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RESEARCH ARTICLE

Antidiabetic Effects of Methanolic Extract of *Trigonella foenumgraecum* Seeds in Diabetic Rats

Alduwish M. Abduallah[1]*, Ahmed Ezzat Ahmed[2], Majed A. Bajaber[3] and Ahlam A. Alalwiat[3]

[1]Department of Biology, College of Science and Humanities in Al-Kharj, Prince Sattam Bin Abdulaziz University, Alkharj 11942, Saudi Arabia; [2]Department of Biology, College of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia; [3]Department of Chemistry, College of Science, King Khalid University, P.O. Box 9004, Abha 61413, Saudi Arabia
*Corresponding author: m.aldawish@psau.edu.sa

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A B S T R A C T

Herbal products are among the most important sources of medicines being investigated for the control of different diseases, including diabetes. Scientists have found multiple plants that are effective in controlling diabetes. In this experiment, the methanolic extract of *Trigonella foenumgraecum* (fenugreek) seeds at 3 different concentrations (200, 300 and 400 mg/kg body weight) were administered orally to evaluate their effects on the serum and body weight parameters of the diabetic rats compared to the standard medicated and non-diabetic control groups. Therapy of methanolic extract of fenugreek and controls continued for 4 weeks, and the effects were observed on the parameters related to weight gain, liver and renal function, serum glucose levels, and lipid profile. The results showed that the effects of the methanolic extract of *T. foenumgraecum* were dose dependent. There was a significant ($p<0.05$) increase in weight gain in the groups treated with methanolic extract of fenugreek at 400 mg/kg. The serum parameters, including glucose levels, serum lipid profile, and serum enzymes of liver and kidney functions in the group treated with methanolic fenugreek extract at 400 mg/kg were comparable to those of non-diabetic group and the standard medicated group. These results showed that the fenugreek methanolic extract effectively controlled hepatic damage and improved serum parameters at 400 mg/kg dose rate. In conclusion, the methanolic extract of fenugreek should be analyzed for its components to determine effective antidiabetic agents. Further research should be done to determine a therapeutic dose of fenugreek seeds for application in the future.

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INTRODUCTION

Metabolic problems are among the most under-reported problems in animals and humans (Díaz *et al.*, 2023). These problems remain an issue of major concern because of their long-term effects on the health and life of humans and animals (Vallianou *et al.*, 2021). They may be caused because of genetic issues, malnutrition, environmental issues and some infectious agents (Dahiya and Nigam, 2023). Diabetes is one of the most common and serious metabolic issues (Misra *et al.*, 2019). Diabetes mellitus is of great concern for health professionals because of its ever-increasing occurrence (Rumbold *et al.*, 2020). Pets are also reported to have diabetes because of lack of proper nutrition, overfeeding, feeding items not suitable for a specific species and unawareness of owners about metabolic issues in the animals (Yu *et al.*, 2023).

Diabetes is a chronic issue that may lead to severe complications (Kumar *et al.*, 2020; Özaydin and Aydin, 2023). It is characterized by high blood glucose level, which is the critical sign of this problem (Awuchi *et al.*, 2020). This increase in blood glucose level may be due to the absence or impaired insulin production, hormonal imbalance and hepatic or renal injuries (Mukhtar *et al.*, 2020). The body becomes unable to put glucose into the body tissues and hyperglycemia leads to hyperglycosuria, which disturbs the osmotic balance of the urine. Increased blood glucose levels alter the blood pH, physiological activities and osmoregulation (Saenkham *et al.*, 2020). These issues can lead to cardiovascular failure, which may be a cause of death in diabetic patients (Amiel *et al.*, 2019). Wounds of diabetic patients become chronic and untreatable because of the retention of glucose in the blood. Retention of the glucose inside the body and its failure to be released into the tissues leads to energy deficiency in the body. These issues make diabetes a major lethal disease in animal and human life (Özaydin *et al.*, 2018).

Scientists are investigating some permanent solutions to diabetes because there is no fully effective treatment of this problem to date (Fujikawa, 2021). Insulin therapy in insulin-dependent diabetes mellitus is the only way to manage the blood glucose level in the body (Fujikawa, 2021). Gene therapy is being investigated to manage insulin production in the body, but it has been unsuccessful for use in humans or animals (Zhong *et al.*, 2023). Multiple glucose level-maintaining substances are being searched to manage diabetes and its complications.

