALB level was ascertained by the Colorimetric bromocresol green method using commercial kits (41253, CliniChem company) (Doumas *et al*., 1971).

### Histopathology analysis

Tissue specimens were obtained from the liver and pancreas, immersed in 10% formalin. Following proper fixation, subjected to dehydration using ethyl alcohol, followed by clearing in xylol. Subsequently, the samples were embedded in paraffin, followed by slicing thin sections and staining them with hematoxylin and eosin (Stevens and Bancroft, 1990).

### Semi-quantitative scoring

Lesion scores of pancreas and liver lesions were performed (Gibson-Corley *et al*., 2013). The mean was computed by blindly inspecting lesions in 10 fields randomly picked from each slide for each animal. Lesions Score scale: 0 denotes no change in the tissue; 1 denotes <10%; 2 denotes 11-25%; 3 denotes 26-45%; 4 denotes 46-75%; and 5=76-100%.

### Statistical analysis

Data analysis was carried out through the SPSS package (version 20, SPSS Inc., Chicago), expressing the data as Mean ± SE (n = 5). The one-way ANOVA succeeded by the Duncan test was utilized, considering $P < 0.05$ statistically significant. One-way ANOVA followed by Tukey's HSD post-hoc test was used to compare differences between treatment groups. Polynomial regression was performed to assess potential non-linear dose-response relationships.

## RESULTS AND DISCUSSION

### Body weight

Unlike the Control, diabetic rats had significantly reduced final body weights ($P \leq 0.05$; Table 1, Figure 1), which was restored in the FSE- and FSP-treated diabetic group. Although DAPA treatment increases body weight significantly compared to diabetic ones, the body weight is still significantly lower than normal.

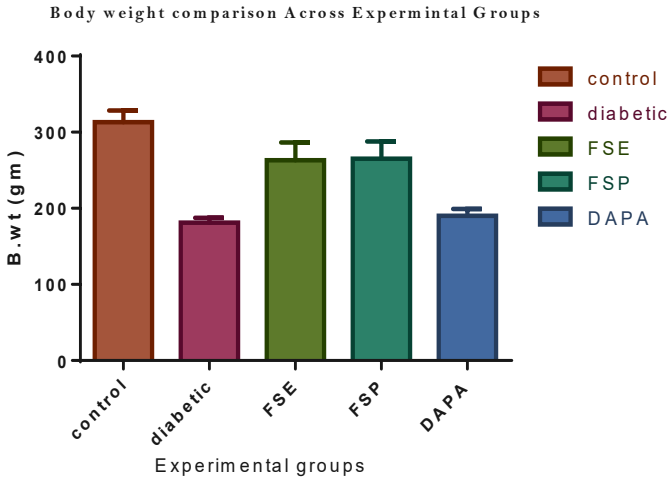


**Figure 1:** Body weight across the experimental groups.

### Serum glucose

Unlike the Control, the Diabetic Group experienced significantly increased glucose and reduced insulin levels ($P \leq 0.05$). These effects were partially reversed in the FSE, FSP, and DAPA groups. However, the FSE or FSP-treated group showed better results than DAPA (Table 2).

Analysis revealed that FSE treatment at 250 mg/kg resulted in significantly lower glucose levels than FSP at 100 mg/kg ($p < 0.05$), but no significant difference was observed between FSE and DAPA treatments Figure 2.

### Serum lipid profile levels

Diabetic rats showed significantly raised TG, TC, and LDL levels ($P \leq 0.05$) and lowered HDL levels ($P \leq 0.05$) compared to the Control. Administrating FSE, FSP, and DAPA to diabetic rats improved lipid profile parameters but were still significantly different than normal, except HDL of FSE and FSP treated group restored to normal. However, the FSE or FSP-treated group showed significant improvement in HDL and LDL results compared to the DAPA group (Table 3).

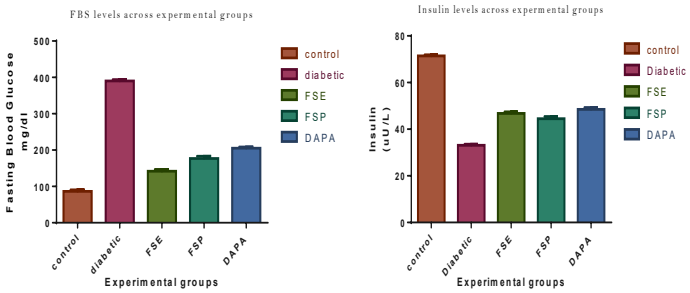


**Figure 2:** Fasting blood glucose (left plot) across the experimental groups, Insulin level (right plot) across the experimental groups.

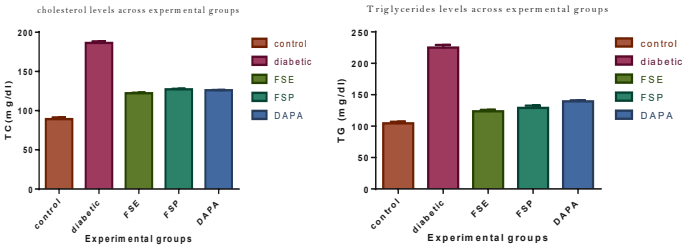


**Figure 3:** Total cholesterol (left plot) across the experimental groups, with error bars showing the standard deviation. Triglyceride level (right plot) across the experimental groups, also with error bars.

