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EFFECT OF FENUGREEK AND METFORMIN ON LIPASE AND SOME OF BIOCHEMICAL PARAMETERS INDUCED - ALLOXAN DIABETIC RATS

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Abstract: This current study was conducted to evaluate the influence of alkaline fenugreek extract and metformin on diabetic male albino rats based on biochemical changes. The results of lipase enzyme measurements indicated a slight but non-significant increase in the fenugreek and metformin-treated groups compared to the diabetic positive control groups. Regarding the measured biochemical concentrations, a considerable reduction was observed in glucose, urea, and creatinine levels in the modified groups relative to the diabetic positive control groups, except for creatinine, where a non-significant decrease was observed between the fenugreek extract-treated group and the diabetic active standard group. Similarly, a remarkable reduction was noted in triglycerides (TGs), total cholesterol (TC), and low-density lipoprotein (LDL) cholesterol levels in the treated groups compared with the diabetic standard reference group, except for the metformin-treated group, where the decrease in triglycerides was non-significant. The results for HDL and VLDL cholesterol levels showed non-significant increases and decreases relative to the diabetic positive control group.

The obtained results exhibited a modest reduction in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) level, however, the observed changes in reductions and increases were not statistically significant in the groups treated with fenugreek extract and metformin compared to the positive control group, respectively. The results of measuring liver weight showed a non-significant decrease in the group treated with fenugreek extract and a significant increase in the group treated with metformin compared to the positive control group, and a non-significant decrease in total weight for all groups in comparison with the positive control group.

Keywords: Diabetes, Fenugreek, Metformin, Lipase, Total Cholesterol, Triglycerides

Introduction

Fenugreek is a well-known medicinal herb with a long established role in traditional medicinal applications. It is used to treat some diseases such as diabetes and high cholesterol. Its original habitat is India and North Africa (Yao et al., 2020). Fenugreek has exhibited a broad spectrum of pharmacological activities, particularly its anti-inflammatory, anti-diabetic, antioxidant, lipid-lowering, and hepatoprotective properties (Sindhu et al., 2022). The blood sugar-lowering and insulin-sensitive properties of the Fenugreek support its potential applications in the management of various health conditions, including a non-alcoholic fatty liver disease (NAFLED). The biological properties are largely attributed to its

abundant dietary fiber and diverse phytochemical constituents, such as alkaloids, steroids, coumarins, flavonoids, , and different volatile oils (Gavahian *et al*., 2024).

Diabetes mellitus (DM) is a chronic metabolic disease characterized by persistent hyperglycemia associated with insufficient insulin secretion, insulin function, or both (Yedjou *et al*., 2023). Metabolic problems are among the most frequently diagnosed conditions in humans and animals (Diaz et al., 2023). The incidence of diabetes has quadrupled since 1980, making it the leading cause of premature death (Lazic *et al*., 2024).

Diabetes is a widespread disease globally, with millions of people suffering from it as a result of obesity, genetic factors, exposure to chemicals, or an unhealthy diet. All these reasons are factors that increase the risk of developing diabetes (Zheng *et al*., 2018).

Patients with diabetes commonly experience multiple symptoms, including excessive urination and thirst, , headache, visual disturbances, peripheral numbness, and tingling in the extremities, dizziness, irritability, and general fatigue, in addition to digestive disorders and sometimes psychological disorders and neuropathy leading to damage to the heart and blood vessels (Astrid *et al*., 2019).

This disease is classified into several types. The least common type is known as Insulin Dependent Diabetes Mellitus (IDDM). This type results from autoimmune reactions, as the body attacks the beta cells, leading to their destruction. This results in a decrease in the level of insulin. The most common type is Non-Insulin Dependent Diabetes Mellitus (VIDDM), which represents about 90% of all people with diabetes. The main reason for it is the deficiency or absence of insulin as a result of several factors, all of which contribute to damage or harm to the beta cells in the pancreas responsible for secreting this hormone. In addition, there is gestational diabetes, which appears as a result of pregnancy. Also, the disease can be induced by using chemicals such as alloxan (Glovaci *et al*., 2019).