Plants are the most reliable sources for medicine development and drug discovery because they contain multiple bioactive compounds. Various plant preparations have been tested against diabetes in animal models and have proven to be effective in controlling diabetes (Lankatillake *et al.*, 2019). Scientists are working on various preparations of plants, like extracts and powders, to develop a final product for the control of diabetes (Wickramasinghe *et al.*, 2022).

Fenugreek (*Trigonella foenumgraecum*) is a common medicinal plant. It is used to extract multiple medicines for use against various diseases of humans and animals (Kumar *et al.*, 2021). This plant has been used in multiple studies to control metabolic disorders (Kania-Dobrowolska and Baraniak, 2020). Fenugreek is a suitable candidate for anti-diabetic drug development and ethanolic extract of this plant has shown promising anti-diabetic properties.

This study aimed to evaluate the efficacy of different concentrations of methanolic extract of Fenugreek seeds to control diabetes by estimating its effects on blood glucose levels, body weight gain, lipid profile including blood cholesterol, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL) and triglycerides (TG), and hepato-renal functioning enzymes in diabetic male albino Wistar rats.

MATERIALS AND METHODS

Preparation of plant extract: Seeds of the Fenugreek were taken from the verified source, dried to remove the moisture contents, inserted into the Soxhlet's apparatus with 80% methanol, and filtered after extraction following the methods of Ishaq *et al.* (2022). The extract was dried and stored at -4°C for further use.

Animals: In this study, male albino Wistar rats, with an average age of 3 weeks and weighing 150 to 200g, were used. Housing of the rats was done in groups, with 10 rats per cage. The average space provided to each rat was 850cm^2 with a height of 25cm. Experimental rats were fed *ad libitum* Laboratory feed containing proteins (27%), fats (10%), carbohydrates (33%) and vitamins (4%). The room temperature was adjusted between 20-25°C and a relative humidity of 50-60% with 12 hours of light provided to them. All the animal ethics guidelines were followed during the experiment (Prager *et al.*, 2011).

Induction of diabetes: Diabetes in experimental rats was induced by injecting streptozotocin intraperitoneally (streptozotocin at 60 mg/Kg body weight of the rat was administered). The injection was administered after measuring fasting blood glucose level. To determine fasting glucose level, the blood of rats was analyzed at 3, 7 and 10-day intervals. The rats exhibiting blood glucose levels exceeding 250 mg/dL on the 10th day were chosen for treatment (Gajdosik et al., 1999).

The experiment layout: The random division of the 150 diabetic rats was done into 5 groups, labeled with letters A to E, each having 3 replicates with 10 rats in each replicate. Group A contained diabetic rats with no medication, which served as a negative control. Groups B, C and D had diabetes that received the methanolic extract of fenugreek (MEF) at 200, 300 and 400 mg/kg body weight, respectively. Group E contained diabetic rats which received an oral dose of Glibenclamide at a rate of 10 mg/kg body weight to serve as a standard control, while group F was non-diabetic, non-medicated served as control. All the treatments started 10 days post streptozotocin (considered as day 0 of treatment). The experiment continued for 4 weeks, and the observations were recorded. The blood samples were collected humanely through the direct cardiac puncture.

Experimental parameters: The following parameters were recorded for rats of each experimental group:

Weight gain: Effects of treatments on the weight gain of Wistar rats were estimated every week. The percentage weight gain was calculated following the work of Oyedemi *et al.* (2011). The formula used is given below:

Percent weight gain= (Final weight- initial weight)÷Initial wieght x 100

Fasting glucose level: Fasting blood glucose levels in experimental rats were estimated every week using the *EZ II* glucometer of onCall® Inc. following the method of Muzaffar *et al.* (2019).

Lipid profile: For the estimation of lipid profile, blood was collected from the hearts of rats at the end of the study and put into Gel-Clot vacutainers for separation of serum. The serum was then processed by spectrophotometry for the estimation of values of lipid parameters i.e., high (HDL), low (LDL) and very-low-density lipids (VLDL) following the methods of Gobinath *et al.* (2022).

