



Received on 27 August 2024; received in revised form, 27 September 2024; accepted, 29 September 2024; published 31 October 2024

EFFECT OF FENUGREEK SUPPLEMENTATION IN DIABETES

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Keywords:

Hypoglycemia, Immunomodulatory, Pancreatic

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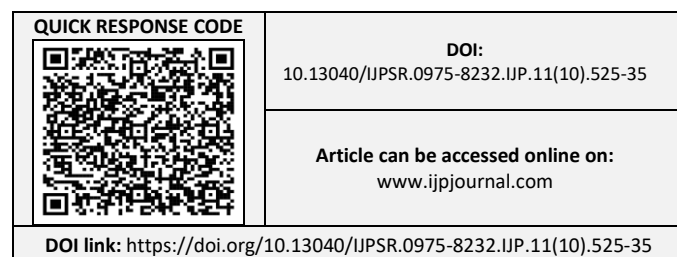
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ABSTRACT: Dietary health supplements have been increasingly utilized in the prevention and treatment of chronic disorders. Over the past few decades, fenugreek (*Trigonella foenum-graecum*) seeds have been reported to exhibit hypoglycemic and cholesterol-lowering effects in both type 1 and type 2 diabetes mellitus patients, as well as in experimental diabetic animal models. The aim of this paper is to review the research findings on the use of fenugreek in diabetes management. There is compelling research evidence indicating that fenugreek can improve hyperglycemia and offer protection against diabetic complications through its immunomodulatory, insulin-stimulating, and antioxidant effects, as well as by enhancing adipocyte differentiation, reducing inflammation in adipose tissues, and preventing or mitigating pancreatic and renal damage. Many of the beneficial effects of fenugreek in diabetes have been attributed to four key bioactive components: diosgenin, 4-hydroxyisoleucine, furostanolic saponins, and its fiber content.

INTRODUCTION: Diabetes is a metabolic disorder characterized by hyperglycemia resulting from abnormalities in insulin secretion and action¹. Chronic hyperglycemia leads to both microvascular (e.g., neuropathy and nephropathy) and macrovascular (mainly cardiovascular) complications, which arise due to increased production of reactive oxygen species and reduced antioxidant defenses². Herbal products have been widely used throughout history to treat various diseases. As the characterization and exact mechanisms of action of these natural products remain unclear, researchers continue to evaluate their beneficial effects on human health, as well as any potential adverse effects³.

Despite the availability of several treatments for diabetes, including insulin analogs, sulphonylureas, biguanides, dipeptidyl peptidase-4 inhibitors, thiazolidinediones, and α -glucosidase inhibitors, many patients prefer using botanicals due to the rising costs and side effects associated with these medications². Many plants, such as cinnamon and ginseng, reduce glucose and lipid levels through mechanisms such as stimulating insulin secretion, delaying gastric emptying, inhibiting glucosidase activity, increasing GLUT4 expression⁴, activating the AMP-activated protein pathway⁵, and inhibiting gluconeogenesis⁶. Several medicinal plants, including ginger, garlic, fenugreek, and cumin, are used for diabetes management^{7,8}.

Fenugreek (*Trigonella foenum-graecum*) is a historically used herbal medicinal plant that is popular in Africa, India, and South and Central Asia⁹. Traditionally, it has been used to treat various conditions, including diabetes and obesity. Fenugreek is known for its antioxidant, antihyperlipidemic, antibacterial, antifungal, anti-



DOI:

10.13040/IJPSR.0975-8232.IJP.11(10).525-35

Article can be accessed online on:
www.ijpjournal.com

DOI link: [https://doi.org/10.13040/IJPSR.0975-8232.IJP.11\(10\).525-35](https://doi.org/10.13040/IJPSR.0975-8232.IJP.11(10).525-35)

inflammatory, and galactagogic properties¹⁰. Fenugreek's pharmacological effects are attributed to a range of bioactive compounds such as polyphenols, steroids, lipids, alkaloids, saponins, flavonoids, hydrocarbons, carbohydrates, galactomannan fiber, and amino acids. Several scientific studies have examined its antidiabetic effects. One study showed that fenugreek increased glucose uptake in HepG2 cells by overexpressing glucose transporter (GLUT-2)¹² and sterol regulatory element-binding protein (SREBP1C) mRNA levels¹¹. Another report by Pradeep and Srinivasan¹³ demonstrated that when fenugreek was combined with 3% onion, it produced better antidiabetic results. A potential fenugreek-based drug (Fenfuro®) was compared to Metformin in a clinical trial, where results showed that Fenfuro combined with Metformin was more effective than Metformin alone¹⁴.

Diosgenin, a saponin, is considered the most bioactive substance in fenugreek. It has antioxidative effects and plays a crucial role in improving diabetic status through several mechanisms^{1, 15}. These mechanisms include β -cell renewal and stimulation of insulin secretion. Additionally, diosgenin elevates the mRNA transcription levels of CCAAT/enhancer-binding protein (C/EBP δ) and peroxisome proliferator-activated receptor- γ (PPAR- γ)^{10, 12}.

Other components in fenugreek include 4-hydroxyisoleucine, an amino acid that enhances insulin secretion and decreases plasma triglyceride and total cholesterol levels¹. Galactomannan, a carbohydrate representing 45-60% of the fenugreek seed, has been shown to block carbohydrate and lipid-hydrolyzing enzymes in the digestive system, leading to lower postprandial glucose levels¹.