Alloxan is a toxic glucose analog with a specific toxic effect on pancreatic beta cells. One of its mechanisms of action is beta cell damage through increased free radical formation (Danilova et al., 2017). Treatments for diabetes vary and include dietary management, pharmaceutical therapies, herbal remedies, and dietary modifications (Magkos *et al*., 2020).

Material and Methods

The alkaloids were extracted from the fenugreek plant. 200 g of the dried plant powder was weighed and 800 ml of 10% glacial acetic acid was added to the powder, and it was left to stand in the acid for 24 hours. The extract was then filtered, and the filtrate was diluted with ionized water. 200 ml of chloroform at a ratio of 1:2 was added to the diluted filtrate, and the filtrate containing the chloroform was separated. This filtrate was then washed with 15% aqueous sodium bicarbonate. Anhydrous sodium sulfate was then added to the chloroform-containing filtrate, and it was filtered again. The filtrate was evaporated in a rotary evaporator containing a water bath at a temperature of 45 °C (Harborne,1993 : Aziz *et al*., 2016).

Laboratory animals

In this experiment, male white rats of the (albino) type were used. The experimental rats were bred and maintained under controlled conditions at the animal facility of the College of Veterinary Medicine/ Mosul University, in plastic cages after the floor of the cages was covered with wood shavings that were changed approximately every three days to maintain the cleanliness of the rats in conditions of a temperature of (25°C). They were provided with

water and food by the supervisors responsible for the animal house. The weights of the rats ranged between (300-180) grams for each rat.

Experimental Design:

Twenty male albino rats were categorized and divided into four experimental groups as follows:

1. Negative Control Group (C^-), comprising of (5) untreated rats.
2. Positive Control Group (C^+), comprising of (5) rats injected with alloxan to induce diabetes at a dose of 150 mg/g body weight.
3. Treatment Group with Fenugreek Extract, consisting of (5) rats. The resulting extract was solubilized in distilled water and administered orally at a dose of 0.2 mg/g body weight daily for four weeks.
4. A metformin-treated group, consisting of (5) rats, was orally administered (0.2 mg/g of body weight) daily for (4) weeks. After this procedure, blood samples were drawn from these groups into test tubes and centrifuged at a speed of (3000 rpm) for approximately 15 min to afford the blood serum. The extracted serum was then frozen, and when used for biochemical measurements, it was thawed before use.

Statistical Analysis

The results of all levels of biochemical variables, weights, and others were analyzed for equal numbers of groups, where the acceptable probability level was $P< 0.05$ using the Statistical Package for the Social Sciences (SPSS) (Urbano *et al.*, 2019).

Results and Discussion

The concentration of lipase enzyme was measured. The obtained data (Table 1) of the positive control group demonstrated insignificant reduction (29 ± 2.0) relative to the negative control group (30±1.0). A slight increase was also observed in both fenugreek (30±3.0) and metformin- treated group (31 ± 4.0) relative to the positive control group 29). (2.0 ± However, these differences were not significant at the statistical level.

Table 1. Measurement of the concentration level of lipases in the blood serum of male rats.

Groupe / Parameter	C^-	C^+	Fenugreek	Metformine
Lipase enzyme	30±1.0	29±2.0	30±3.0	31±4.0

* Letters that are the same horizontally indicate a non-significant difference at $p < 0.05$.
* Letters that are different horizontally indicate a significant difference at $p < 0.05$.
* **Values expressed as (Mean ±SD)**

It is believed that the decrease is due to a decrease in the function of the ommatidium cells in the periphery of the insulin-depleted islets of Langerhans (Skrha *et al.*,1983).

Administration of fenugreek to diabetic rats significantly restored intestinal lipase activity to levels close to those of undiagnosed rats (Hamden *et al.*,2008; Hamden *et al.*,2009). Metformin may cause hepatotoxicity, such as acute hepatitis and hepatitis C. Notably, metformin may provide direct protection to pancreatic beta cells from oxidative damage and endoplasmic reticulum (ER) stress, both of which act essential roles beta cells destruction under metabolically stressful conditions (Moon et al., 2017).