**Table 1:** Impacts of FSE, FSP, and DAPA on body weight in STZ-triggered diabetic rats.

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | Control | Diabetic | FSE | FSP | DAPA |
| Body weight (g) | $313.15^{a} \pm 15.27$ | $181.0^{b} \pm 6.55$ | $263.33^{a} \pm 23.09$ | $265.00^{a} \pm 22.9$ | $190.33^{b} \pm 8.96$ |

Data represent mean ± SD, $^{abc}$ The different letter in the same row is significant ($P \leq 0.05$).

**Table 2:** Impacts of FSE, FSP, and DAPA on fasting glucose and insulin in STZ-provoked diabetic rats.

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | Control | Diabetic | FSE | FSP | DAPA |
| glucose level (mg/dl) | $86.5^{d} \pm 4.30$ | $390.0^{a} \pm 4.0$ | $141.80^{c} \pm 4.90$ | $177^{bc} \pm 6.10$ | $205.0^{b} \pm 3.50$ |
| Insulin (uU/l) | $71.43^{a} \pm 0.45$ | $33.10^{c} \pm 0.50$ | $46.80^{b} \pm 0.65$ | $44.5^{b} \pm 0.90$ | $48.5^{b} \pm 0.80$ |

Data represent mean ± SD, $^{abc}$ The different letter in the same row is significant ($P \leq 0.05$).

**Table 3:** Effects of FSE, FSP, and DAPA on lipid profile in STZ-provoked diabetic rats.

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | Control | Diabetic | FSE | FSP | DAPA |
| Total cholesterol (mg/dl) | $89.20^{c} \pm 2.15$ | $186.40^{a} \pm 2.04$ | $122.30^{b} \pm 0.59$ | $127.3^{b} \pm 0.6$ | $126.1^{b} \pm 0.39$ |
| Triglycerides (mg/dl) | $104.5^{c} \pm 2.39$ | $225.0^{a} \pm 4.20$ | $123.6^{b} \pm 2.30$ | $129.1^{b} \pm 3.4$ | $139.40^{b} \pm 1.8$ |
| High-density lipoprotein (mg/dl) | $53.30^{a} \pm 0.70$ | $25.20^{c} \pm 1.10$ | $54.8^{a} \pm 1.8$ | $53.08^{a} \pm 0.80$ | $31.3^{b} \pm 1.9$ |
| Low-density lipoprotein (mg/dl) | $15.00^{d} \pm 0.92$ | $116.20^{a} \pm 0.60$ | $42.78^{c} \pm 1.02$ | $48.45^{c} \pm 0.70$ | $66.92^{b} \pm 0.66$ |

Data represent mean ± SD, [abc] The different letter in the same row is significant ($P \leq 0.05$).

**Table 4:** Impact of FSE, FSP, and DAPA on liver function tests in STZ-provoked diabetic rats.

| Parameters | Experimental groups | | | | |
|---|---|---|---|---|---|
| | Control | Diabetic | FSE | FSP | DAPA |
| ALT (IU/L) | $41.0^{d} \pm 1.5$ | $151.2^{a} \pm 1.9$ | $45.0^{c} \pm 0.9$ | $46.3^{c} \pm 1.1$ | $49.0^{b} \pm 1.0$ |
| AST (IU/L) | $140.2^{c} \pm 9.5$ | $230.0^{a} \pm 10.1$ | $155.5^{b} \pm 8.6$ | $162.4^{b} \pm 10.2$ | $165.1^{b} \pm 12.1$ |
| Albumin (g/dl) | $4.03^{a} \pm 0.15$ | $2.6^{c} \pm 0.1$ | $3.3^{b} \pm 0.10$ | $3.26^{b} \pm 0.08$ | $3.15^{b} \pm 0.06$ |
| Total Protein (g/dl) | $8.3^{a} \pm 0.10$ | $4.8^{c} \pm 0.10$ | $7.5^{b} \pm 0.15$ | $7.3^{b} \pm 0.10$ | $6.8^{b} \pm 0.13$ |

Data represent mean ± SD, [abc] The different letter within the same row is significant ($P \leq 0.05$)

Analysis revealed that FSE treatment at 250 mg/kg resulted in significantly lower lipid profile levels than FSP at 100 mg/kg ($p < 0.05$), but no significant difference was observed between FSE and DAPA treatments, Figures 3, 4.

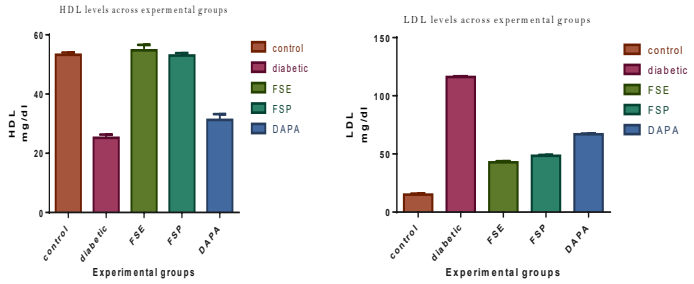


**Figure 4:** HDL (left plot) across the experimental groups, with error bars showing the standard deviation. LDL (right plot) across the experimental groups, also with error bars.

