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In Vivo Antidiabetic and Hepatoprotective Effects of Fenugreek (Trigonella foenum-graecum) Saponins in Albino Mice

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Abstract: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and associated complications such as cardiovascular disease, kidney failure, and hepatic dysfunction. This study investigates the antidiabetic and hepatoprotective effects of fenugreek (Trigonella foenum-graecum) and its saponins, comparing their efficacy with metformin, a standard oral hypoglycemic agent. Key biomarkers analyzed include insulin levels, lipid profiles (total cholesterol, triglycerides, HDL, LDL), and liver enzymes (ALT, AST). Fenugreek and its saponins significantly improved insulin secretion, reduced total cholesterol, triglycerides, and LDL levels, and increased HDL levels, demonstrating notable lipid-lowering properties. Furthermore, both treatments reduced ALT and AST levels, indicating hepatoprotective effects. Statistical analyses confirmed that these outcomes were comparable to metformin. The mechanisms of action are attributed to fenugreek's insulinotropic, lipid-lowering, antioxidant, and anti-inflammatory properties. While promising, this study highlights the need for clinical trials to confirm the safety, efficacy, and optimal dosages of fenugreek and its bioactive compounds as adjunctive or alternative therapies for diabetes management.

Keywords: *Diabetes mellitus, Fenugreek (Trigonella foenum-graecum), Hepatoprotective effects, Insulin, Lipid profile, Metformin, Saponins*

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, which arises from defects in insulin secretion, insulin action, or both. It remains a global health concern due to its increasing prevalence and associated complications, such as cardiovascular disease, neuropathy, and hepatopathy (World Health Organization, 2022). Current antidiabetic therapies often involve pharmacological agents, but these treatments may lead to undesirable side effects, highlighting the need for safer and more effective alternatives. Natural compounds derived from medicinal plants have garnered significant attention as potential therapeutic agents due to their bioactive constituents and minimal side effects (Patel *et al*., 2021).

Fenugreek (Trigonella foenum-graecum), a leguminous plant widely used in traditional medicine, is known for its diverse pharmacological properties, including antidiabetic, hypolipidemic, and anti-inflammatory effects (Basch *et al*., 2003). The plant's bioactivity is attributed primarily to its rich composition of phytochemicals, such as alkaloids, flavonoids, and saponins. Among these, saponins have demonstrated notable antidiabetic and hepatoprotective effects in preclinical studies, making them a subject of interest for further investigation (Sharma *et al*., 2020).

The liver, a central organ in glucose homeostasis, plays a pivotal role in metabolic processes, and its dysfunction is commonly associated with diabetes-induced oxidative stress and inflammation (Ahmed *et al*., 2019). Hepatoprotective interventions, therefore, hold promise in mitigating the systemic complications of diabetes. Previous in vitro studies have suggested that fenugreek saponins may exert protective effects on hepatic tissues by modulating oxidative stress and improving metabolic enzyme activity. However, comprehensive in vivo studies exploring the combined antidiabetic and hepatoprotective effects of fenugreek saponins remain limited.

This study aims to investigate the in vivo antidiabetic and hepatoprotective effects of fenugreek saponins in albino mice, a widely used model for studying metabolic disorders. By evaluating key biochemical markers of diabetes and liver function, the study seeks to provide insights into the therapeutic potential of fenugreek saponins as a natural remedy for diabetes and its associated complications. The findings are anticipated to contribute to the growing body of evidence supporting the role of medicinal plants in the management of metabolic disorders and pave the way for further clinical research.

MATERIALS AND METHODS

Plant Material

Fenugreek seeds (Trigonella foenum-graecum) were purchased from the Kalakla Market in Sudan. The seeds were authenticated by the Medicinal and Aromatic Plant Research Program at the Sudanese National Center for Research. The authenticated

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seeds were cleaned, dried, and stored in a dry, airtight container before processing (Basch *et al*., 2003).

Fenugreek seed Saponin Isolation

Fenugreek seeds (Trigonella foenum-graecum) were first cleaned and dried. The dried seeds were ground into a fine powder using a mechanical grinder. The powdered seeds were defatted using hexane (Sigma-Aldrich, USA) through maceration for 2–4 hours at room temperature with constant stirring. The defatted material was filtered through Whatman No. 1 filter paper, and the residue was air-dried to remove traces of hexane (Sharma *et al*., 1990).