In Table (2), the glucose concentration level was measured, and the results shown indicated a significant increase among the infected positive control group (176±45.2) mg/dl compared to the healthy negative control group (123±2.0) mg/dl and a significant decrease among the group treated with fenugreek (151± 21.2) mg/dl compared to the infected positive control group (176±45.2) mg/dl, as well as a significant decrease among the group treated with metformin (140± 9.8) mg/dl compared to the infected positive control group (176± 45.2) mg/dl.

Urea concentration was also measured, and the results shown in the table above indicate a significant increase in the positive control group (88.5± 19.0) compared to the healthy negative control group (33± 2.0) mg/dl. A remarkable decrease was also noted in the fenugreek-treated group (54.5± 3.5) relative to the positive standard control group (88.5± 19.0) mg/dl, and a significant decrease was observed in the metformin-treated group (37.5 ± 7.7) compared to the positive control standard group (88.5± 19.0) mg/dl. Creatinine concentration was also measured, and the results shown in the table above indicate a significant increase in the positive control group (0.40± 0.2) relative to the healthy negative control group (0.16± 0.01) mg/dl, with a non-significant decrease observed. There was a significant decrease in the fenugreek-treated group (0.28± 0.05) mg/dl compared to the positive control standard group (0.40± 0.2) mg/dl, as well as a significant decrease in the metformin-treated group (0.13±0.11) mg/dl compared with the positive control standard group (0.40± 0.2) mg/dl.

Table 2. Evaluation of glucose, urea, and creatinine levels in the blood serum of male rats.

Groupe / Parameter	C^-	C^+	Fenugreek	Metformine
Glucose mg/dl	123 ± 2.0 **a**	176 ± 45.2 **b**	151 ± 21.2 **a**	140 ± 9.8 **a**
Urea mg/ dl	33 ± 2.0 a	88.5 ± 19.0 b	54.5 ± 3.5 a	37.5 ± 7.7 a
Creatinine mg/dl	0.16 ± 0.01 **a**	0.40 ± 0.2 **b**	0.28 ± 0.05 **ab**	0.13 ± 0.11 **a**

* Letters that are the same horizontally refer insignificant difference at $p < 0.05$.
* Letters that are different horizontally refer a significant difference at $p < 0.05$.
* **Values expressed as (Mean ±SD)**

Early research demonstrated that fenugreek is rich in biologically active compounds, such as 4-hydroxy isoleucine, trigonelline, and dietary fiber, which may enhance insulin responsiveness and slower glucose absorption in the intestines (Abedin et al., 2024). Fenugreek's ability to lower blood sugar levels is attributed to its high galactomannan content (Mathur et al., 2020). A previous study concluded that fenugreek's effectiveness in treating diabetes is due to its high fiber content (Luo *et al.*, 2023; Bafadam *et al.*, 2021).

Fenugreek extract mitigates kidney damage by improving kidney function indicators such as blood urea nitrogen (BUN), creatinine, and oxidative stress markers. This underscores fenugreek's role in reducing kidney damage through its antioxidant and anti-inflammatory properties (Massoud *et al.*, 2022; Hadi *et al.*, 2020). Fenugreek also improves kidney function by lowering urea and creatinine levels (Alsuliam *et al.*, 2022).

Metformin is believed to stimulate pancreatic beta cells to produce insulin. It also works through phenolic compounds to stimulate glucose production in the liver, thus reducing the amount of sugar the body produces. Metformin also increases the body's sensitivity to insulin, thereby lowering blood sugar levels (Tao *et al.*, 2019). It improves insulin sensitivity and reduces gluconeogenesis in the liver (Rena and Hardie, 2017).

The decrease in urea and creatinine concentrations in metformin-treated mice may be attributed to its significant improvement in kidney function, suggesting it could be a useful drug in mitigating the progression of chronic kidney disease in diabetic mice (Al-Za'abi *et al.*, 2021).

Metformin may also be effective in mitigating the progression of chronic kidney disease in both diabetic and non-diabetic mice (Kadhim and Lateef, 2024).