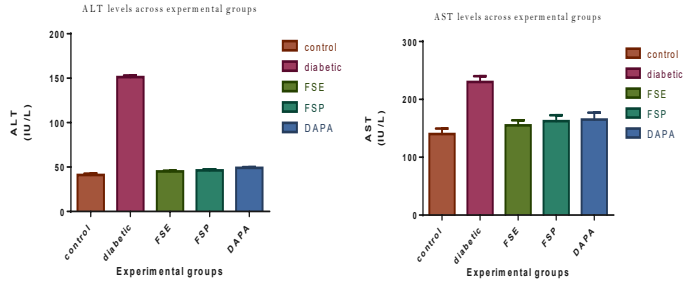


**Figure 5:** ALT (left plot) across the experimental groups, with error bars showing the standard deviation. AST (right plot) across the experimental groups, also with error bars.

## Liver function

The Diabetic group displayed significantly escalated serum ALT and AST levels in contrast to the other groups ($P \leq 0.05$), with no significant variation between the DAPA, FSE, and FSP groups. Furthermore, the Diabetic group exhibited significantly lowered serum TP and ALB levels compared to the other groups ($P \leq 0.05$), with no significant change between the DAPA, FSE, and FSP groups (Table 4 and Figures 5, 6).

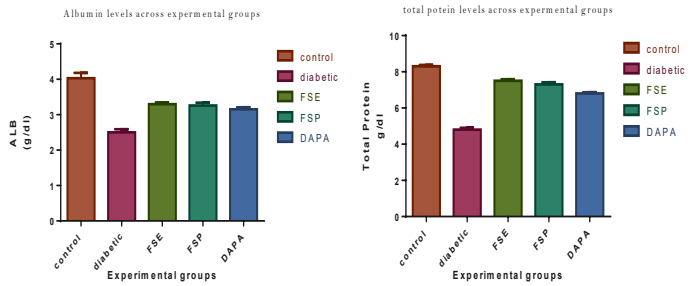


**Figure 6:** Albumin (left plot) across the experimental groups, with error bars showing the standard deviation. Total protein (right plot) across the experimental groups, also with error bars.

## Histopathological analysis

### Pancreas

The control rats had normal size of acinar cells and islets of Langerhans. The cells of islets had homogenous, eosinophilic cytoplasm with round to oval nuclei (Figure 7a). In contrast, the pancreatic tissue of the diabetic group possessed few small-sized islets of Langerhans, with few degenerated cells showing nuclear shrinkage and cytoplasmic vacuolation (Figure 7b). In the DAPA group, pancreatic tissue showed moderate congestion of vessels, mild degeneration and edema of islets cells (Figure 7c). In the FSE group, pancreas tissue had a small size, and few numbers of islets of Langerhans with shrinking of nuclei and moderate cytoplasmic vacuolation (Figure 7d). For FSP group, pancreatic tissue showed no inflammation nor degenerative changes. In the islets center, the cells had normal

eosinophilic cytoplasm with round nuclei (Figure 7e).

