



Research Article



Effects of Fenugreek (*Trigonella foenum-graecum*) Seed Extract on Glycemic Control, Physiological Parameters, Hematological Profile and Hormonal Changes in Alloxan-Induced Diabetic Mice

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KEY WORDS:

Diabetes mellitus, alloxan-induced diabetes, fenugreek (*Trigonella foenum-graecum*), hematological parameters, blood glucose, hormonal alterations, glibenclamide, experimental mice

Abstract: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia and is frequently accompanied by hematological, physiological, and hormonal disturbances. The present study aimed to evaluate the antidiabetic and protective effects of fenugreek (*Trigonella foenum-graecum*) seed extract on blood glucose levels, body weight, hematological indices, and selected hormonal parameters in alloxan-induced diabetic mice, in comparison with the standard antidiabetic drug glibenclamide. Diabetes was induced in mice using alloxan monohydrate, and the animals were allocated into four groups: normal control, diabetic control, diabetic mice treated with fenugreek seed extract, and diabetic mice treated with glibenclamide. Fasting blood glucose levels and body weight were monitored throughout the experimental period. Hematological parameters, including hemoglobin concentration, red and white blood cell counts, and packed cell volume, as well as serum levels of growth hormone, cortisol, and progesterone, were assessed at the end of the study. Alloxan-induced diabetic mice exhibited significant hyperglycemia, body weight loss, anemia, leukocytosis, reduced packed cell volume, and pronounced hormonal imbalance compared with normal controls. Treatment with fenugreek seed extract significantly reduced blood glucose levels, prevented body weight loss, and markedly improved hematological parameters. Moreover, fenugreek administration effectively modulated diabetes-associated hormonal alterations, as evidenced by decreased cortisol levels and normalization of growth hormone and progesterone concentrations. These effects were comparable to those observed with glibenclamide treatment. In conclusion, fenugreek seed extract demonstrated significant antidiabetic, hematological, and endocrine protective effects in alloxan-induced diabetic mice, supporting its potential as a natural therapeutic agent for the management of diabetes mellitus and its associated complications.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic and lifelong condition, resulting from lack of insulin or insulin independent effect. It is associated with many complications of systemic nature including: physiologic, hematological or hormonal alterations.

Continuous hyperglycemia leads to oxidative stress, immune dysfunction and endocrine disbalance even in terms of hormone levels as growth hormone, cortisol and principally progesterone. Conventional drugs like sulfonylureas (e.g. glibenclamide) still ensure effective for blood sugar control, however,

issues concerning side effects and expensive pricing preclude their routine and habitual usage particularly in areas of developing world^[1]. Hence, herbal medicine has emerged as an area of great interest in the aspect of alternative and complementary treatment for diabetes. Of these medicinal plants, fenugreek (*Trigonella foenum-graecum*), known to be used in the traditional medicine of Asian countries and some Middle Eastern areas has been reported to have significant antidiabetic properties.

Medicinally active parts Fennel seeds contains many bioactive molecules such as soluble fibre, flavonoids and 4-hydroxyisoleucine (an amino acid derivative)^[1,3]. These constituents are reported to enhance the glucose utilization, insulin action and carbohydrate metabolism. Experimental studies and clinical trials have shown that addition of fenugreek to dietary regimen lowers fasting serum glucose, postprandial glucose and glycosylated hemoglobin (HbA1c) in T2DM individuals. The hypoglycemic effect of fenugreek is reported to be multi-mechanistic i.e., improved insulin sensitivity, regulation of intestinal carbohydrate absorption and increase in insulin secretion^[1,2]. Fenugreek extracts have been shown to reduce elevated blood glucose concentrations and oxidative stress markers in alloxan-induced diabetic animal models, reflecting protective activity towards pancreatic β -cells as well as other metabolic enzymes^[3,4]. However, despite such well-documented effects in antidiabetes, little or no information was available as to whether fenugreek has any effect in other physiological systems known to be affected by diabetes such as haematological indices and endocrine parameters [growth hormone (GH), cortisol and progesterone]. This prompted us to investigate the effect of fenugreek seed extract on glycaemia, body weight changes, hematological indices (hemoglobin concentration [Hb], red blood cell count [RBC], white blood cell count [WBC] and packed cell volume [PCV]) and selected hormonal profiles in alloxan monohydrate-induced diabetic mice both when compared with that of glibenclamide which is an established antidiabetic drug.