Although the detailed mechanisms underlying fenugreek's antidiabetic activity have yet to be fully elucidated, many studies suggest that its antioxidant activity plays a significant role in hepatoprotection. It is also possible that fenugreek reverses protein glycation caused by hyperglycemia¹⁶. Further investigation into the molecular mechanisms of action and active components of fenugreek is needed. In many parts of the world, fenugreek is commonly consumed as a beverage. In the current study, we aim to compare different

routes of administration of fenugreek at a clinically feasible dose (100 mg/kg) in a diabetic rat model induced by streptozotocin (STZ).

MATERIALS AND METHODS:

Animals: All experiments were conducted in accordance with the NIH guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of the October University for Modern Sciences and Arts (2018). Male Sprague Dawley rats, weighing 175-200 g, were obtained from the Theodore Bilharz Research Institute (Cairo, Egypt). The rats were randomly divided into the following groups: Group 1: diabetic (DM) rats receiving 100 mg/kg of fenugreek extract (HERB-PHARM) every other day (EOD). Group 2: DM rats receiving 100 mg/kg of fenugreek intraperitoneally (IP) daily. Group 3: DM rats receiving 100 mg/kg of fenugreek orally daily. Group 4: untreated diabetic group. Group 5: healthy nondiabetic control group.

Diabetes Model: The animals received streptozotocin (STZ; 75 mg/kg in sterile citrate buffer) *via* intraperitoneal injection. Diabetes was confirmed one week after the STZ injection by measuring blood glucose levels. Rats with fasting glucose levels of 270 mg/dl (15 mmol/l) or higher were included in the study (3). At the end of the experiment, histological examination of the rat pancreatic tissues further confirmed the presence of diabetes.

Sample Collection: At the end of the 4-week treatment period, the rats were anesthetized using a combination of ketamine and xylazine (ketamine 80-100 mg/kg, xylazine 10-12.5 mg/kg, administered intraperitoneally).

Blood was collected via cardiac puncture, and the rats were then dissected to obtain tissue samples from the pancreas, kidney, and liver for biochemical and histological analysis. There was no difference in the sample size, as animals lost due to mortality were replaced.

Biochemical Analysis: The collected serum was divided into aliquots to evaluate liver and kidney functions, as well as serum glucose levels and the lipid profile, as previously described³.

Antioxidant Activity: Frozen liver tissue samples (0.2 g) were homogenized in phosphate-buffered saline. The resulting suspension was centrifuged at 4,400 rpm, and the supernatants were collected for testing antioxidant enzyme levels.

Catalase Enzyme Activity: Catalase enzyme activity was measured according to the method originally described by Aebi¹⁷. In brief, the assay involves catalase reacting with a known quantity of hydrogen peroxide (H_2O_2), with the reaction being stopped after 1 minute by adding a catalase inhibitor. The remaining H_2O_2 then reacts with 4-aminophenazone and 3,5-dichloro-2-hydroxybenzene sulfonic acid to form a chromophore, which is measured at an absorbance of 510 nm using a spectrophotometer.

Glutathione Peroxidase: Glutathione peroxidase activity was measured using the method originally described by Paglia and Valentine¹⁸. The assay principle is based on the indirect measurement of cellular glutathione peroxidase activity. The enzyme reduces an organic peroxide using cellular glutathione, resulting in the production of oxidized glutathione (GSSG). The rate of decrease in absorbance at 340 nm is directly proportional to the glutathione peroxidase activity in the sample.

Glutathione S-transferase (GST) Enzymatic Activity: The enzyme activity was measured using the method developed by Habig *et al.*¹⁹. The GST enzyme catalyzes the conjugation of reduced

glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) through the -SH group of glutathione. This reaction produces the conjugate S-(2,4-dinitrophenyl)-L-glutathione, which can be detected. Absorbance is measured at 340 nm.

Peroxidase Activity: Peroxidase activity was assessed based on enzyme inhibition (20). Sulfite is added to inhibit the enzyme before the addition of hydrogen peroxide. Freshly prepared 1% o-phenylenediamine and 0.3% hydrogen peroxide are added to the liver tissue homogenate. The reaction is stopped after 5 minutes by adding sodium bisulfite. Absorbance is measured at 430 nm, and enzyme activity is expressed as the change in absorbance at 430 nm (ΔOD_{430}) per minute per milligram of protein.

Histological Analysis: Formaldehyde-fixed tissue samples were paraffin-embedded, and 5 μm sections were cut and stained with hematoxylin and eosin. The slides were examined and photographed using a BX51 light microscope equipped with an Olympus digital camera (DP20) (Olympus).

RESULTS:

Blood Glucose Levels: Fasting blood glucose levels in rats treated with fenugreek extract were significantly reduced compared to the diabetic control group across all treatment groups **Fig. 1**. No significant differences were observed between the different treatment groups.