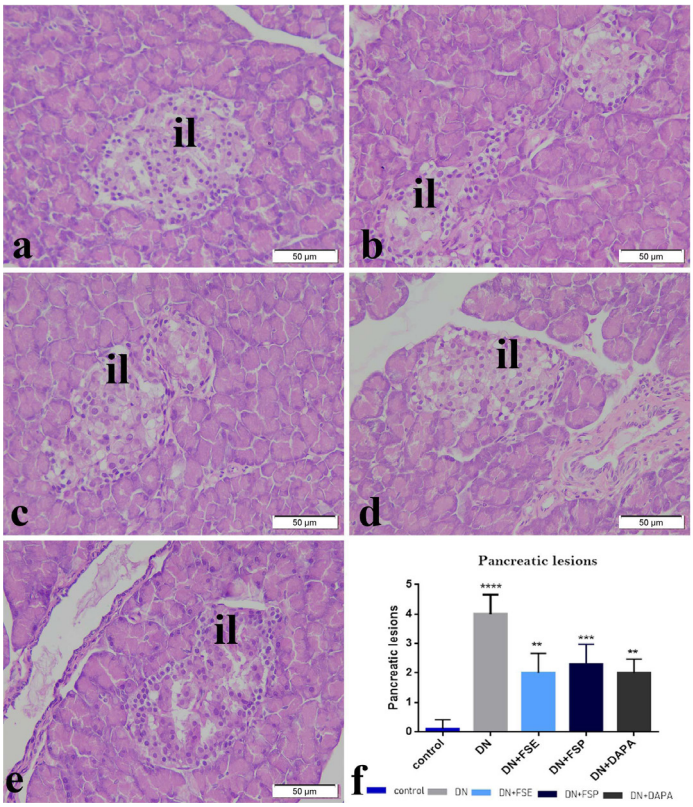


**Figure 7:** Representative images of histopathology of pancreas. a: Control group: The pancreas shows normal islets of Langerhans and acinar cells. The β-cells have homogeneous, eosinophilic cytoplasm with round to oval nuclei. b: Diabetic group: The pancreas shows few small-sized islets of Langerhans, with few degenerated cells showing nuclear shrinkage and cytoplasmic vacuolation. c: FSE group: The pancreas shows no inflammation or degeneration changes. In the islets center, the cells had normal eosinophilic cytoplasm with round nuclei. d: FSP group: The pancreas shows a small size and few number of islets of Langerhans with shrinking of the nucleus and moderate cytoplasmic vacuolation. e: DAPA group: The pancreas shows moderate congestion of vessels and mild degeneration of islet cells with mild edema f: Score of pancreatic lesions. Islet of pancreas (il). H and E. X 400 (Scale Bar = 50 µm).

## Liver

The hepatocytes of control rats showed normal lobular structure with granular or eosinophilic cytoplasm and vesicular rounded or oval nuclei (Figure 8a). The liver in the diabetic group revealed eosinophilic vacuolated cytoplasm with relatively condensed nuclei. The hepatocytes also showed hydropic degeneration and focal lymphocytic inflammatory infiltrate (Figure 8b). In the liver of the DAPA group, the hepatocyte showed a mild eosinophilic reaction in the cytoplasm (Figure 8c). For FSP and FSE groups, liver tissues were almost normal with mildly dilated sinusoids and minimal focal hydropic degeneration (Figure 8d-e).

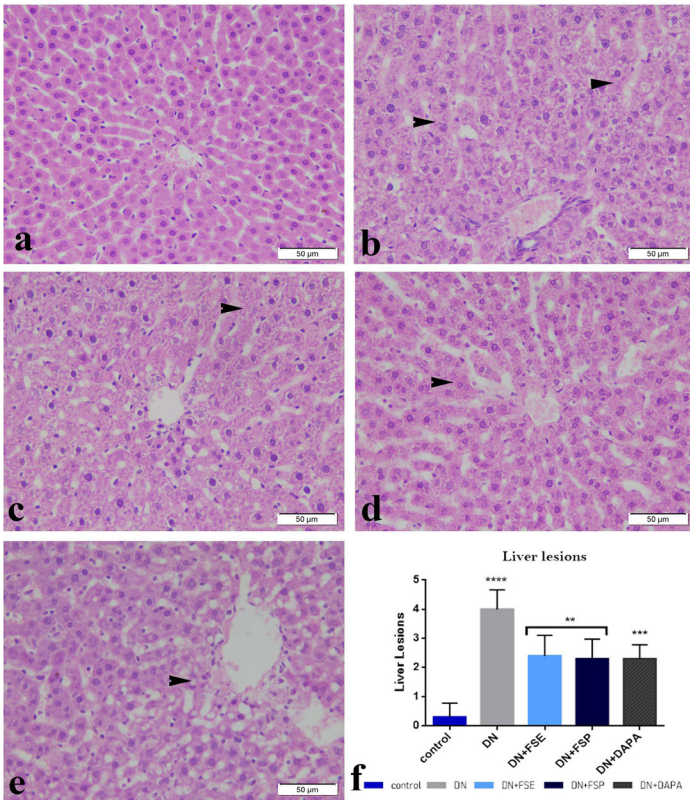


**Figure 8:** Representative images of histopathology of liver. a: Control group: The liver shows a normal lobular structure with granular or eosinophilic cytoplasm and vesicular rounded or oval nuclei. b: Diabetic group: The Liver shows diffuse degeneration of hepatic cells. and mild congestion of central veins. c: FSE group: The Liver shows normal hepatocytes with normal nuclei and mildly dilated sinusoids. d: FSP group: The Liver shows mild degeneration of hepatic cells along with dilated sinusoids e: DAPA group: The Liver shows mild vacuolation in the cytoplasm of some hepatocytes. f: Score of hepatic lesions. arrow refers to hepatic cells. H and E. X 400 (Scale Bar = 50 µm).

Globally, DM and its associated complications present a noteworthy worldwide public health concern. The occurrence and prevalence of DM are increasing, contributing to a substantial impact on both health and the economy (Li *et al.*, 2019) and approaching epidemic levels. The prevalence of DM among Egyptian adults, as published by the International Diabetes Federation, is 15.2%, a potentially underestimated estimate (Al-Rubeaan, 2010; Hegazi *et al.*, 2015).

The most prevalent type of diabetes, known as type-2 diabetes, accounts for over 90–95% of all diabetes and is characterized by irregularities in insulin synthesis and insulin resistance (Gheibi *et al.*, 2017).

Currently there is no viable treatment without side effects, despite the advancement of therapeutic agents; as a result, new preventive techniques and improved therapeutic

interventions are required (Gheibi *et al*., 2017).

Accordingly, this study aimed to induce type 2 diabetes rat model using fructose and low dose STZ then to compare the protective impacts of DAPA (a therapeutic drug act by inhibiting sGLT2) and fenugreek Medicinal plant using its extract (FSE) and powder (FPS) in STZ-provoked T2DM rat model. This was done by assessing serum glucose, insulin, lipid profiles and liver functions, besides examining the histopathological alternations in the pancreas and liver tissues of treated rats.

