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Determining the health effects of Greek how fenugreek seed extract laboratory rats with affects type 2 diabetes in some of its biochemical parameters

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Abstract
The aim of this research was to assess the effect of fenugreek seed extract on the appearance of health for individuals within the College of Veterinary Medicine, Tikrit University, between January 22 2025 and February 21 2026. The investigators studied whether or not there was an effect of fenugreek on blood glucose levels and insulin and lipid profiles in adult male albino rats with type two diabetes administered via an injection. After 28 days of consuming fenugreek seed extract, the blood glucose levels in these rats had decreased, whereas the blood insulin level increased, and their lipid profile decreased from baseline levels.

Keywords: Fenugreek, diabetes induction, serum glucose levels, lipid profiles

Introduction
The legume fenugreek (*Trigonella foenum-graecum*) has several health and medicinal advantages. Its efficacy in controlling blood sugar and cholesterol levels has been demonstrated by scientific studies. Nutrients like fiber, protein, vitamins, and minerals are abundant in fenugreek seeds. Fenugreek is an old plant that has been used both medicinally and culinarily for thousands of years. It is utilized in traditional medicine for a variety of health concerns.

Due to its various health and nutritional advantages, fenugreek, which originated in the Mediterranean and Middle East, is today grown all over the world. With trifoliate leaves and white or yellow flower buds that mature into pods with tiny brown seeds, this herbaceous shrub can reach a height of 30 to 60 cm, Both natural medicine and culinary employ fenugreek seeds. They are distinguished by their powerful scent and rather bitter flavor. The fenugreek plant has many health and therapeutic benefits, which are within the permissible concentrations between 200-400 mg/kg daily. Fenugreek can provide various health benefits, including reducing blood glucose levels and improving the body's insulin response in people with type 2 diabetes. Fenugreek is also beneficial for the heart; the fiber and antioxidants naturally found in fenugreek can help to lower LDL cholesterol levels and promote good heart health. In addition, because it contains compounds that possess anti-inflammatory properties, fenugreek can help to reduce pain and swelling in the body.

The Chemical Composition of Fenugreek
The many biological uses of fenugreek are due to the high content of active chemical constituents. The use of fenugreek will depend on the large number of active chemicals found in fenugreek and how they have been proven to have health benefits (Yasothai 2021) [20]. These substances include fibrous foods, which help people digest their food better and reduce their cholesterol, protein foods, which are a major part of the building blocks of the human body, flavonoids, which are antioxidants that prevent oxidative damage and reduce inflammation in the body, as well as volatile oils, such as sesquiterpenes, diosgenin, that are responsible for the distinctive and medicinal smells of fenugreek. It also contains vitamins C and B6 that enhance health and minerals, including iron, calcium, phosphorus, and potassium, that are important for bone and blood health and many other bodily functions (Billaud and Adrian 2021) [4].

Induced Diabetes

The induction of diabetes in laboratory animals through experimentation has dramatically advanced our knowledge and understanding of science. Many effective treatments for diabetes have been developed and the main causes of diabetes are well known. Historically, numerous experimental induction methods with laboratory animals have been used to produce conditions similar to those experienced by human beings. The work of Romanian physician Paulesco and Canadian physicians Banting and Best were significant contributions to our knowledge of methods of chemically producing diabetes. In 1921, they did so by surgically removing a dog's pancreas then chemically inducing diabetes in that same dog subsequently. Other chemicals that can be used to produce diabetes include Alloxan (ALX) and Streptozotocin (STZ). (Panneerselvam and Govindasamy, 2004) [15].

Brugnatelli made the initial discovery of alloxan in 1818. Alloxan's capacity to specifically kill pancreatic beta cells was initially reported by Dunn. That was in 1943. It is a naturally occurring metabolite of purines and a derivative of uric acid. Alloxan is unstable and has an estimated half-life of 1.5 minutes. It is important to note that, in contrast to rats, humans are resistant to the effects of alloxan (Szkudelski, 2001) [18]. Alloxan's mode of action has been suggested by a number of studies, including its interaction with fundamental biological substances that contain sulfhydryl groups (SH), such as glutathione and the enzyme superoxide dismutase.

These compounds are reduced to dialluric acid, which reacts with oxygen to form superoxide (O2). The resulting superoxide then undergoes dismutation to form hydrogen peroxide (H_2O_2). This reaction occurs spontaneously and is catalyzed by the enzyme SOD. After the formation of hydrogen peroxide, it reacts with ferrous ions (Fe+2) to form high-energy hydroxyl radicals (OH•), which lead to lipid oxidation and DNA strand damage, thus inhibiting proinsulin synthesis as a mechanism for destroying pancreatic beta cells (β-cells). esearch shows reactive oxygen species (ROS) are key players in both diabetes development and disease complications. ROS has been shown to lead to pancreatic beta cell dysfunction and impairments in the glucose transport machinery. Alloxan can be given IV, IP, or SC (Inzucchi, 2005) [9].