Serum biochemistry for liver and kidney functions: Serum albumins, globulins, albumin to globulin ratio, total proteins, alkaline phosphatase (ALP), total bilirubin, urea, creatinine, ALT and AST were measured using spectrophotometric kits following the methods of Gobinath *et al.* (2022).

Statistical analysis: Mean values (±SE) were computed for each parameter. The recorded data was analyzed using Minitab® software. Analysis of variance (generalized linear model) combined with the Tuckey's test for means comparison was applied to estimate statistical differences at 95% significance level.

RESULTS

Effect on weight gain: The effect of methanolic extract of fenugreek (MEF) on body weight gain of rats was recorded every week for 4 weeks and compared statistically. The results showed that rats receiving MEF at 300 and 400 mg/kg or an oral dose of Glibenclamide at10 mg/kg body showed significantly higher weight gain ($p<0.05$) than the untreated diabetic control rats, those receiving MEF at 200 mg/kg or non-medicated non-diabetics (Fig. 1). However, differences in weight gain of rats receiving MEF at 300 and 400 mg/kg or an oral dose of Glibenclamide at10 mg/kg were non-significant. Furthermore, a reduction in body weight was noted in the rats of the untreated diabetic control group during 4 weeks and in 200 mg/kg treated diabetic rats during the first 2 weeks of the experiment.

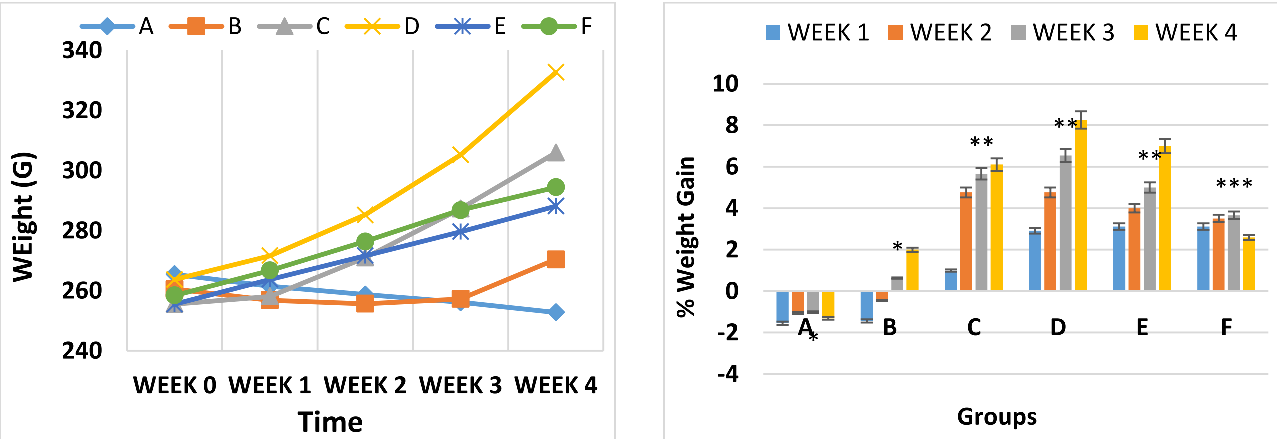
Blood glucose levels: The results of the experiment showed that the effects of the MEF on blood glucose levels were in a dose-dependent manner and the MEF at 400 mg/kg had a glucose-lowering efficiency comparable ($p>0.05$) to standard medicated control group (Table 1).

Serum lipid contents: The results suggested that the MEF showed a dose-dependent effect on the serum cholesterol, TG, LDL, VLDL and HDL. The HDL tended to increase to normal level by the MEF at 400 mg/kg dose rate. The VLDL, LDL, cholesterol and TG values were reduced to normal ranges by the highest concentration (400 mg/kg) of the MEF, as well as by an oral dose of Glibenclamide at10 mg/kg (Table 2).

Renal and hepatic parameters: Renal and hepatic function enzymes, urea, albumins, globulins, TSP, A/G ratios, ALKP, AST, ALT, Bilirubin and creatinine were determined to evaluate the hepatoprotective effects of MEF in Wistar rats. Statistically significant ($p<0.05$) differences were observed for the standard medicated control and the MEF at 400 mg/kg concentration compared to untreated controls (Fig. 2).