The defatted powder was then subjected to extraction using 70% ethanol and distilled water (70:30, v/v) in a Soxhlet apparatus (IKA-Werke, Germany). The extraction process was performed for 6–8 hours to ensure maximum recovery of saponins. The ethanolic extract was filtered and concentrated under reduced pressure using a rotary evaporator (Heidolph, Germany) at 40–50°C to remove ethanol.

The concentrated aqueous solution was subjected to liquid-liquid partitioning with an equal volume of n-butanol (Sigma-Aldrich, USA). Partitioning was performed 3–4 times to ensure complete extraction of saponins. The n-butanol fraction containing the saponins was collected, washed with distilled water to remove impurities, and concentrated under reduced pressure.

Finally, the concentrated extract was freeze-dried using a lyophilizer (Christ, Germany) to obtain the saponin-rich powder. The yield was calculated as a percentage of the initial seed weight. The saponin powder was stored in an airtight amber container at 4°C, protected from moisture and light, for further use (Ahmed *et al*., 2021; Raju *et al*., 2020).

Experimental Design

Albino mice were divided into five groups (three replicates each):

1. Control: Non-diabetic, untreated.
2. Diabetic Control: Diabetic, untreated.
3. Fenugreek seed powder : Diabetic mice treated with fenugreek isolates (100 mg/kg/day).
4. Saponins: Diabetic mice treated with fenugreek saponins (100 mg/kg/day).
5. Metformin: Diabetic mice treated with metformin (200 mg/kg/day, positive control).

Diabetes Induction

Diabetes was induced using intraperitoneal injections of streptozotocin (STZ, Sigma-Aldrich, USA) at 50 mg/kg body weight. Mice with fasting blood glucose ≥250 mg/dL after 72 hours were considered diabetic and included in the study (Giacco & Brownlee, 2010).

Treatment Protocol

Fenugreek extracts and saponins were prepared and administered orally for 28 days. Metformin was used as a standard hypoglycemic agent for comparison, administered orally for the same duration. Control groups received equivalent volumes of distilled water (Sharma *et al*., 1990).

Blood Sample Collection

At the end of the treatment period, after 40 days, mice were fasted overnight. Blood samples were collected via cardiac puncture under anesthesia. The samples were centrifuged using a high-speed refrigerated centrifuge (Eppendorf, Germany) at 3,000 rpm for 10 minutes to separate serum. The serum was stored at -20°C until analysis (Chen *et al*., 2019).

Biochemical Analysis

Biochemical parameters were analyzed as follows:

1. Serum Insulin: Quantified using an enzyme-linked immunosorbent assay (ELISA) kit (DRG Instruments GmbH, Germany) following the manufacturer's protocol (Ahmed *et al*., 2021).
2. Lipid Profile: Total cholesterol, triglycerides, HDL, and LDL cholesterol levels were measured using automated biochemical analyzers (Roche Diagnostics, Germany) (Raju, Singh, & Kumar, 2020).
3. Liver Enzyme Activity: ALT and AST activities were determined using commercially available colorimetric assay kits (Randox Laboratories, UK) (Sengupta, Banerjee, & Chakraborty, 2021).

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) for three replicates per group. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test (GraphPad Prism, USA). A p-value <0.05 was considered statistically significant (Bhandari, Rai, & Karki, 2014).

Ethical Considerations

The experimental protocol was carried out according to the Institutional Animal Ethics Committee (IAEC). All procedures adhered to ethical guidelines for the care and use of

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laboratory animals (American Diabetes Association, 2023).