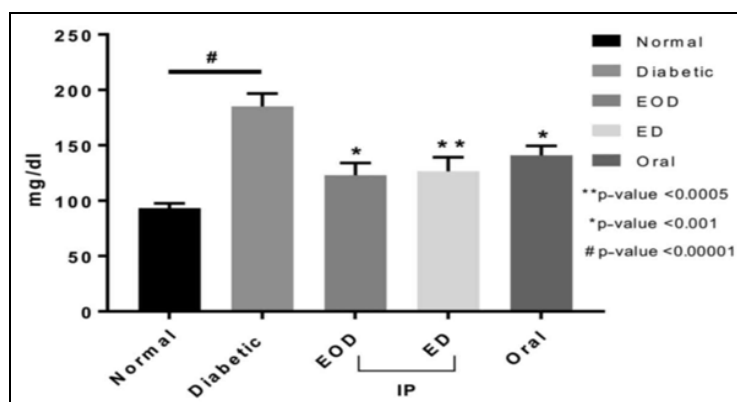


FIG. 1: EFFECT OF FENUGREEK TREATMENT ON BLOOD GLUCOSE LEVELS IN DIFFERENT GROUPS. ALL GROUPS SHOWED A SIGNIFICANT DECREASE IN GLUCOSE LEVELS COMPARED TO DIABETIC RATS

Renal Function: Urea levels were elevated in diabetic rats **Fig. 2**. No significant reduction in urea levels was observed with fenugreek treatment, except in the daily injection group **Fig. 2**.

Creatinine levels also increased in untreated diabetic rats. Fenugreek injection did not lower creatinine levels; however, a significant decrease in creatinine was observed with oral treatment **Fig. 2**.

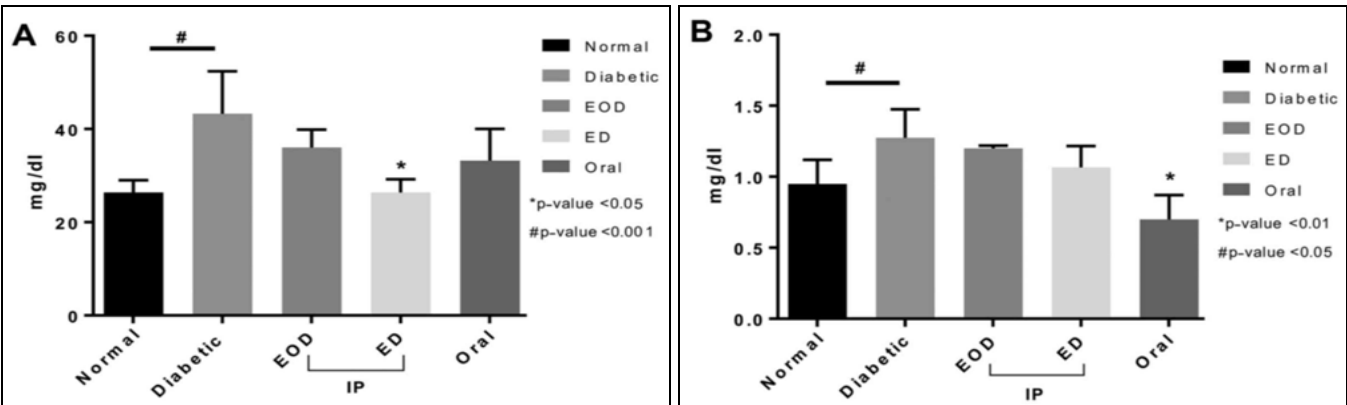


FIG. 2: EFFECT OF FENUGREEK TREATMENT ON KIDNEY FUNCTION: (A) UREA LEVEL SIGNIFICANTLY INCREASED IN DIABETIC RATS COMPARED TO THE NORMAL GROUP. ED INJECTION GROUP WAS THE ONLY ONE TO SHOW A SIGNIFICANT DECREASE COMPARED TO DIABETIC RATS, WHICH WAS BACK TO NORMAL. (B) CREATININE LEVELS WERE INCREASED IN DIABETIC RATS. ORAL TREATMENT SHOWED A SIGNIFICANT REDUCTION IN CREATININE LEVELS COMPARED TO DIABETIC RATS. DATA ARE SHOWN AS MEAN ± SD, N=5.

Liver Functions and Lipid Profile: Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were elevated in diabetic rats. Fenugreek treatment, both orally and *via* daily injections, significantly reduced AST and ALT levels compared to the diabetic control group.

Protein levels were significantly increased in all treated groups compared to untreated diabetic rats, with the highest levels and significance observed in the daily injection (ED) group compared to the diabetic control **Fig. 3**.