DISCUSSION

Researchers have widely used plant extracts to control various infectious and non-infectious diseases of veterinary and human origin (Abbas *et al.*, 2020). Herbal extracts contain multiple compounds i.e., phenolics, flavonoids, alkaloids, terpenes, and their derivatives (Saeed and Alkheraije, 2023). These compounds have been proved to be effective in controlling various diseases. Herbal extracts have been widely used for the treatment of diabetes (Rahman *et al.*, 2022). The phenolic compounds have been proven effective in maintaining blood glucose levels (Ahmed *et al.*, 2022). Similarly, phenolics and terpenes have been proven effective in maintaining lipid contents and other serum parameters (Guo *et al.*, 2022). These compounds of phenolic origin determine the antidiabetic effects of the plants (Wojdyło and Nowicka, 2021). Previous studies have also shown that MEF has a variety of phenolic compounds that are responsible for its biological activities (Wojdyło and Nowicka, 2021).



(a) (b)

Fig. 1: (a) Effect of methanolic extract of Fenugreek on body weight of Wistar rats compared to control groups. (b) Effect of methanolic extract of Fenugreek on percent weekly weight gain compared to control groups. Groups with similar "*" signs are statistically comparable ($p>0.05$). A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Fenugreek at 200 mg/kg; C: Diabetic rats receiving the methanolic extract of Fenugreek at 300 mg/kg; D: Diabetic rats receiving the methanolic extract of Fenugreek at 400 mg/kg; E: Diabetic rats receiving an oral dose at10 mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats.

Table 1: The effects of methanolic extract of Fenugreek seeds on fasting glucose levels (mg/dL) in experimental rats on different weeks of treatment

Groups	Week 0	Week 1	Week 2	Week 3	Week 4
A	267.66±16.16a	263.66±14.18ab	262.66±11.59ab	261±12.12a	260.66±11.23a
B	265.66±8.32a	276.29±8.65a	281.81±8.83a	284.63±8.92a	281.79±8.83a
C	270±3.6a	261.9±3.49ab	248.8±3.32b	228.9±3.05b	206.01±2.75b
D	266.66±10.26a	253.33±9.75ab	235.6±9.06bc	214.39±8.25c	190.81±7.34b
E	269.33±14.64a	232.33±10.26b	210.33±10.06c	193±7.21c	182.66±3.78b
F	151.33±25.54b	154±23c	153±19.07d	152.66±21.1d	153.33±19.6c

A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Fenugreek at 200 mg/kg; C: Diabetic rats receiving the methanolic extract of Fenugreek at 300 mg/kg; D: Diabetic rats receiving the methanolic extract of Fenugreek at 400 mg/kg; E: Diabetic rats receiving an oral dose at10 mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats. Groups with common superscripts in a column are statistically comparable ($P>0.05$).