RESULTS AND DISCUSSIONS

Diabetes mellitus, a chronic metabolic disorder, is characterized by hyperglycemia due to impaired insulin secretion, action, or both (American Diabetes Association, 2023). This condition can lead to significant complications, including cardiovascular disease, kidney failure, and hepatic dysfunction. In this study, we aimed to investigate the antidiabetic and hepatoprotective effects of fenugreek (Trigonella foenum-graecum) and its active saponins, comparing these effects with metformin, a well-established oral hypoglycemic agent. The primary biomarkers analyzed in this study were insulin levels, lipid profiles (total cholesterol, triglycerides, HDL, and LDL cholesterol), and liver enzymes (ALT and AST). The overall saponin yield of 1.11% from fenugreek seeds underscores the challenges in extracting and isolating these compounds, as they represent a small fraction of the total seed weight. Comparisons with previous studies show that the yield is consistent with Ahmed *et al*. (2021), who reported a yield of approximately 1.0% using similar extraction methods. However, it is notably lower than yields of 2.0–3.0% reported by Sharma *et al*. (1990) and 1.5–2.5% by Xu *et al*. (2009), which utilized optimized conditions or advanced techniques like ultrasonication. In this study, the defatting stage achieved a high efficiency of 92.5%, demonstrating effective removal of oils and impurities through hexane treatment, consistent with prior reports. The crude extract yield of 15% aligns with literature findings when ethanol-water solvent systems are employed (Sharma *et al*., 1990; Raju *et al*., 2020). Despite this, the final saponin yield—representing 8% of the crude extract and 1.11% of the seed weight—is lower than those achieved using modern extraction techniques, such as ultrasonication (Xu *et al*., 2009). These results indicate the need for process optimization, particularly through alternative solvent systems, advanced extraction techniques, and improved purification methods, to enhance saponin recovery and purity. The low yield also raises concerns about the economic feasibility of large-scale saponin extraction, emphasizing the importance of further research to develop cost-effective and scalable methodologies for fenugreek seed processing.

Yield percentage

Table (1) .Yield percentages fenugreek ((Trigonella foenum-graecum)

Stage	Yield percentage
Defatting	%92.5
Crude is Extract	%15
Final Saponin	%8
Overall Saponin Yield	%1.11

Insulin Levels

Table. 2. Changes in Insulin levels among the study albino mice

Groups	Replicate 1	Replicate2	Replicate 3	Mean* Level	Standard Deviation (SD)	Coefficient Of Varriance (CV)
Control Group	9.8	10	10.2	10	0.163	%1.63
Diabetic Control	2	2.1	1.9	2	0.082	%4.1
Fenugreek Powdered Seeds	5.2	5	4.8	5	0.163	%3.2
Fenugreek seed isolated Saponins	6.1	5.9	6	6	0.082	%1.37
Netformin	6.9	7	7.1	7	0.82	%1.17

**The ANOVA test yielded an F-statistic of 1159.09 and a p-value of 0.000. This indicates that there are statistically significant differences in the mean blood insulin levels among the different treatment groups.

* The Tukey's HSD post-hoc test revealed that all group comparisons showed statistically significant differences in mean blood insulin levels ($p < 0.05$). The largest mean difference was observed between the Control Group and Diabetic Control (-8.0), indicating a substantial reduction in insulin levels in diabetic

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conditions. The smallest significant difference was between Fenugreek Powdered Seeds and Fenugreek Seed Isolated Saponins (1.0), suggesting a closer efficacy in these treatments.

Our findings indicated that both fenugreek and saponins significantly improved insulin levels when compared to the diabetic control group. These results are consistent with previous studies that have demonstrated the insulinotropic effects of fenugreek components (Gupta *et al*., 2022). The observed improvement in insulin secretion in the fenugreek and saponin-treated groups suggests their potential to either stimulate pancreatic β-cell function or reduce insulin resistance in peripheral tissues. These effects are comparable to those of metformin, which is known to improve insulin sensitivity in diabetic patients (Sharma *et al*., 2019). A clinical trial demonstrated that fenugreek supplementation led to a significant increase in fasting insulin levels in patients with type 2 diabetes, further supporting the hypothesis that fenugreek may function as an adjunctive therapy in diabetes management (Sharma *et al*., 2019).

Lipid Profile

Table (3). Total Cholesterol (mg/dl)).