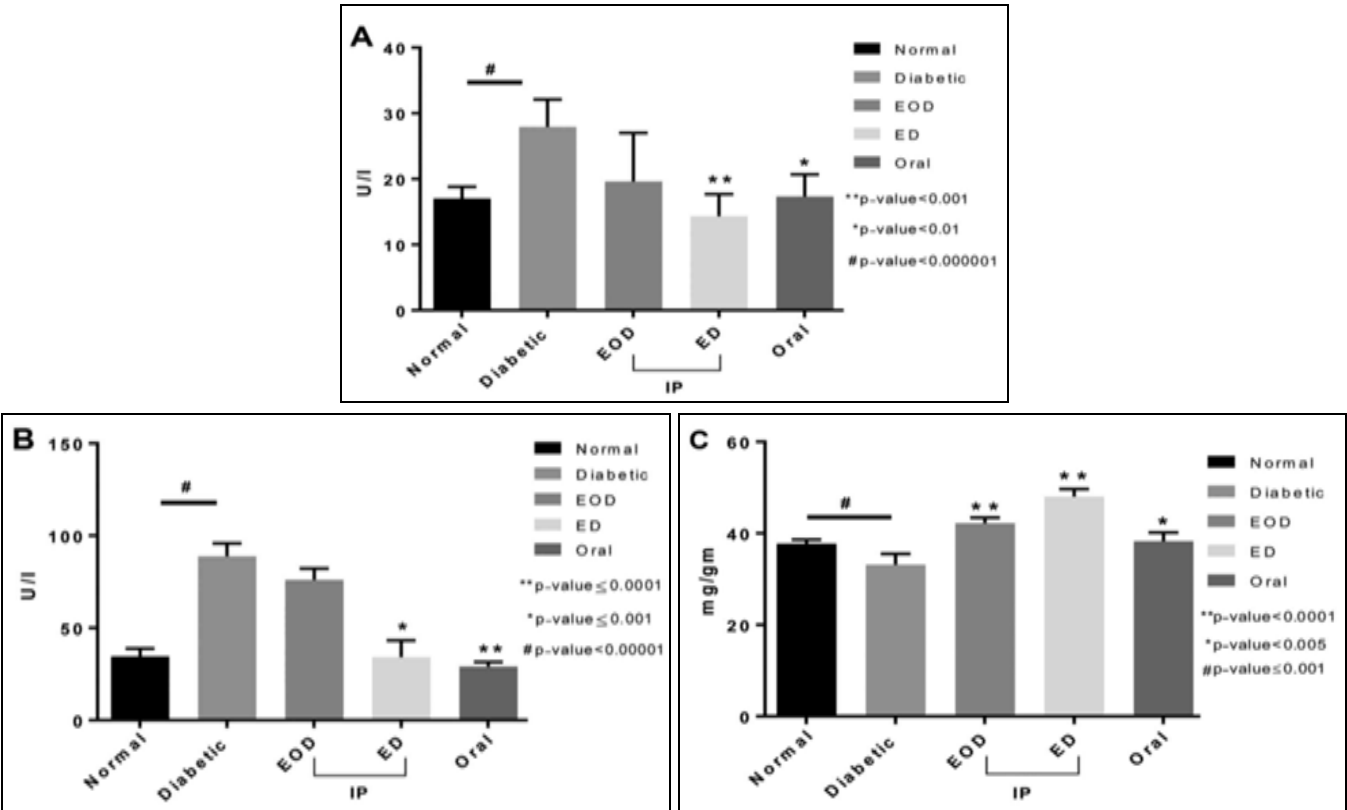


FIG. 3: EFFECT OF FENUGREEK TREATMENT ON LIVER FUNCTION TEST: LIVER ENZYMES (A) ASPARTATE AMINOTRANSFERASE (AST) AND (B) ALANINE AMINOTRANSFERASE (ALT) LEVELS INCREASED IN DIABETIC RATS. ORAL AND ED INJECTION GROUPS SHOWED A SIGNIFICANT DECREASE IN BOTH AST AND ALT LEVELS COMPARED TO DIABETIC RATS. (C) ON PROTEIN LEVELS, FENUGREEK ADMINISTRATION CAUSED A SIGNIFICANT INCREASE IN PROTEIN LEVELS IN ALL TREATED GROUPS COMPARED TO UNTREATED DIABETIC RATS. DATA ARE SHOWN AS MEAN ± SD, N=5. ED, DAILY.

High-density Lipoprotein (HDL) Levels: Did not increase in diabetic rats compared to normal controls. A significant increase in HDL was observed only with daily fenugreek injections. Conversely, triglyceride levels were significantly elevated in diabetic rats. All treatment groups

demonstrated a significant decrease in triglyceride levels compared to the diabetic rats receiving daily injections, with the highest significance seen in these treated groups. Cholesterol levels increased following diabetes induction and were not reduced by fenugreek treatment **Fig. 4**.