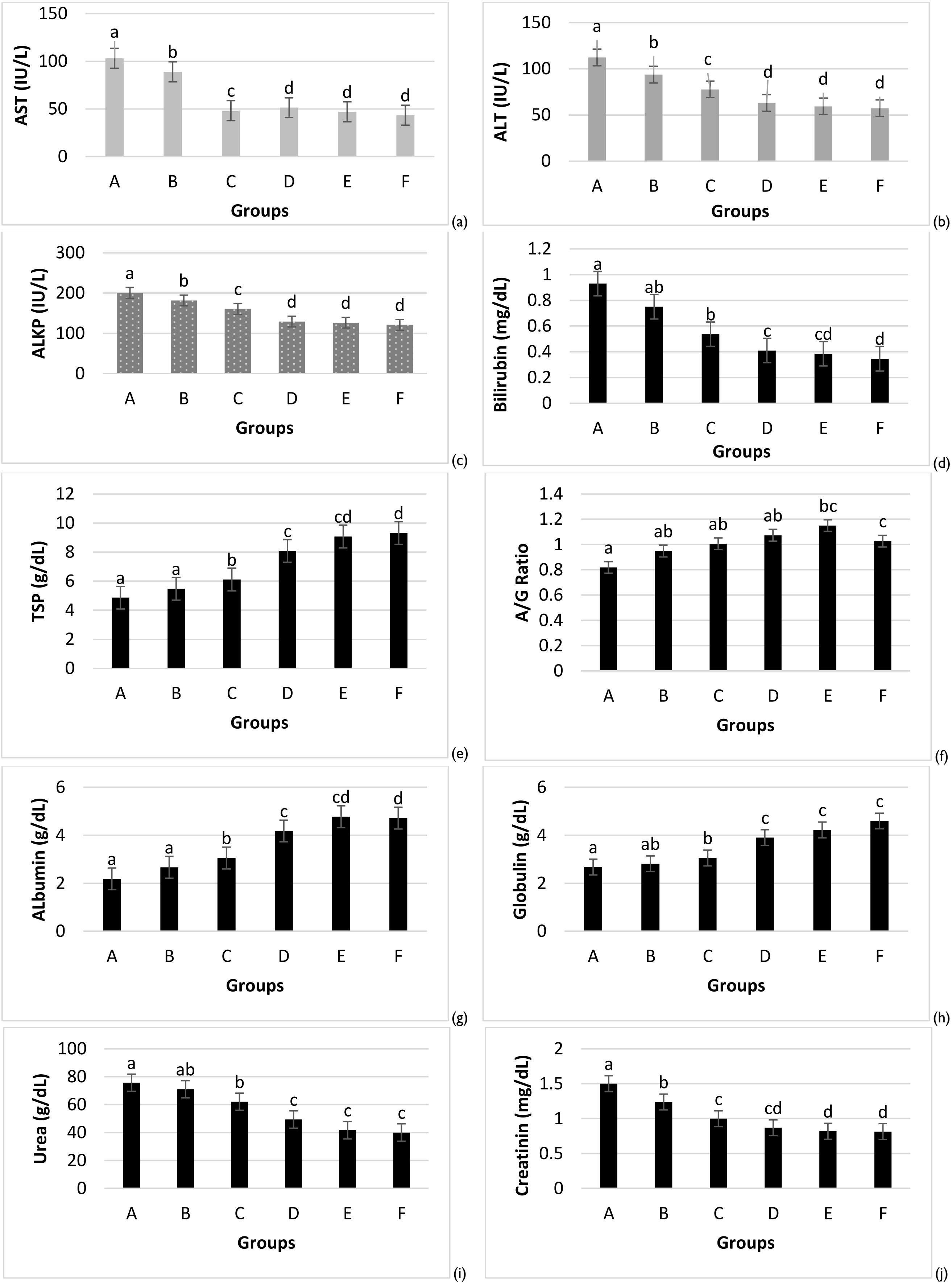


Fig. 2: Liver and serum parameters (a) ALT, (b) AST, (c) ALKP, (d) Bilirubin, (e) TSP, (f) A/G ratio(g) Albumin, (h) Globulin, (i) Urea, and (j) Creatinine of methanolic extract treated diabetic Wistar rats compared to the control groups. Groups with common superscripts are statistically comparable (P>0.05). A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Fenugreek at 200 mg/kg; C: Diabetic rats receiving the methanolic extract of Fenugreek at 300 mg/kg; D: Diabetic rats receiving the methanolic extract of Fenugreek at 400 mg/kg; E: Diabetic rats receiving an oral dose at10 mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats; ALT: alanine aminotransferase; AST: Aspartate transferase; ALKP; Alkaline phosphatase; TSP: total serum proteins; A/G ratio: Albumins to globulins ratio.

Table 2: Lipid profile of methanolic extract treated diabetic Wistar rats compared to control groups.

Parameters / Groups	VLDL	LDL	HDL	Cholesterol	Triglycerides
A	43.33±2.08[a]	105.66±4.04[a]	18±2[a]	183.66±18.03[a]	170.66±4.04[a]
B	34.9±1.94[b]	91.84±3.72[ab]	18.85±1.65[ab]	125.16±3.86[b]	147.69±10.16[b]
C	32.16±2.38[b]	82.09±13.55[b]	25.52±2.34[bc]	119.58±10.83[b]	143.28±7.02[b]
D	20.83±1.66[c]	42±1.83[c]	30.02±1.82[c]	80.62±5.49[c]	91.95±3.23[c]
E	19±1[c]	43±2.64[c]	34.33±1.52[d]	86±3.6[c]	101.66±2.88[c]
F	16.33±1.52[c]	37.33±2.51[c]	36.66±0.57[d]	74±6.55[c]	97.33±2.51[c]

A: Control group of Diabetic rats; B: Diabetic rats receiving the methanolic extract of Fenugreek at 200 mg/kg; C: Diabetic rats receiving the methanolic extract of Fenugreek at 300 mg/kg; D: Diabetic rats receiving the methanolic extract of Fenugreek at 400 mg/kg; E: Diabetic rats receiving an oral dose at10 mg/kg body weight of Glibenclamide as a standard control; F: Non-medicated nondiabetic Wistar rats. Groups with common superscripts in a column are statistically comparable (P>0.05).