Materials and Methods

Materials (Sample Preparation)

Fenugreek seeds were purchased from local stores in the city of Tikrit. The seeds were clean and ready for extraction using the method outline by Eman B. (2022). 10 grams of seed were placed into 20 ml of water within a clean glass container at a temperature between 40°F to 45°F for a duration of 10-15 minutes. Once the extraction was complete, the resulting liquid was poured through a piece of filter paper into another glass container and refrigerated until needed.

Preparation of Laboratory Pets

Adult male Sprague-Dawley rats (4-5 weeks old, 175-180 grams) were chosen for use in this experiment. The rats are from the College of Veterinary Medicine at Tikrit University and were housed in metal cages with metal lids and wood shavings for bedding. The cages were kept clean and sterile, changing the wood shavings twice a week. For environmental control, the rats were exposed to laboratory conditions including a (12) hour light/dark cycle and a temperature of (25) °C. The rats were allowed (2) days to adapt to the new conditions and checked for disease. Food and water (adlibitum) were available during the entire experiment.

Experimental Diabetes Induction

Male rats were given subcutaneous injections of Alloxan (BDH, UK) to produce experimental diabetes. At the time of the injection, 1 g of alloxan was prepared by dilution in 10 mL of freshly prepared normal saline and was equivalently administered at 100 mg/kg body weight (Owoyele *et al*., 2005) [14]. Prior to inducing diabetes, the animals were deprived of food for 24 hours. After receiving the injection, they were again given food, 5% glucose in water, and drinking water for 24 hours to minimize the risk of rapid decreases in blood glucose levels due to pancreatic beta-cell death causing a great deal of released insulin which would lower blood glucose levels. The control animals received only 1 mL of normal saline and had free access to food and water. Each of the diabetic animals was confirmed to be diabetic with a urine reagent test strip (MACHEREY-NAGEL, Germany) every 48 hours for a period of 10 days to confirm the presence of glucose in the urine. Animals that showed a glucose level higher than 200 mg/100 ml were considered to have diabetes as they showed signs of extreme fatigue and frequent urination (Alarcon-Aguilara *et al*., 1998) [2].

Experimental Design: Nine adult rats were randomly divided into three groups of three animals each:

Group 1: Control Group

Group 2 (Diabetes-Induced Control Group): Throughout the experiment, this group had untreated diabetes and continued to have access to food and water.

Group 3 (Diabetes-Induced): This group received a regular diet plus 400 mg/kg of fenugreek seed extract every day

The animals were fasted for approximately 12 hours prior to administration. The temperature was maintained between 20-25°C, and the lighting was maintained for at least 12 hours daily during the 28-day experiment.

Blood Sampling

The animals were sedated with chloroform and starved for ten hours right after the experiment. The animals were put in a glass container that was sealed, and a syringe was used to draw blood from their hearts. Four to five milliliters of blood were extracted and put in disposable tubes. Serum was extracted from these tubes by centrifuging them for 15 minutes at 3000 rpm, and it was kept at 2°C until analysis.

Results and Discussion

Effect of Fenugreek Seed Extract on Blood Glucose in Induced Diabetes Rats

Table (1) shows the effect of fenugreek seed extract on glucose concentration. The results showed a significant increase ($P< 0.05$) in serum glucose concentration in untreated G2 induced diabetic male rats throughout the experimental period compared to the healthy G1 control group.

This occurs because the alloxan has attacked the pancreatic β-cells of mice, causing them to produce an excess of free radicals which build up in the body and harm β-cells. When insulin resists the action of insulin by blocking the receptors on cells to utilize glucose for energy, glycogenolysis will respond to this disruption by converting and fully converting glucose into glycogen, while gluconeogenesis will occur to provide additional energy from glucose that has been created from non-carbohydrate substrates. (Alhazza, 2007) [3].

Table 1: Effect of fenugreek seed extract on serum glucose concentration (mg/100 ml) in rats with induced diabetes

Groups	72 hours after infection	First week	Second week	Third week	Fourth week
G1	107±1.2 c	105±1.00 c	98±1.00 c	102±1.05 c	104±1.00 c
G2	2311.00 a ±	239±1.00 a	304±1.03 a	318±1.00 a	329±1.04 a
G3	212±1.00 b	192±1.02 b	189±1.00 b	172±1.00 b	141±1.00 b

Different letters in the same column indicate significant differences between the mean scores at a probability level of 0.05.

Over the course of a four-week treatment period, feeding with fenugreek seed extract raises serum insulin levels. These findings align with those of Rita Kiss *et al*. (2018) [16], who linked enhanced beta cell function islets in the of Langerhans of diabetic in the pancreas rats.to notable rise in insulin concentration. Fenugreek seed extract's flavonoids successfully decreased free radical-induced cell damage, and its antioxidant activity helped to increase serum insulin levels, stop more beta cell death, and somewhat improve beta cell function.

These results are also consistent with those of Fatemeh *et al*. (2024), who reported that fenugreek seed extract has an effect on glucose balance in diabetic patients and stimulates the pancreas to produce insulin.