In This study, diabetic rats showed a significantly lowered body weight in contrast to the Control, possibly due to poor glycemic control and elevated protein catabolism and muscle atrophy caused by insulin insufficiency (Haidari *et al*., 2012). In addition, with elevated serum glucose level and insufficient insulin, carbohydrates were not available as a source of energy, result in increasing breaking down proteins and fat as an alternate (Rossmeisl *et al*., 2003).

On the other hand, the DAPA group showed significantly reduced body weight than the other groups, which may be related to the continuous catabolic state. The body weight in the FSE and FSP groups was significantly increased compared to Diabetic rats, which was non-significant compared to the Control. The progressive escalation in weight suggests that FSE can attenuate the toxicity of STZ, particularly at high doses. It might be possible that treatment with FG can lead to better utilization of nutrients in the diet by enhancing the intestinal microbiota (Man *et al*., 2023) and, thus, a gain in weight.

A rat model with induced peripheral insulin resistance, followed by low dose STZ to target the pancreatic β-cells would closely mimic not only the phenotype but also the pathogenesis of human T2DM (Asrafuzzaman *et al*., 2017). insulin resistance induced by high fructose feeding in both humans and animal models (Basciano *et al*., 2005) are commonly characterized by profound metabolic dyslipidemia, which appears to result from hepatic and intestinal overproduction of atherogenic lipoprotein particles.

Streptozotocin is derived from Streptomyces achromogenes, a well-known selection to trigger experimental DM and is essentially composed of a nitrosourea analogue and deoxy glucose moiety. Its deoxy glucose moiety is responsible for transporting the native molecule across the cell membranes using glucose transporter 2 (GLUT2) for transportation into the pancreatic β-cells. While, Its nitrosourea moiety causes β-cell damage by alkylation or breakage of DNA strands and a consequent increase in poly-ADP-ribose synthetase activity while (Ghasemi *et al*., 2014).

These explanations support our finding that unlike the control, the diabetic group displayed significantly decreased insulin levels and hyperglycemia because of STZ capability to cause selective pancreatic beta cell necrosis, which causes degranulation and loss of capability to release insulin, (Lenzen, 2008; Zafar and Naqvi, 2010). This is consistent with the pancreas's histological findings, which showed few small-sized islets of Langerhans with few degenerated cells showing nuclear shrinkage and cytoplasmic vacuolation. These results agreed with the finding of (Sayed *et al*., 2020).

In our experiment, The Diabetic group displayed significantly escalated TG, TC, and LDL serum levels and lowered HDL levels compared to Control. In consistent with many investigations which stated that Dyslipidemia is a common feature of T2DM. The characteristic dyslipidemia profile consists of elevated TG, TG-rich lipoproteins (TRLs), small dense LDLs (sdLDL), and reduced HDL levels (Revathy *et al*., 2014; Vergès, 2015). Several factors such as hyperglycemia, insulin resistance, abnormalities in adipokines and adipocytokines have been implicated in pathophysiology of dyslipidemia in T2DM (Galicia-Garcia *et al*., 2020). T2DM dyslipidemia occurs due to insulin resistance (IR) which leads to an impaired adipose tissue fat storage, accompanied by increased FFA release from the intracellular TG stores of adipocytes. The elevated fatty acid released from fat stores in the body's periphery occurs because of low or not acting insulin (normally insulin inhibits hormone-sensitive lipase, the enzyme which hydrolyzes adipose tissue TG and release FFA) (Revathy *et al*., 2014). The released FFAs are taken up by hepatocytes, where they can be directed to the mitochondria and undergo β-oxidation; be re-assimilated into TG to assemble new VLDL particles; increase rate of gluconeogenesis resulting in a worsening of hyperglycemia; or stored as TG leading to hepatic steatosis. T2DM and IR impair metabolism and clearance of chylomicrons and very low-density lipoprotein (VLDLs). Activation of Cholesteryl Ester Transfer Protein (CETP) promotes an exchange of TG out of remnant like particles (RLPs) and incorporates Cholesteryl Ester (CE) from HDL and LDL particles leading to reduced levels of circulating HDL-C and an increase in the more atherogenic sdLDL particles (Galicia-Garcia *et al*., 2020). A serious complication of TG-rich lipoproteins elevation and their remnants contribute to atherogenesis and cardiovascular diseases (CVD) risk and experimental studies indicated a connection between cholesterol deposition and inflammation as a result of LDL entry into the artery wall.

Treatment of diabetic rats with FSE, FSP or DAPA improve the glycemic index and lipid profile by decreasing glucose levels, TG, TC and LDL-c and increasing insulin level and HDL-c. These findings were in consistent with the findings of (Haeri *et al*., 2012).

The mechanism by which fenugreek seeds have anti-diabetic properties is delaying gastric emptying time and decreasing glucose uptake in the small intestine because of their high fiber content, in that way reducing metabolism of carbohydrate and lowering blood glucose levels. Moreover, there is a renewal of pancreatic cells by preserving β-cells and an increase in levels of serum insulin by stimulation of islet cell regeneration. Fenugreek also stimulates glycogen synthase activity and promotes the production of liver and muscle glycogen. Additionally, fenugreek may increase insulin sensitivity by enhancing insulin action at the cellular level, lowering HbA1c levels by using glucose in the peripheral tissues and maintaining blood glucose levels (Shabil *et al*., 2023).