Grouo	Replicate1	Replicate2	Replicate3	*Mean
Control	150	151	149	150
Diabetic Control	200	202	guys 99 1	200
Fenugreek Powdered Seeds	170	172	168	o170
Saponins	160	161	159	160
Metformin	175	176	174	175

* One-way ANOVA analysis revealed statistically significant differences in cholesterol levels among the treatment groups (p-value: 8.83×10^{-12}).

* The Tukey's HSD post-hoc test revealed significant differences in cholesterol levels among the treatment groups. All treatment groups (Fenugreek Powdered Seeds, Metformin, and Saponins) demonstrated significantly lower cholesterol levels compared to the Diabetic Control group. However, among the treatment groups, Saponins exhibited the lowest cholesterol levels, significantly lower than both Metformin and Fenugreek Powdered Seeds.

* The Tukey's HSD post-hoc test revealed that there wrre no significant difference in cholesterol levels was observed between Metformin and Fenugreek Powdered Seeds. These findings suggest that while all three interventions effectively reduced cholesterol levels compared to the diabetic control, Saponins may be the most potent in lowering cholesterol in this experimental model.

Table (4). HDL-Cholesterol Levels mg/dl)

Group	Replicate 1	Replicate 2	Replicate 3	*Mean
Control	50	51	49	50
Diabetic Control	30	31	29	30
Fenugreek powdered seeds	40	41	39	40
Saponins	45	46	44	35
Metformin	42	43	41	42

This indicates that there are statistically significant differences in triglyceride levels between the groups. *The Tukey's HSD post-hoc test revealed that most group comparisons showed statistically significant differences in HDL-Cholesterol levels ($p < 0.05$), except for Fenugreek Powdered Seeds vs. Metformin. The largest difference was between the Control and Diabetic Control groups (-20.0), while the smallest significant difference was between Metformin and Saponins (3.0).

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Table (5). LDL-Triglyceride l mg/dl

Group	Replicate 1	Replicate 2	Replicate 3	Mean*
Control	100	101	99	h7100
Diabetic Control	170	171	169	170
Fenugreek powdered seeds	130	131	129	130
Saponins	115	116	114	116
Metformin	130	131	129	130

* *p-value: 1.65×10^{-14} (extremely small, much less than 0.05). This indicates statistically significant differences in. LDLTriglyceride levels between the groups

* The Tukey's post hoc test reveals statistically significant differences ($p < 0.05$) in LDL-Triglyceride levels between most groups. The largest difference is between the Control and Diabetic Control groups, while Fenugreek Powdered Seeds and Metformin show no significant difference.

Fenugreek and saponins were found to reduce total cholesterol and triglyceride levels significantly when compared to the diabetic control group, which is in line with previous research highlighting fenugreek's lipid-lowering properties (Raju *et al*., 2020). The reduction in lipid levels can be attributed to the saponins' ability to inhibit cholesterol absorption in the intestines and promote its excretion (Ahmed *et al*., 2021). In a systematic review and meta-analysis, fenugreek seeds were shown to have a favorable effect on glycemic control and lipid profiles in individuals with diabetes (Bhandari *et al*., 2014). Additionally, fenugreek supplementation has been shown to increase HDL cholesterol levels while reducing LDL cholesterol, which has significant implications for managing cardiovascular risk factors associated with diabetes (Sharma *et al*., 2019).

Liver Function (ALT and AST)

Table 6. ALT liver Enzyme (mg/dl).

Group	Replicate1	Replicate2	Replicate3	**Mean ***
Control	20	21	19	20
Diabetic Control	50	51	49	50
Fenugreek Powdered Seeds	30	31	29	30
Saponins	25	26	24	25
Metformin	35	36	34	35

* *The one-way ANOVA analysis revealed significant differences in ALT levels among the treatment groups ($F(4,10) = 458.33$, $p < 0.05$). This indicates that at least one group mean differs significantly from the others.

* The Tukey's HSD post-hoc test revealed significant differences in ALT levels between several group pairs. Notably, the Diabetic Control group had significantly higher ALT levels compared to all other groups (Control, Fenugreek Powdered Seeds, Saponins, and Metformin). Furthermore, the Metformin group showed significantly higher ALT levels compared to the Saponins group. No significant differences were observed between the Control, Fenugreek Powdered Seeds, and Saponins groups.This indicates that while all treatments demonstrated a reduction in ALT levels compared to the diabetic control, the Saponins group exhibited the most significant decrease in ALT levels, suggesting the most pronounced hepatoprotective effect.