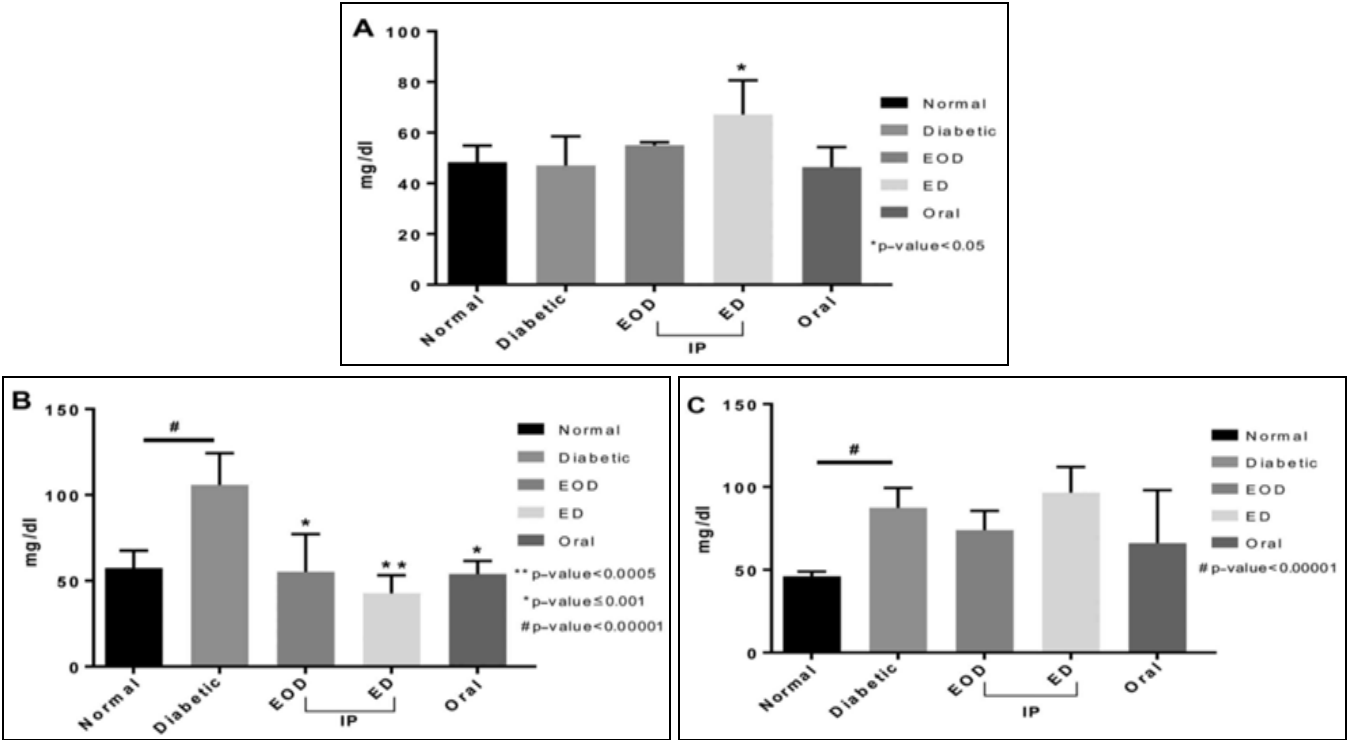
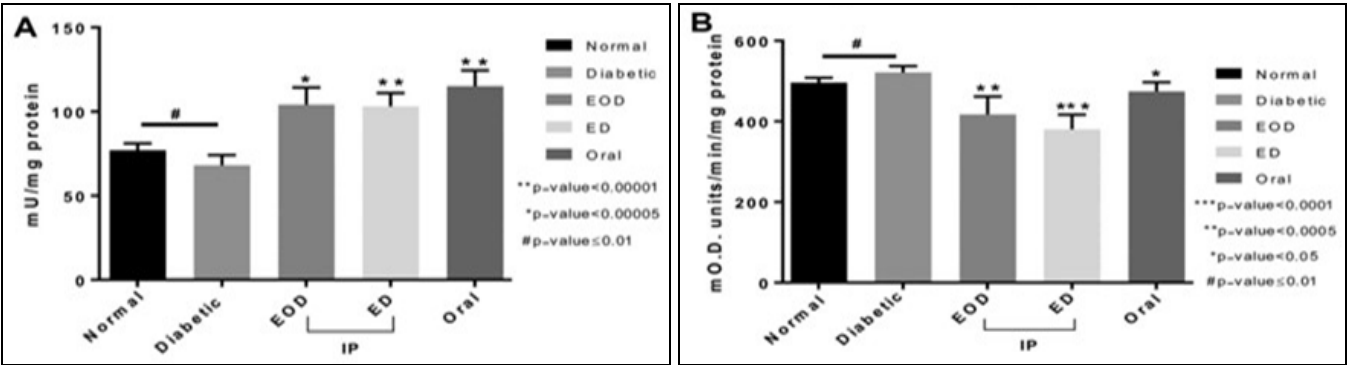


FIG. 4: EFFECT OF FENUGREEK TREATMENT ON LIPID PROFILE: (A) HIGH-DENSITY LIPOPROTEIN (HDL). (B) CHOLESTEROL LEVELS WERE NOT INCREASED IN DIABETIC RATS COMPARED TO NORMAL, ONLY AFTER ED INJECTION OF FENUGREEK A SIGNIFICANT INCREASE WAS SEEN IN HDL LEVELS. (C) TRIGLYCERIDES WERE SIGNIFICANTLY INCREASED IN DIABETIC RATS. ALL TREATMENT GROUPS SHOWED A SIGNIFICANT DECREASE IN TRIGLYCERIDES. FENUGREEK TREATMENT DID NOT CAUSE ANY SIGNIFICANT CHANGE IN CHOLESTEROL LEVELS. Data are shown as mean ± sd, n=5. ed, daily.

Antioxidant Enzymes: Catalase enzyme activity in the liver tissue of untreated diabetic rats was slightly lower compared to normal rats. However, catalase levels increased significantly in all treatment groups compared to both normal and untreated diabetic rats, with oral fenugreek treatment showing the highest levels **Fig. 5A-B**.

Quantitative analysis of peroxidase activity in liver tissue revealed elevated peroxidase levels in diabetic rats compared to normal controls. All treated groups exhibited a significant reduction in peroxidase levels compared to untreated diabetic rats, with the daily injection group show highest and most significant reduction **Fig. 5C-D**.