Effect of Fenugreek Seed Extract on Insulin Concentration

Table (2) shows a decrease in serum insulin concentration in rats in the experimentally induced diabetic group (G2) compared to the control group (1G). The table also shows that after 28 days of oral administration of fenugreek seed extract, insulin levels increased compared to the untreated group.

Table 2: Effect of Fenugreek Seed Extract on Serum Insulin Concentration in In the category of healthy male laboratory and mice with diabetes

Groups	Insulin concentration
G1	32.20±0.05
G2	2.04±1.00
G3	24.19±1.00

Different letters in the same column indicate significant differences between the mean scores at a probability level of 0.05.

During the treatment period, the study animals' serum insulin levels increased as a result of being fed fenugreek seed extract. These findings align with those published by Kumer Dipto *et al*. (2024). Improved pancreatic beta cell function is responsible for the third group's higher insulin concentration as compared to the second group, which received no treatment. This improvement is due to the presence of flavonoids in fenugreek seeds, which possess antioxidant properties. These antioxidants help protect the body from oxidative stress and inflammation, thus reducing cell damage caused by free radicals and consequently raising serum insulin levels (Billaud and Adrian, 2021) [4].

Effect of fenugreek seed extract on the lipid profile in diabetic rats

The results in Table (3) show the effect of fenugreek seed extract on the lipid profile, as the increase in CHO, TG, LDL, and VLDL in diabetic rats is very common (Mohammed, 2020) [11].

Table 3: Effect of fenugreek seed extract on the lipid profile in serum blood of male rats with induced diabetes healthy and those

Groups	COH	TG	HDL	LDL	VLDL
	(Mg/dl)				
G1	90.01±0.09c	83.02±0.11c	62.05±0.02a	16.02±0.05c	18.02±0.15c
G2	160.02±0.22a	164.11±0.07a	25.10±0.05c	87.04±0.05a	37.09±0.08a
G3	121.11±0.03b	88.02±0.14b	49.11±0.05b	40.10±0.05b	14.08±0.21b

Different letters in the same column indicate statistically significant differences between the mean scores at a probability level of 0.05.

Oral fenugreek seed extract stimulation of lipoprotein lipase activity is responsible for the decrease in LDL, CHO, TG, and VLDL levels and the rise in HDL levels in diabetic rats. This stimulation reduced TG and may have increased the uptake of triglycerides from plasma by adipose tissue and skeletal muscle, which decreased body fat percentage and increased satiety. Research indicates that the accumulation of fat, especially saturated fatty acids and free cholesterol, may be significantly influenced by apoptosis in hepatocytes. This could trigger both intrinsic and extrinsic apoptotic pathways by impairing hepatocyte function. Fenugreek seed extract's active ingredients have several health advantages, such as strong antioxidant qualities, liver protection, and cholesterol lowering (Zargham Faisal *et al*., 2024) [22].

Furthermore, fenugreek seed extract strengthens the immune system, provides energy, and protects cells from damage. Fenugreek seeds have been shown to lower cholesterol, decrease obesity, and aid in maintaining a healthy weight. These qualities of fenugreek seed are due to its high vitamin and mineral content, which contributes to cholesterol lowering by reducing oxidation damage to cells, preventing

heart disease, and enhancing the function of a liver enzyme (7α-hydroxylase) in the blood (Billaud & Adrian, 2021) [4]. The effects of fenugreek seed extract on liver enzymes in serum from healthy, adult, male rats and insulin-resistant, diabetic male rats were evaluated to assess the influence of fenugreek in healthy and insulin-resistant rats for 28 days.

Table (4) shows a significant increase (P<0.05) in the levels of the liver enzymes AST, ALT, and ALP in the control group compared to the healthy control group throughout the experimental period.

Table 4: Effect of fenugreek seed extract on liver enzyme levels in the blood serum of healthy male rats and those with induced diabetes over 28 days

Group	AST	ALT	ALP
	(IU/L)		
G1	42.02±0.1c	44.10±1.5c	136.02±0.4 c
G2	66.04±0.2 a	61.12±1.2 a	167.00±1.50 a
G3	45.05±0.4b	49.20±1.50b	141.02±1.2b

Different letters on the values in the same column indicate significant differences between the values at a probability level of 0.05.

The results presented here agree with those obtained by Leila *et al* (2023), where they reported an increase in the levels of the enzymes AST, ALT and ALP measured in the serum of diabetic mice. The increase in these enzyme levels is likely attributed to the death of liver cells, which subsequently results in damage to the heart and impairment of metabolism through the impaired function of the liver, as a result of diabetes. AST, ALT, and ALP enzyme levels rise as a result of enhanced liver cell metabolism.

The results obtained and shown in Table (2) also agreed with what Syeda and Javeria (2021) stated regarding the decre the enzymes AST, ALT and ALP. The amount of activity seen with this decrease of active contains; The cause of the decrease will be because the active contain affects the activity of the enzymes AST, ALT and ALP. The cause for this decrease in activity will be from the active contains found in the product in the extract of fenugreek seeds, which are a natural antioxidant that works to break down free radicals and reduce their activity and effect, thus preventing the destruction of cell membranes through the oxidation process and thus maintaining the level of liver enzyme values.

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