Other different mechanisms implicated the antidiabetic properties of fenugreek have been investigated in several researches (Khound *et al*., 2018), glucagon suppression (Shabil *et al*., 2023).

Additionally, fenugreek seeds have an ability to improve insulin sensitivity in adipocytes (Li *et al*., 2018), which is crucial for regulating blood sugar levels and mitigating the risk of excessive insulin secretion. Fenugreek seeds could inhibit intestinal sodium-dependent glucose uptake in vitro using rabbit intestinal brush border membrane vesicles. Fenugreek seeds have also been shown to regulate glucagon-like peptide-1 (GLP-1) activity, which is important for secreting insulin after food intake (King *et al*., 2015).

Fenugreek seeds are rich in bioactive compounds e.g. alkaloids (trigonelline), flavonoids (quercetin, luteolin), saponins and steroidal sapogenins. Additionally, fenugreek seeds are palatable and nutritionally dense, containing carbohydrates, proteins, lipids and volatile oils (Alu'datt *et al*., 2024). Some of these bioactive compounds is responsible for fenugreek antidiabetic effect by different pathways. Diosgenin, a saponin (steroidal glycoalkaloid) showed increased regeneration of pancreatic beta cells and insulin granules (Kalailingam *et al*., 2014a).

Trigonelline comprises 0.1–0.3% of fenugreek seeds. Trigonelline can increase insulin sensitivity due to its ability to improve the insulin signaling pathway by increasing insulin receptor autophosphorylation (IR-PH) and the translocation of effector molecules pT308-Akt, and glucose transporter 4 to the cell surface, thus increasing glucose uptake (Aldakinah *et al*., 2017). One of the most studied fenugreek compounds is 4-Hydroxyisoleucine, (4-HIL), classified as an amino acid that constitutes 1–3% of fenugreek seeds. It has been shown to have antidiabetic effects by, stimulating insulin secretion and improving glucose tolerance (Singh *et al*., 2022). It has been proposed that 4-HIL works by negatively regulating TNF-alpha production, resulting in insulin sensitivity and increasing GLUT4 (glucose transporter type 4) and p-IRS-1 (insulin receptor substrate (1) in the insulin-signaling pathway. It is thought to work directly on pancreatic cells and may enhance glucose uptake in peripheral tissues such as skeletal muscle and adipose tissue, contributing to improved glycemic control.

Additionally, fenugreek can help modulate the action of insulin, making cells more responsive to it. Fenugreek possesses anti-inflammatory properties that may help mitigate inflammation in the body. By reducing levels of pro-inflammatory cytokines, fenugreek can support better insulin sensitivity and metabolic health (Al-Mosawi, 2021).

Fenugreek is modulating the lipid profiles by reducing total cholesterol, LDL cholesterol and triglycerides, referring another significant property of diabetes management (Shaikh *et al*., 2022). Diosgenin may regenerate pancreatic beta cells and insulin granules. Trigonelline increases insulin sensitivity and improves insulin signaling pathways. Galactomannan can improve lipid profiles and glycemic control, and 4-HIL stimulates insulin secretion and has anti-oxidative and anti-inflammatory effects (Singh *et al*., 2022).

The lipid-lowering effects of fenugreek are likely influenced by the bioactive compounds present in the extract, which may include saponins, flavonoids, and alkaloids. These compounds are known to have distinct effects on lipid metabolism compared to the whole powder form, which may contain additional components that could counteract some of the lipid-lowering benefits. Moreover, the extraction process can concentrate specific phytochemicals, enhancing their bioavailability and efficacy in modulating lipid profiles (Ríos *et al*., 2015). This could explain the lower LDL levels observed in the FSE group compared to the FSP group. The potential factors such as differences in absorption rates and metabolic pathways in the two forms of fenugreek, which may contribute to the observed differences in LDL levels.

Fenugreek also appears to decrease oxidative stress and lipoprotein levels, which also antidiabetic.

In agreement with our results, (Abdel-Wahab *et al*., 2018; Oraby *et al*., 2019a), and Sayed *et al*. (2020) stated that An SGLT2 inhibitor, DAPA, decrease blood glucose levels and increase insulin level thereby improving the BGLs of diabetic patients without causing hypoglycemia by blocking SGLT2 in the kidney's early proximal tubules and consequently, limit renal glucose reabsorption and increase glucose excretion in the urine. These results accompanied by enhancement of the pancreas histopathology, which showed moderate congestion of vessels and mild degeneration of

islet cells (Wei *et al*., 2020) as shown also in our pancreatic histopathology.