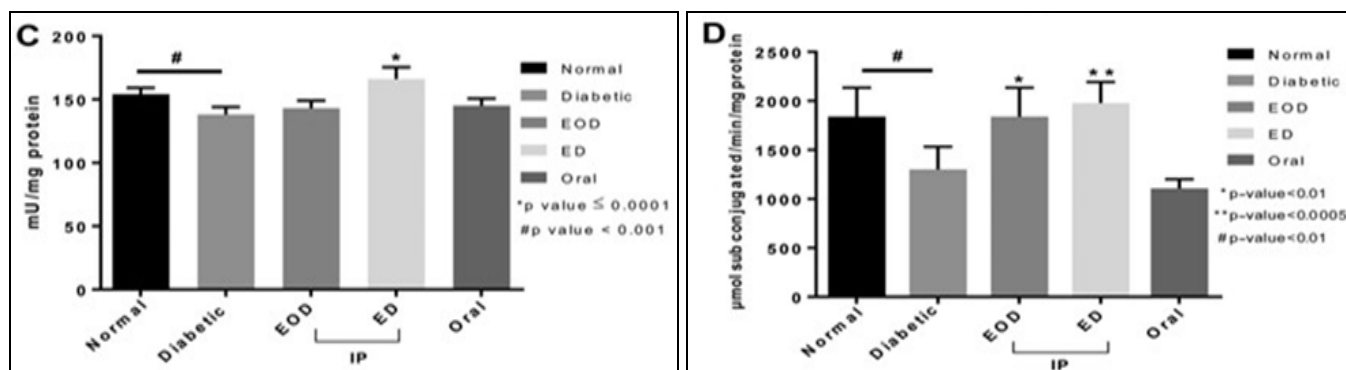


FIG. 5: EFFECT OF FENUGREEK TREATMENT ON LIVER ANTIOXIDANT ENZYMES. (A) CATALASE ENZYME LEVELS WERE NOT SIGNIFICANTLY DIFFERENT BETWEEN NORMAL AND DIABETIC RATS. THESE LEVELS INCREASED SIGNIFICANTLY IN ALL TREATED GROUPS. (B) PEROXIDASE LEVELS WERE INCREASED IN DIABETIC RATS COMPARED TO NORMAL. ALL TREATED GROUPS SHOWED A SIGNIFICANT REDUCTION IN PEROXIDASE LEVELS COMPARED TO DIABETIC UNTREATED RATS. (C) GLUTATHIONE PEROXIDASE LEVELS WERE NOT CHANGED IN DIABETIC UNTREATED OR EOD AND ORAL TREATED RATS. THE ONLY GROUP SHOWING A SIGNIFICANT INCREASE WAS THE ED INJECTION TREATED GROUP. (D) GLUTATHIONE S-TRANSFERASE ENZYME WAS SIGNIFICANTLY REDUCED IN DIABETIC UNTREATED RATS. NO SIGNIFICANT CHANGE WAS SEEN WITH THE ORAL ADMINISTRATION OF FENUGREEK, WHEREAS BOTH EOD AND ED INJECTED GROUPS SHOWED A SIGNIFICANT INCREASE IN THE ENZYME LEVELS COMPARED TO THE DIABETIC CONTROL GROUP. Data are shown as mean $\pm$ SD, n=5. ED, daily, EOD, every other day.

Histological Changes in the Pancreas: Normal rats displayed the typical healthy architecture of the

pancreatic islets of Langerhans, with no signs of cell injury.