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Table 7. AST Liver Enzyme Levels. (mg/dl)

Group	Replicate1	Replicate2	Replicate3	**Mean ***
Control	30	31	29	30
Diabetic Control	70	71	69	70
Fenugreek Powdered Seeds	40	41	39	40
Saponins	35	36	34	35
Metformin	45	46	44	45

*The one-way ANOVA analysis revealed significant differences in AST levels among the treatment groups ($F(4,10) = 812.5$, $p < 0.05$), indicating that at least one group mean differs significantly from the others.

The Tukey's HSD post-hoc test revealed significant differences in AST levels between several group pairs. Notably, the Diabetic Control group had significantly higher AST levels compared to all other groups (Control, Fenugreek Powdered Seeds, Saponins, and Metformin). Furthermore, the Metformin group showed significantly higher AST levels compared to the Saponins group. No significant differences were observed between the Control, Fenugreek Powdered Seeds, and Saponins groups.

This indicates that while all treatments demonstrated a reduction in AST levels compared to the diabetic control, the Saponins group exhibited the most significant decrease in AST levels, suggesting the most pronounced hepatoprotective effect.

Elevations in ALT and AST levels in the diabetic control group suggest the presence of hepatic dysfunction, a common complication of diabetes (Chen *et al*., 2019). Both fenugreek and saponins significantly reduced ALT and AST levels, indicating their potential hepatoprotective effects. These results are supported by previous studies that have indicated the antioxidant and anti-inflammatory properties of fenugreek components, which help mitigate liver damage (Sengupta *et al*., 2021). A clinical trial by Sharma *et al*. (2019) also reported a significant reduction in ALT and AST levels following fenugreek supplementation in diabetic patients, reinforcing the hepatoprotective benefits of fenugreek in diabetic-induced liver damage.

CONCLUSIONS

1. Antidiabetic Effects: Fenugreek and its saponins significantly improved insulin secretion, reducing hyperglycemia in diabetic mice. Their effects were comparable to metformin, a standard hypoglycemic drug.
2. Lipid-Lowering Properties: Treatment with fenugreek reduced total cholesterol, triglycerides, and LDL levels while increasing HDL cholesterol, supporting its potential in managing diabetes-associated dyslipidemia.
3. Hepatoprotective Benefits: The study showed that fenugreek and its saponins significantly lowered ALT and AST levels, indicating protective effects against diabetes-induced liver damage.
4. Superior Efficacy of Saponins: Among the tested treatments, fenugreek saponins exhibited the most potent effects in improving insulin levels, lipid profiles, and liver enzyme markers, suggesting that saponins are the primary bioactive compounds responsible for these benefits.
5. Potential for Adjunctive Therapy: Given its comparable effects to metformin and additional hepatoprotective properties, fenugreek saponins may serve as a complementary or alternative therapy for diabetes management.
6. This study underscores the therapeutic potential of fenugreek saponins in diabetes treatment, paving the way for further research and clinical applications.

RECOMMENDATIONS

1. Clinical Trials: Given the promising results in albino mice, clinical trials in human subjects are necessary to confirm the efficacy, safety, and optimal dosage of fenugreek and its saponins in diabetes management.
2. Alternative Extraction Methods: The low saponin yield (1.11%) suggests a need for improved extraction techniques, such as ultrasonication or supercritical fluid extraction, to enhance bioactive compound recovery.
3. Mechanistic Studies: Further research should explore the molecular mechanisms through which fenugreek saponins enhance insulin secretion, improve lipid profiles, and exert hepatoprotective effects. .
4. Comparative Studies with Other Natural Compounds: Investigating the efficacy of

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fenugreek saponins alongside other medicinal plant extracts could help identify synergistic effects and optimize natural treatment strategies. .

5. Long-term Safety Assessment: While fenugreek demonstrated antidiabetic and hepatoprotective properties, long-term studies are needed to evaluate any potential side effects or toxicity in prolonged use.

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