MATERIAL AND METHODS

Experimental Animals: Adult male albino mice weighing 25-30g and aged 8-10 weeks were used in this study. The animals were obtained from the animal house of the College of medicine Baghdad university under standard laboratory conditions (temperature $22 \pm 2^\circ\text{C}$, relative humidity $55 \pm 5\%$, and a 12h light/dark cycle). Mice were provided with a standard pellet diet and water ad libitum. All experimental procedures were conducted in

accordance with institutional guidelines for the care and use of laboratory animals and were approved by the local ethical committee.

Chemicals and Drugs: Alloxan monohydrate was purchased from a certified commercial supplier and freshly dissolved in sterile normal saline prior to use. Glibenclamide was obtained from a local pharmaceutical source and prepared in distilled water. All other chemicals and reagents used in this study were of analytical grade.

Induction of Experimental Diabetes: After overnight fasting (12 h), diabetes mellitus was induced in mice by a single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg body weight. To prevent initial hypoglycemic shock, mice were provided with 5% glucose solution for 24 h after alloxan administration. Seventy-two hours after injection, fasting blood glucose levels were measured using a glucometer via tail vein puncture. Mice with fasting blood glucose levels = 200 mg/dL were considered diabetic and included in the study.

Preparation of Fenugreek Seed Extract: Fenugreek (*Trigonella foenum-graecum*) seeds were obtained from a local market and authenticated by a botanist. The seeds were washed, shade-dried, and finely powdered. One hundred grams of the powdered seeds were soaked in one liter of distilled water and stirred continuously for 24 h at room temperature. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator. The resulting extract was stored at 4°C until use.

Experimental Design: Mice were randomly divided into four groups (n = 6 per group):

- Group I (Normal Control): received distilled water only.
- Group II (Diabetic Control): alloxan-induced diabetic mice receiving no treatment.
- Group III (Diabetic + Fenugreek): diabetic mice treated orally with fenugreek seed extract at a dose of 400 mg/kg body weight/day.
- Group IV (Diabetic + Glibenclamide): diabetic mice treated orally with glibenclamide at a dose of 5 mg/kg body weight/day.

All treatments were administered orally using a gastric gavage for 28 consecutive days.

Measurement of Blood Glucose: Fasting blood glucose levels were measured at baseline and weekly intervals

during the experimental period using a portable glucometer. Blood samples were collected from the tail vein after overnight fasting.

Body Weight and Physiological Parameters: Body weight was recorded weekly throughout the experimental period. Food and water intake, as well as general behavior, were monitored daily to assess physiological changes associated with diabetes and treatment.

Hematological Analysis: At the end of the experimental period, mice were anesthetized, and blood samples were collected via cardiac puncture into EDTA-coated tubes. Hematological parameters including red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hb), and packed cell volume (PCV) were determined using an automated hematology analyzer.

Hormonal Profile: Serum levels of growth hormone (GH), cortisol, and progesterone were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Blood samples were allowed to clot at room temperature and centrifuged at 3000 rpm for 15 minutes to obtain serum, which was stored at -20°C until analysis. The assays were performed in duplicate, and hormone concentrations were calculated based on standard calibration curves provided with each kit. The selected hormones were chosen to reflect metabolic, stress-related, and endocrine alterations associated with diabetes mellitus.

Statistical Analysis: Data were expressed as mean \pm standard deviation (SD). Statistical analysis was performed using SPSS software. Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant. Data normality was assessed using the Shapiro-wilk test.

RESULTS AND DISCUSSIONS

Alloxan administration induced a significant elevation in fasting blood glucose levels in diabetic mice compared with the normal control group throughout the experimental period ($p < 0.05$). In contrast, mice treated with fenugreek seed extract exhibited a progressive and significant reduction in blood glucose levels from the first week of treatment, with the most pronounced decrease observed at week 4. The hypoglycemic effect of fenugreek was comparable to that of the standard antidiabetic drug glibenclamide.