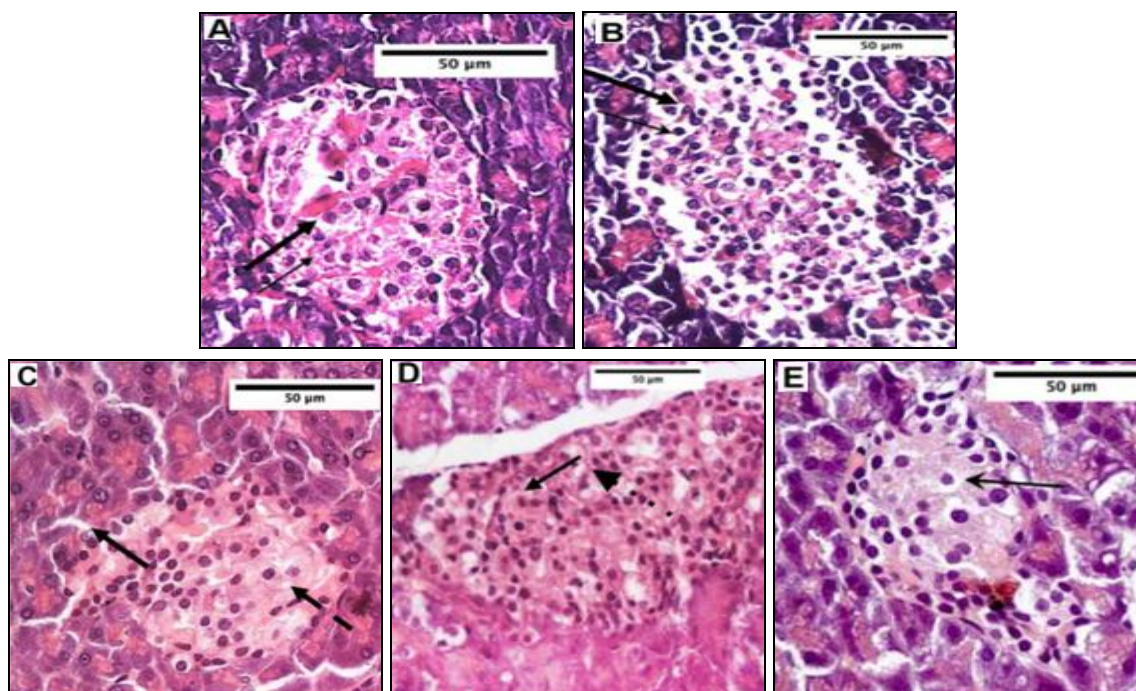


FIG. 6: HISTOLOGICAL CHANGES IN THE PANCREAS. (A) NORMAL SAMPLES SHOWED NORMAL HISTOLOGICAL STRUCTURES OF ENDOCRINE CELLS OF PANCREATIC ISLETS WITHOUT ABNORMAL ALTERATIONS. (B) DIABETIC RATS SHOWED SEVERE DEGENERATIVE AND VACUOLAR CHANGES OF THE OUTER CELLULAR ZONE OF PANCREATIC ISLETS WITH KARYO-PYKNOSIS OF MOST OF THE CELLS (ARROW). (C) ANIMALS RECEIVING ORAL FENUGREEK SHOWED NO PROTECTIVE EFFECTS AND DEGENERATIVE CHANGES IN CELLULAR ELEMENTS OF PANCREATIC ISLETS WITH MANY CELLS DEMONSTRATING PYKNOTIC NUCLEI (ARROW) WITH VARIABLE RECORDS OF CYTOPLASMIC VACUOLATION (DASHED ARROW). (D) EVERY OTHER DAY INJECTION OF FENUGREEK SHOWED DETERIORATION CHANGES IN CELLULAR COMPONENTS OF PANCREATIC ISLETS IN MOST OF THE TISSUE SECTIONS WITH NUMEROUS CELLS DEMONSTRATING PYKNOTIC NUCLEI (ARROW). THE DASHED ARROW SHOWS IRREGULAR RECORDS OF CYTOPLASMIC VACUOLATION. THE PANCREAS SHOWS MILD DEGENERATIVE CHANGES IN CELLULAR COMPONENTS OF PANCREATIC ISLETS IN MOST OF THE TISSUE SECTIONS WITH NUMEROUS CELLS DEMONSTRATING PYKNOTIC NUCLEI. THE DASHED ARROW SHOWS IRREGULAR RECORDS OF CYTOPLASMIC VACUOLATION. (E) EVERY DAY INJECTION OF FENUGREEK SHOWED MILD PYKNOSIS WITH CYTOPLASMIC VACUOLAR CHANGES (ARROW). (ARROW) (N=4).

In contrast, untreated diabetic rats exhibited severe alterations in both the acini and islets of the pancreas. Many cell nuclei appeared thickened and enlarged, indicative of karyopyknosis and numerous vacuoles were observed **Fig. 6A and B**. In rats receiving oral fenugreek extract, the pancreas showed no signs of protection, with destructive and degenerative changes in the pancreatic islets in almost all tissue sections, with many cells demonstrating pyknotic nuclei with variable records of cytoplasmic vacuolation **Fig. 6C**. Treatment with fenugreek injections (ED and EOD) **Fig. 6D and E** showed no histological signs of improvement with changes in cellular components of pancreatic islets with numerous cells demonstrating pyknotic nuclei and cytoplasmic vacuolation.

Histological Changes in the Kidneys: Normal rats exhibited typical histological features of the renal cortex and medulla, with intact corpuscles. In contrast, diabetic rats showed extensive damage, including intraluminal cast formation, numerous degenerated tubular cells, and congested inter-tubular blood vessels **Fig. 7A and B**.

Oral fenugreek extract resulted in focal areas of degenerative vacuolar changes in the lining cells and pyknotic nuclei **Fig. 7C**. Fenugreek injections led to pronounced damage in the renal corpuscles, severe vacuolar changes in mesangial cells, and extensive necrotic changes in tubular lining cells, with most cells losing their structural details **Fig. 7D and E**.