The DAPA significantly decreased TC and TG, which came in agreement with Yang *et al*. (2022). This finding demonstrated the ability of DAPA to control glucose metabolism and blood lipids. In the present investigation, the impact of FSE and FSP on lipid profile was significantly decreased TG and TC levels and increased HDL levels compared to diabetic rats. These findings align with the results reported by Sharma and Choudhary (2014). Fenugreek appeared to exerts hypolipidemic effects, improve lipid profile (triglyceride, total cholesterol and high-density lipoprotein), which are known risk factors for T2DM (Shaikh *et al*., 2022) analogous to results of lipid profile of our study which show partial adjustment by significant decreasing TG, TC and LDL and increasing HDL.

Increased ALT and AST activities along with decreased total protein and albumin levels of diabetic rats compared to control in our study clearly indicate liver damage. These findings were supported with the histopathological finding of hepatic tissue of diabetic rats which showed necrosis of hepatocytes around the portal regions, accompanied by enlarged and strained portal arteries, as well as regions of inflammatory cell infiltration and depletion of glycogen in the pericentral zones. These results come in agreement with Xin *et al*. (2015) and Salih *et al*. (2014), who suggest that these alterations may be ascribed to the hepatic damage resulting from STZ induction. Since STZ uses glucose transporter 2 (GLUT2) for transportation across cell membrane organs which express this transporter such as the kidney, liver, and intestine are affected (Galicia-Garcia *et al*., 2020).

However, type 2 diabetes associated with elevated blood glucose (hyperglycemia), excess of non-esterified free fatty acids (FFAs–NEFA) and circulating very low density lipoproteins (VLDLs), chylomicrons remnants (CMRs) that are rich in triglycerides (TG) causing glucotoxicity and lipotoxicity. This induces a spike in reactive oxygen species (ROS) concentrations, which in turn leads to an abnormal generation of inflammatory molecules. Given that inflammation is a recognized inducer of oxidative stress, a synergistic interaction occurs between the two processes, with consequent amplification of harmful effects. The sustained and marked increase in levels of ROS contributes significantly to the pathogenesis of T2DM and IR. Therefore, a pro-oxidant environment leads to mitochondrial dysfunction, endoplasmic reticulum (ER) stress, activation of NADPH oxidase (NOX) and superoxide ($O_2^-$) production. The increase in ($O_2^-$) production activates the five major pathways involved in the pathogenesis of diabetes complications: enhancement of the polyol pathway, increased formation of advanced glycation end products (AGEs), increased expression of AGEs receptor and its activating ligands, activation of protein kinase C (PKC) isoforms, and overactivity of the hexosamine pathway (Dali-Youcef *et al*., 2012). Through these pathways, increased intracellular ROS causes defective angiogenesis in response to ischemia, activates a number of proinflammatory pathways, and cause long-lasting epigenetic changes which drive persistent expression of proinflammatory genes even after glycemia is normalized (Committee, 2023; Lu *et al*., 2024).

Liver function tests (liver enzymes, Total protein and albumin) were improved significantly in diabetic rats treated with FSE (250 mg/ kg.bwt), FSP (100 mg/ kg.bwt) or DAPA (1 mg/ kg.bwt) comparing to diabetic rats. But the measured values still significantly different when compared with the normal control which means that liver functions tests restored partially to normal.

On the other hand, some studies suggest that fenugreek may help improve liver function. It has antioxidant properties that can protect liver cells from damage, potentially leading to reductions in ALT, AST levels. Fenugreek has hepatoprotective properties, which may help improve overall liver health. Since the liver is responsible for producing albumin, better liver function could potentially lead to improved albumin and total protein levels (Hadi *et al*., 2020).

In consistent with our finding, some studies reported that DAPA can lead to reductions in ALT and AST levels in patients with type 2 diabetes, particularly those with non-alcoholic fatty liver disease (NAFLD). This suggests potential benefits for liver health. The antioxidant properties of DAPA may also play a role in protecting liver cells from damage, further supporting its potential to lower ALT and AST levels. DAPA may influence serum albumin levels indirectly through its effects on kidney function and fluid balance. Improved glycemic control can lead to better kidney health, potentially stabilizing albumin levels in the bloodstream. Since albumin is a significant component of total protein, the relationship between DAPA and albumin levels also affects total protein assessments. DAPA's ability to lower albuminuria can help stabilize total protein levels, indicating improved kidney function (Harreiter *et al*., 2021).

Another explanation, dapagliflozin improved liver dysfunction and steatosis by inhibiting de novo lipogenesis in the liver, since, by using a mouse model of NASH with diabetes, there was a significantly lower expression of fatty acid synthase and acetyl-CoA carboxylase 1, two genes involved in denovo lipogenesis (Takase *et al*., 2017), Fatty acid synthase is a key enzyme in the hepatic biosynthesis

of fatty acids and is believed to determine the maximal liver capacity for producing fatty acids Thus, dapagliflozin may contribute to improvement of hepatic steatosis in patients with type 2 diabetes and NAFLD by inhibiting fatty acid production through promotion of urinary glucose excretion (Shimizu *et al*., 2019). Consequently, suppress lipotoxicity, lipid accumulation and hepatic tissue undesirable alteration which accompanied T2DM.

In a previous study, genes involved in cell death, stress response, inflammation, T-cell response, and fibrosis were substantially downregulated in diabetic patients treated with another sGLT2 inhibitor; Tofogliflozin (Takeshita *et al*., 2022). These results indicated that sGLT2 inhibitors may alleviate liver inflammation, hepatocellular damage, and liver fibrosis and exert unique pleiotropic effects beyond glucose lowering.