Table 2: Regarding body weight, one-way ANOVA revealed a significant difference among the experimental groups at the end of the study period

($p < 0.05$). Diabetic control mice exhibited a marked reduction in body weight compared with the normal control group, indicating metabolic deterioration associated with uncontrolled hyperglycemia. In contrast, treatment with fenugreek seed extract significantly prevented body weight loss and maintained body weight near normal values. Notably, no significant difference was observed between the fenugreek-treated group and the glibenclamide-treated group, suggesting a comparable protective effect.

One-way analysis of variance (ANOVA) demonstrated a statistically significant difference among the experimental groups for all measured hematological parameters (Hb, RBC, WBC, and PCV) ($p < 0.05$). Post hoc analysis using Tukey's multiple comparison test revealed the following:

Hemoglobin (Hb) levels were significantly reduced in the diabetic control group compared with the normal control group ($p < 0.05$), indicating anemia associated with diabetic status. Treatment with fenugreek seed extract significantly increased Hb concentration compared with the diabetic control group ($p < 0.05$), restoring values toward normal levels. A similar improvement was observed in the glibenclamide-treated group, with no significant difference between the two treated groups. Red blood cell (RBC) counts showed a significant decrease in diabetic control mice compared with controls ($p < 0.05$). Fenugreek administration significantly ameliorated this reduction, resulting in RBC values closer to those of the normal control group. Glibenclamide treatment produced comparable effects, and no statistically significant difference was detected between the fenugreek- and glibenclamide-treated groups. White blood cell (WBC) counts were significantly elevated in diabetic control mice compared with normal controls ($p < 0.05$), reflecting an inflammatory response associated with diabetes. Fenugreek treatment significantly reduced WBC counts compared with the diabetic control group ($p < 0.05$), indicating attenuation of systemic inflammation. Glibenclamide exerted a similar anti-inflammatory effect. Packed cell volume (PCV) values were significantly lower in diabetic control mice than in the normal control group ($p < 0.05$). Administration of fenugreek seed extract significantly increased PCV values relative to the diabetic control group ($p < 0.05$), while glibenclamide treatment resulted in a near-complete restoration of PCV toward normal levels.

The hormonal profile analysis revealed significant alterations in growth hormone, cortisol, and progesterone levels in alloxan-induced diabetic mice compared to the normal control group ($p < 0.05$).

Table 1: Effect of Fenugreek Seed Extract and Glibenclamide on Blood Glucose Levels (mg/dL) in Mice

Study Groups	Day 0	Week 1	Week 2	Week 4
Normal Control	95 ± 5	97 ± 6	96 ± 4	98 ± 5
Diabetic Control	260 ± 15*	275 ± 18*	290 ± 20*	306 ± 22*
Diabetic + Fenugreek	255 ± 14*	210 ± 16#	175 ± 14#	140 ± 12#
Diabetic + Glibenclamide	258 ± 16*	190 ± 15#	156 ± 13#	120 ± 10#

Values are expressed as Mean ± SD (n = 6)

* Significant difference compared with normal control group (p < 0.05)

Significant difference compared with diabetic control group (p < 0.05)

Table 2: Effect of Fenugreek Seed Extract and Glibenclamide on Body Weight (g) in Mice

Study Groups	Day 0	Week 4
Normal Control	28 ± 2	30 ± 2
Diabetic Control	27 ± 2	22 ± 1*
Diabetic + Fenugreek	27 ± 2	26 ± 2#
Diabetic + Glibenclamide	27 ± 2	28 ± 2#

Values are expressed as Mean ± SD (n = 6)

* Significant difference compared with normal control group (p < 0.05)

Significant difference compared with diabetic control group (p < 0.05)

Table 3: Effect of fenugreek seed extract and glibenclamide on hematological parameters in alloxan-induced diabetic mice

Test	Control	Diabetic Control	Diabetic + Fenugreek	Diabetic + Glibenclamide
Hb (g/dL)	14.2 ± 0.8	10.5 ± 0.7*	12.8 ± 0.6	13.5 ± 0.7
RBC (×10 ⁶ /μL)	7.1 ± 0.4	5.3 ± 0.3*	6.5 ± 0.4	6.8 ± 0.5
WBC (×10 ³ /μL)	6.2 ± 0.5	9.8 ± 0.7*	7.1 ± 0.6	6.8 ± 0.5
PCV (%)	43 ± 3	32 ± 2*	39 ± 2	41 ± 3