The total glycerol (TC) concentration level was measured, and the results shown in Table (3) indicate a significant decrease among the positive control group (79±0.5) mg/dl compared to the healthy negative control group (108±6.0) mg/dl, a significant decrease among the fenugreek-treated group (53.5±4.9) mg/dl compared to the positive control group (79±0.5) mg/dl, and a significant increase among the metformin-treated group (94±4.2) mg/dl compared to the positive control group (79±0.5) mg/dl.

Regarding the level of triglyceride (TG) concentration, the results indicated a significant increase among the infected positive control group (78.5±6.3) mg/dl compared to the healthy negative control group (57±2.5) mg/dl and a non-significant decrease for the fenugreek-treated group (61±9.8) mg/dl and the metformin-treated group (71±1.0) mg/dl compared to the infected positive control group (78.5±6.3) mg/dl.

As for the level of (HDL) concentration, the results indicated a significant decrease among the positive control group (38±3.0) mg/dl compared to the healthy negative control group (69±2.0) mg/dl, a non-significant increase among the group treated with fenugreek (42±1.4) mg/dl compared to the infected positive control group (38±3.0) mg/dl, and a significant increase among the group treated with metformin (55±5.7) mg/dl compared to the infected positive control group (38±3.0) mg/dl.

The level of (LDL) concentration was observed to be significantly higher among the infected positive control group (33±5.5) mg/dl compared with the healthy negative control group (24±1.5) mg/dl, and to be significantly lower among the fenugreek-treated group (15±3.0) mg/dl compared with the infected positive control group (33±5.5) mg/dl, and to be significantly lower among the metformin-treated group (21±2.5) mg/dl compared with the infected positive control group (33±5.5) mg/dl.

Regarding the level of (VLDL) concentration, the results indicated a significant increase among the infected positive control group (19±4.1) mg/dl compared to the healthy negative control group (13±3.2) mg/dl, and a non-significant decrease among the fenugreek-treated group (14±2.0) mg/dl and a decrease in metformin treatment (17 ±1.7) mg/dl compared to the infected positive control group (19±4.1) mg/dl.

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Table 3. Measurement of the concentration level of (TC, TG, HDL, LDL, VLDL) in the blood serum of male rats.

Groupe / Parameter	C^-	C^+	Fenugreek	Metformine
TC mg/dl	6.0±108 **d**	0.5±79 **b**	4.9±53.5 **a**	4.2±94 **c**
TG mg/dl	2.5±57 **a**	6.3±78.5 **c**	61 9.8± **ab**	1.0±71 **bc**
HDL mg/dl	0.2±69 **c**	3.0±38 **a**	42 1.4± **a**	5.7±55 **a**
LDL mg/dl	1.5±24 **b**	5.5±33 **c**	3.0±15 **a**	2.5± 21 **a**
VLDL mg/dl	3.2 ± 13 **a**	4.1±19 **a**	2.0±14 **a**	1.7±17 **a**

* Letters that are the same horizontally reflect insignificant difference at $p < 0.05$.
* Letters that are different horizontally reflect significant difference at $p < 0.05$.
* **Values expressed as (Mean ±SD)**

Dyslipidemia is a major complication of diabetes, characterized by elevated levels of triglycerides (TC), triglycerides (TG), and LDL, and decreased levels of HDL (Lokpo et al., 2022). TC and TG are indicators of lipids damage and are used in the assessment of cardiovascular disease and other health conditions by measuring blood lipid levels (Lee and Siddiqui, 2023). After using fenugreek extract, we observed a decrease in all types of lipids except HDL, which showed an increase in its concentration.

The decrease in total cholesterol may be attributed to a decrease in cholesterol and fatty acid synthesis (Bopanna et al.,1997). This effect is due to the saponin factor, which lowers serum cholesterol levels by increasing bile cholesterol secretion (Amin *et al.*, 1988; Ali *et al.*, 1995). In addition, fenugreek seeds contain mannose, which leads to a decrease in cholesterol synthesis (Roberts,2011). The decrease in triglyceride (TG) concentration after exposure to alloxan is due to the therapeutic effects of plants in improving insulin release levels and regulating fat and carbohydrate metabolism (Al-Mehdar and Al-Battah,2016).