The pancreatic tissue structural organization is directly related to the stage and severity of DM (Murao *et al*., 2008). Here, diabetic rats exhibited impaired pancreatic islet cells and significantly altered exocrine and endocrine components following STZ injection, which aligns with prior results reported by Zin *et al*. (2019). The FG-treated rats exhibited enhanced pancreatic tissue function, which may be attributed to diosgenin, which is hypothesized to regenerate pancreatic β-cells and increase insulin secretion Kalailingam *et al*. (2014b). Additionally, diosgenin enhances BGLs and maintains the integrity of the pancreas, liver, and skeletal muscle tissues. A prior study on FG oil's impact on the pancreas in a rat model of diabetes demonstrated that the administration of FG oil resulted in a partial reversal of pancreatic cell damage and renal function (Hamden *et al*., 2010). Furthermore, the histological analysis of the liver in diabetic rats revealed necrosis of hepatocytes around the portal regions, accompanied by enlarged and strained portal arteries, as well as regions of inflammatory cell infiltration and depletion of glycogen in the pericentral zones. The administration of FG normalized these variations. Novel metabolomics investigations have revealed that FG flavonoids can reduce IR, enhance glycolysis and gluconeogenesis, and provide protection to pancreatic islet and liver cells against injury (Jiang *et al*., 2017).

Oxidative stress and inflammation play crucial roles in the pathogenesis of T2DM and its complications. 4-Hydroxyisoleucine 4-HIL exerts its antioxidant and anti-inflammatory effects by decreasing oxidative stress and the inflammatory response via activation of nuclear factor erythroid 2-related factor 2 (Nrf2 gene, a free radical scavenger and antioxidant enzyme stimulator) and the transforming growth factor β1 signaling pathway (Avalos-Soriano *et al*., 2016; Singh *et al*., 2022).

Prescribed antidiabetic synthetic drugs may have side effects, The incidences of adverse side effects were recorded in diabetic patient treated with tofogliflozin, another SGLT2 inhibitor which attributed to the increased incidence of genital and urinary tract symptoms (Takeshita *et al*., 2022). This adverse effect may be due to the excessive excretion of glucose in urine.

However, in comparison to prescribed synthetic drugs, Fenugreek is of natural origin and have been consumed through diet for many generations with noted health benefits (Alu'datt *et al*., 2024) and are likely to have a lower risk of unwanted side effects. Animal studies using controlled administration of doses (up to 1000 milligrams of fenugreek seed per kilogram of weight) have been shown to be non-toxic to mammals.

## CONCLUSIONS AND RECOMMENDATIONS

This study underscores the potential of FSP and FSE as effective treatments for DM, particularly in managing hyperglycemia and dyslipidemia. The findings demonstrate that both FSP and FSE significantly improved insulin levels, reduced BGLs, and enhanced lipid profiles in STZ-induced diabetic rats. Additionally, these treatments mitigated the STZ-induced damage to liver and pancreas tissues, highlighting their protective effects. Their contents of alkaloids may cause the mode of action of FG. The results suggest that FSP and FSE could serve as safe and effective herbal alternatives to conventional antidiabetic drugs. Accordingly, it is recommended to investigate the underlying mechanisms by which FSP and FSE exert their antidiabetic effects, focusing on their impact on insulin signaling pathways and glucose metabolism. Determining the optimal dosages of FSP and FSE for maximum therapeutic benefit with minimal side effects, considering the different stages of DM and patient demographics is also crucial. Additionally, conducting human clinical trials is crucial to verify the FSP and FSE efficiency and safety in diabetic patients, ensuring the results are applicable to a broader population. By implementing these recommendations, the potential of FSP and FSE as viable treatments for DM can be fully realized, contributing to better management of this chronic condition.

The study was conducted on a relatively small sample size of 50 male albino rats, which may limit the generalizability of the findings to larger and more diverse populations, including humans. The eight-week duration of the therapeutic intervention may not be sufficient to observe the long-term impacts and possible side effects fenugreek. The study primarily focused on the outcomes of fenugreek treatment without delving deeply into the underlying mechanisms of action. Understanding the precise biochemical and molecular pathways involved

would provide a more comprehensive understanding of how these treatments work. Addressing these limitations in future research will help validate and extend the findings, ensuring that the potential benefits of fenugreek for DM treatment are fully understood and effectively translated into clinical practice.

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## NOVELTY STATEMENT

The present study compare for the first time between the effect of fenugreek extract and dapagliflozin, addressing their potential role in controlling glucose level in STZ induced diabetes in tat along with modulating liver degeneration and steatosis.

## AUTHOR'S CONTRIBUTION

JAEH, AGAH, AMA, AAD: Idea and research design. BME: Sample collection. BME, AAD: Methodology, chemical analysis and data curation. BME: Statistical analysis. AAD, HAS: Writing the original draft and revising the final version. AGAH: Performed the histopathology. All authors review, editing, read and approved the final manuscript.

### Conflict of interest

The authors have declared no conflict of interest.

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