Values are expressed as Mean ± SD (n = 6)

* Significant difference compared with normal control group (p < 0.05)

Significant difference compared with diabetic control group (p < 0.05)

Table 4: Hormonal Profile in Diabetes Mice

Hormone	Normal Control	Diabetic (Alloxan)	Diabetic + Fenugreek	Diabetic + Glibenclamide
Growth Hormone (ng/mL)	2.58 ± 1.97	10.15 ± 2.34*	2.40 ± 1.43#	2.50 ± 1.38#
Cortisol (μg/dL)	9.82 ± 1.53	18.82 ± 6.67*	8.80 ± 1.62#	8.17 ± 1.15#
Progesterone (ng/mL)	1.32 ± 0.72	4.25 ± 1.30*	2.13 ± 0.83#	1.58 ± 0.36#

Values are expressed as Mean ± SD (n = 6)

* Significant difference compared with normal control group (p < 0.05)

Significant difference compared with diabetic group (p < 0.05)

Diabetic mice exhibited markedly elevated levels of growth hormone and cortisol, along with a significant disturbance in progesterone levels. Treatment with fenugreek seed extract significantly modulated these hormonal changes, restoring hormone levels toward normal values. The effects of fenugreek were comparable to those observed in the glibenclamide-treated group. The results showed a significant elevation (p < 0.05) in growth hormone levels in alloxan-induced diabetic mice compared to the normal control group. Treatment with fenugreek seed extract markedly reduced growth hormone levels compared to the diabetic group, restoring them to values comparable to those observed in the glibenclamide-treated group.

The present study demonstrated that fenugreek (*Trigonella foenum-graecum*) seed extract significantly ameliorated the detrimental effects of alloxan-induced diabetes on glycemic control, physiological parameters, hematological indices, and hormonal balance in mice. These findings align with accumulating evidence suggesting that fenugreek possesses multi-faceted therapeutic properties relevant to diabetes management. Consistent with previous meta-analyses and randomized studies, fenugreek supplementation significantly reduced

fasting blood glucose levels compared to untreated diabetic controls. Recent clinical meta-analyses report significant improvements in fasting plasma glucose and glycated hemoglobin with fenugreek supplementation in type 2 diabetic patients, supporting its glycemic regulatory potential in vivo^[5,13]. Fenugreek's hypoglycemic effects are often attributed to its high soluble fiber content, galactomannan polysaccharides, and the amino acid derivative 4-hydroxyisoleucine, which have been shown to enhance peripheral glucose uptake and insulin secretion^[6]. The significant prevention of weight loss in fenugreek-treated mice may reflect improved metabolic balance and reduced protein catabolism. This observation is supported by clinical findings showing stabilization of anthropometric indices with fenugreek supplementation^[7]. The diabetic control group exhibited significant anemia (low Hb and RBC), leukocytosis (elevated WBC), and reduced packed cell volume (PCV), which are common complications of uncontrolled hyperglycemia and oxidative stress. These disturbances may stem from chronic inflammation and red blood cell membrane fragility triggered by elevated glucose levels and oxidative damage^[8]. Fenugreek seed extract significantly improved hematological indices, as evidenced by