As for the increase in HDL, the use of some plants, especially those containing flavonoids, leads to an increase in its concentration in the blood serum. This may be attributed to the inhibition of the cholesterol ester transfer protein (CETP), as a number of studies have indicated that humans with a sexual dysfunction in the (CETP) protein have been found to have an increase in HDL (Oboh *et al.*,2015).

The reduction in LDL and VLDL may be due to improved metabolic activity following dosing with the extracts. It is clear that the flavonoids and phenols present in the plant components play a significant role in reducing LDL and VLDL concentrations. Additionally, fenugreek reduces lipid peroxidation, which has antioxidant properties, and both of these properties contribute to the treatment of dyslipidemia (Gong *et al.*, 2016).

Similarly, the reduction in lipid levels following metformin treatment is agreement with previous report by (Angadi *et al.*,2026: Solymár *et al.*,2018), who linked this effect to the modulation of hepatic lipid metabolism and enhanced insulin responsiveness (Solymár *et al.*,

2018). In conclusion, it can be hypothesized that metformin, in addition to its primary blood glucose-lowering effect, exhibits multiple effects that modulate lipid metabolism and blood lipid levels in patients with type 2 diabetes. The improvement in blood lipid profiles associated with metformin treatment may contribute to a reduced risk of cardiovascular complications among diabetic patients (Dettlaff-pokora and Swierczynski,2026).

The level of the enzyme concentration (AST) was measured, and the results shown in Table (4) indicated a non-significant increase among the infected positive control group (99±3.5)UL compared with the healthy negative control group (98±3.0)UL, a non-significant decrease among the group treated with fenugreek (97.5±10.6)UL compared with the infected positive control group (99±3.5)UL, and a non-significant increase among the group treated with metformin (111±6.2) UL compared with the infected positive control group (99±3.5) UL.

The level of the enzyme (ALT) concentration was also measured, and the results shown in the same table above indicated a non-significant increase among the infected positive control group (40±1.0) UL compared with the healthy negative control group (35.6±2.51 UL, as well as a non-significant increase among the group treated with fenugreek (39.5±4.9) UL compared with the infected positive control group (40±1.0) UL, and a significant increase among the group treated with metformin (45±2.0) UL compared with the infected positive control group (40±1.0) UL.

Table 4. Evaluation of AST and ALT levels in the blood serum of male rats.

Groupe / Parameter	C^-	C^+	**Fenugreek**	**Metformine**
AST U/L	3.0±98 **a**	3.5±99 **a**	10.6±97.5 **a**	6.2± 111 **a**
ALT U/L	2.51±35.6 **a**	1.0±40 **ab**	4.9±39.5 **ab**	2.0±45 **b**

* Letters that are the same horizontally reflect insignificant difference at $p < 0.05$.
* Letters that are different horizontally reflect significant difference at $p < 0.05$.
* **Values expressed as (Mean ±SD)**

The increased activity of serum AST and ALT enzymes may suggest the liver tissue injury associated with alloxan exposure, resulting in the leakage intracellular enzymes into the bloodstream (Navarro et al., 1993) and potential damage to liver cells (Aldayel and El-Semary, 2020; Al-jaff *et al.*, 2023). Fenugreek, on the other hand, reduces serum liver enzyme activities, indicating, potentially reflecting improved hepatocyte integrity (Baset *et al.*,2021; Elmnan *et al.*,2012). This is likely due to its antioxidant properties and high nutritional value (Tohamy *et al.*,2012; Middha *et al.*, 2011), as well as its alkaloids that inhibit oxidative stress and help restore normal liver and kidney function (El-mnan et al., 2012). As for metformin, the reason for the increase in (AST, ALT) is most likely due to the effect of the drugs on liver cells, as it leads to the destruction and release of the enzyme into the bloodstream (Jasim and Abdul-Razzaq, 2024).

The liver weight was measured, and the results shown in Table (5) indicated a significant increase among the infected positive control group (7.39±0.31) gm/kg compared with the healthy negative control group (6.95±0.05) gm/kg, a non-significant decrease among the

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fenugreek-treated group (7.33±0.29) gm/kg compared with the infected positive control group (7.39±0.31) gm/kg, and a significant increase among the metformin-treated group (8.73±0.03) mg/dl compared with the infected positive control group (7.39±0.31) gm/kg.