In the present study, the investigation of the effects of MEF on body weight and weight gain revealed that MEF helped the rats to maintain their weight gain. The groups treated with 400 mg/kg MEF showed a positive weight gain and had similar results to the standard medicated control group. Loss of body weight is a major problem in diabetes (Awuchi *et al.*, 2020). The decrease in body weight is mainly due to the faulty metabolism of glucose and its unavailability for physiological use by the body (Shakib *et al.*, 2019). This problem causes the body to starve and a decline in weight gain is observed. Similar results were observed in previous studies using herbal extracts, where the diabetic rats showed a sharp decline in body weight (Oguntibeju, 2019).

The effects of MEF on the blood glucose levels were also recorded and the results revealed that the effects were in a dose-dependent manner. MEF at 400 mg/kg was effective in decreasing blood glucose, as was also noted in diabetic rats receiving an oral dose at10 mg/kg body weight of Glibenclamide as a standard control. Hyperglycemia is the major indicator of diabetes and is the principal factor in diabetes-related pathologies, so its management has prime importance in diabetes management (Zakir *et al.*, 2023). Mustafa *et al.* (2019) found that extracts of *Curcuma longa*, *Aegle marmelos*, *Glycyrrhiza glabra*, and *Lavandula stroechcas* were effective in reducing blood glucose levels in rats with induced diabetes. Fadzelly *et al.* (2006) and Dey *et al.* (2020) also suggested similar findings while working on *Strobilanthes crispus* and *Swertia chirayita*, respectively. The mechanism of action may include the involvement of phenolic compounds, which trigger multiple pathways leading to increased transfer of the sugar from the blood into tissues (El-Hadary and Ramadan, 2019).

In the present study, effects of MEF on the lipid profile were also investigated. The results showed that lipid profile of the diabetes-affected rats was maintained within normal ranges by the MEF at 400 mg/kg concentration. The lipid profile is very important in diabetes because the levels of cholesterol, TGs, and LDLs are increased in diabetes and their estimation can help to estimate the risks of diabetes (Sheikh and Gallehdari, 2023). Blood levels of HDLs are lowered in diabetes, which leads to increased risks of cardiovascular diseases. The lipid profile is an economical and early tool for the detection of hepatic injuries, including diabetes (Shao *et al.*, 2022). According to Shao *et al.* (2022), the herbal extract of *Acacia pennata* was highly effective in controlling the lipid profile in rats. Many other authors have also claimed similar results in their studies (Gobinath *et al.*, 2022).

Liver and renal function profiles were also estimated, and the protective effects of MEF on these body organs were evaluated in the present study. ALT, ALKP, Bilirubin, and AST are determinants for normal liver functions, while creatinine and urea are the determinants for the function of kidneys. Normal blood levels of albumins, globulins, A/G ratio, and TP are important for functioning of both kidneys and the liver. All these parameters were effectively controlled by the MEF at 400 mg/kg body weight dose rate. These effects were anticipated because many other researchers have claimed similar results using herbal formulations. The hepatoprotective and renal function maintenance effects of MEF can be attributed to the active compounds that have been found effective in controlling hepatic and renal injuries.

Conclusions: The results of the present experiment show that the methanolic extract of Fenugreek seeds can reduce the severity of signs and symptoms of diabetes in the Wistar rats. Similar results can be achieved while working with different species of animals. Methanolic extracts from other plants should also be tested to manage diabetes. Extended trials should be conducted to estimate the toxic effects, suitable concentrations, and active compounds from methanolic extracts of Fenugreek.

Authors contribution: Methodology and conceptualization were carried out by AMA and AEA, while analysis of data and writing up of the paper was done by AMA, MAB and AAA.

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