increased Hb, RBC counts, normalized WBC and improved PCV in treated mice. Similar effects have been demonstrated in rodent models, where fenugreek seed extract enhanced erythrocyte counts and hemoglobin concentration in high-fat diet or diabetic models. These hematological improvements likely reflect fenugreek's antioxidant properties and its capacity to mitigate diabetes-induced oxidative stress, thereby preserving cell membrane integrity and hematopoietic function^[8,13,14]. Diabetic mice exhibited significant hormonal dysregulation, including elevated growth hormone and cortisol, as well as altered progesterone levels. These changes reflect the endocrine disruption associated with chronic hyperglycemia and stress responses. Treatment with fenugreek extract led to significant modulation of these hormonal imbalances, trending toward normal levels and comparable to the effects of glibenclamide^[14]. While specific studies on fenugreek's impact on growth hormone and cortisol in diabetic models are limited, its modulatory effects on metabolic hormones and stress markers have been suggested in related phytotherapeutic research. The improved hormonal profile observed in this study may be due to enhanced glycemic control and reduced systemic stress, consistent with the broader metabolic effects reported for fenugreek bioactive^[8,9]. Although progesterone is classically regarded as a female reproductive hormone, it is also synthesized in males at low concentrations by the adrenal cortex and serves as a key precursor for several steroid hormones, including cortisol and aldosterone. Alterations in progesterone levels in male diabetic animals may therefore reflect dysregulation of adrenal steroidogenesis rather than reproductive function^[15,16]. Chronic hyperglycemia and oxidative stress are known to disrupt adrenal gland function and steroid hormone metabolism, leading to abnormal circulating levels of stress-related and precursor hormones. The observed elevation of progesterone in alloxan-induced diabetic mice in the present study may indicate enhanced adrenal activity or impaired downstream steroid conversion pathways^[13,17]. Fenugreek seed extract significantly normalized progesterone levels, suggesting a modulatory effect on adrenal steroid genesis and endocrine homeostasis. This effect may be secondary to improved glycemic control and reduced systemic stress, as evidenced by the concomitant reduction in cortisol levels. Similar regulatory effects of phytochemicals on steroid hormone balance have been reported in experimental models of metabolic disorders. The concurrent normalization of growth hormone, cortisol, and progesterone levels highlights

the multifaceted endocrine regulatory potential of fenugreek seed extract. These findings suggest that fenugreek not only improves glycemic control but also mitigates diabetes-induced neuroendocrine stress responses, supporting its role as a holistic antidiabetic intervention^[14]. The multifactorial benefits of fenugreek likely arise from its composite phytochemicals, including saponins, flavonoids, and polysaccharides, which exert antioxidant, anti-inflammatory, and insulin tropic effects. These compounds have been shown to improve glucose metabolism, lipid profiles, and oxidative stress biomarkers in diabetic conditions^[9-11]. Our findings support the concept that effective diabetes management should encompass not only glycemic control but also the preservation of hematological and endocrine homeostasis. Fenugreek's ability to modulate blood glucose, hematological indices, and hormonal balance suggests its potential role as a complementary therapeutic agent.

Limitations and Future Directions: Despite the promising findings of the present study, several limitations should be acknowledged. First, the experimental design was limited to an alloxan-induced diabetic mouse model, which primarily reflects insulin-deficient diabetes. Therefore, the results may not fully represent the complex pathophysiology of type 2 diabetes mellitus characterized by insulin resistance. Second, the study duration was relatively short, and long-term effects and safety of fenugreek seed extract administration were not evaluated. Chronic exposure studies are necessary to assess potential cumulative effects and toxicity. Additionally, although significant improvements were observed in glycemic control, hematological indices, and hormonal parameters, the underlying molecular mechanisms were not directly investigated. Measurements of insulin levels, oxidative stress biomarkers, inflammatory cytokines, and gene or protein expression related to insulin signaling pathways (such as GLUT4, AMPK, and PI3K/Akt) would provide deeper mechanistic insights. Furthermore, the hormonal assessment was limited to selected endocrine markers, and broader evaluation of other metabolic hormones may further clarify fenugreek's endocrine regulatory potential. Future studies should focus on dose-response relationships, long-term administration, and comparative evaluations using different diabetic models, including diet-induced insulin resistance models. Isolation and characterization of specific bioactive compounds responsible for the observed effects would also be valuable. Ultimately, well-designed clinical trials are required to validate the translational relevance of fenugreek seed extract

as a complementary therapeutic agent for diabetes management and its associated hematological and endocrine complications

CONCLUSION

In conclusion, fenugreek seed extract demonstrated significant ameliorative effects on hyperglycemia, body weight changes, hematological disturbances, and hormonal imbalances in alloxan-induced diabetic mice. These effects were comparable to those observed with glibenclamide treatment, underscoring fenugreek's therapeutic promise. Further investigations into its molecular mechanisms and clinical relevance are warranted.

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