Table 5. Measurement of liver weight in male rats.

Groupe / Parameter	C^-	C^+	Fenugreek	Metformine
Liver weight gm/kg	0.05 ± 6.95 **a**	0.31 ± 7.39 **b**	0.29 ± 7.33 **ab**	0.03 8.73 ± **c**

* Letters that are the same horizontally indicate a non-significant difference at $p < 0.05$.
* Letters that are different horizontally indicate a significant difference at $p < 0.05$.
* **Values expressed as (Mean ±SD)**

The weight gain observed in the positive control group is similar to that reported by (Aissaoui *et al.*,2017; Otunola and Afolayan,2015).This suggests that alloxan-induced diabetes may have caused an increase in liver weight, attributed to insulin deficiency which reduced the activity of lipolytic enzymes, leading to hepatomegaly due to fat accumulation (Mir *et al.*, 2008).

On the other hand, the results of the statistical analysis of the fenugreek-treated group showed a decrease in liver weight, which is consistent with the study cited by (Bhat *et al.*,2015). Organ weight is considered an indicator of organ damage and its treatment (Adanma,2019). It was found that functions that were halted or lost due to high cholesterol can return to their previous state after improved insulin perfusion in the blood. This role is believed to be played by plant extracts through their active ingredients (Ezekwesili and Gwacham,2015). As for metformin, this is likely attributed to the drug's effect on liver cells, leading to their destruction and the release of enzymes into the bloodstream (Jasim and Abdul-Razzaq,2024).

Kidney weight was measured, and the results shown in Table (6) indicated a non-significant increase among the infected positive control group (0.87±0.15) gm/kg compared with the healthy negative control group (0.7±0.1) gm/kg, a non-significant decrease among the fenugreek-treated group (0.69± 0.05) gm/kg compared with the infected positive control group (0.87±0.15) gm/kg, and a non-significant decrease among the metformin-treated group (0.76±0.02) gm/kg compared with the infected positive control group (0.87±0.15) gm/kg.

Table 6. Measurement of kidney weight in male rats.

Groupe / Parameter	C^-	C^+	Fenugreek	Metformine
Kidney weight gm/kg	0.1 ± 0.7 **a**	0.15 ± 0.87 **a**	0.05±0.69 **a**	0.02±0.76 **a**

* Letters that are the same horizontally indicate a non-significant difference at $p < 0.05$.
* Letters that are different horizontally indicate a significant difference at $p < 0.05$.
* **Values expressed as (Mean ±SD)**

The kidneys are highly active and complex organs that help remove waste products, maintain homeostasis, and regulate acid-base balance. Nephrotoxicity can impair kidney function (Ali *et al.*,2021). Alloxan injections cause numerous functional physiological changes, including an increase in free radicals, which makes renal tissues more susceptible to damage and leads to significant effects on kidney histological structure (Deeba et al., 2020). Kidney enlargement may also result from an increase in protein synthesis and/or a decrease in the breakdown of extracellular kidney components (Chang *et al.*, 2016).
Following treatment with fenugreek, the kidney-to-body weight ratio decreased, indicating that kidney enlargement was reversed in diabetic mice. Elevated lipid levels are associated with diabetic nephropathy (Saraswat *et al.*,2020; Mestry *et al.*,2017). This aligns with the study by (Arbo *et al.*,2009) showing that plant extracts exhibit contrasting and improved results. The reduction in kidney weight observed with metformin treatment may be due to its significant improvement in kidney function, suggesting it could be a beneficial drug in mitigating the progression of chronic kidney disease in diabetic mice (Al-Za'abi *et al.*, 2021).

Conclusions

The study showed that fenugreek extract and metformin have significant effects in lowering blood sugar, urea, creatinine and blood lipids. This plant has a long history of medicinal use and has great and well-known benefits for the human body as a result of containing active compounds from the raw extract of this plant, and this makes us think about developing some drugs in the future.

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