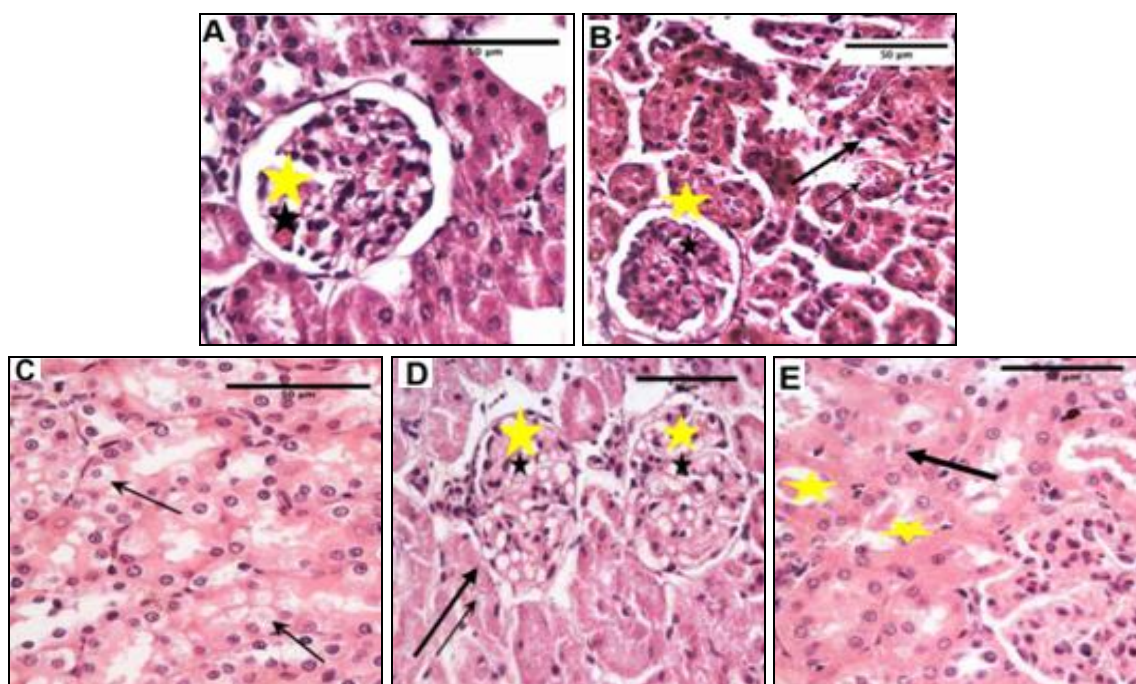


FIG. 7: HISTOLOGICAL CHANGES IN THE KIDNEYS. (A) SAMPLES DEMONSTRATED THE NORMAL HISTOLOGICAL FEATURES OF THE RENAL CORTX AND MEDULLA WITH INTACT RENAL CORPUSCLES (STAR) AND DIFFERENT TUBULAR STRUCTURES IN DIFFERENT REGIONS. (B) DIABETIC SAMPLES SHOWED WIDE AREAS WITH INTRALUMINAL CAST FORMATION IN DIFFERENT SEGMENTS OF NEPHRONS (STAR) WITH MANY DEGENERATED TUBULAR CELLS (ARROW) AND CONGESTED INTER-TUBULAR BLOOD VESSELS (ARROWHEAD). (C) ORAL FENUGREEK SAMPLES SHOWED FOCAL AREAS OF DEGENERATIVE VACUOLAR CHANGES IN LINING CELLS AND/OR PYKNOTIC NUCLEI (ARROW). (D) EVERY OTHER DAY INJECTIONS SHOWED MORE PRONOUNCED DAMAGE OF RENAL CORPUSCLES WITH SEVERE VACUOLAR CHANGES IN MESANGIAL CELLS ACCOMPANIED WITH HYPOCELLULARITY (STAR). SEVERE NECROBIOTIC CHANGES WERE RECORDED IN TUBULAR LINING CELLS. MOST OF THE CELLS LOSING THE CELLULAR DETAILS (ARROW). (E) EVERY DAY INJECTION SHOWED INTRALUMINAL EOSINOPHILIC CASTS (STAR) AND MILD NECROBIOTIC CHANGES (ARROW).

Histological Changes in the Liver: Hepatic tissue samples from normal rats displayed healthy hepatocytes, blood vessels, and sinusoids **Fig. 8A**. Untreated diabetic rats exhibited a high number of

degenerated hepatocytes with karyopyknosis, along with dilatation and congestion of blood vessels and sinusoids **Fig. 8B**. Oral fenugreek extract treatment revealed hepatocytes with shrunken pyknotic nuclei

and loss of cellular detail **Fig. 8C**. Fenugreek injections also showed degenerating hepatocytes

with shrunken pyknotic nuclei and significant loss of cellular detail **Fig. 8D and E**.

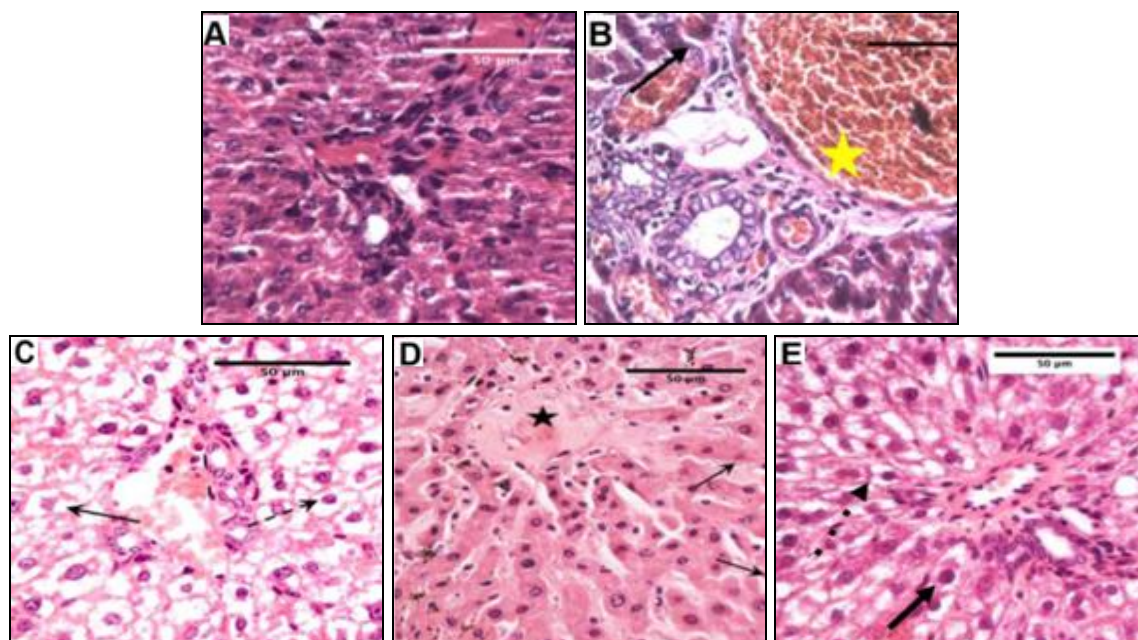


FIG. 8: HISTOLOGICAL CHANGES IN THE LIVER TISSUES. (A) CONTROL SAMPLES SHOWED APPARENT NORMAL HEPATIC TISSUE WITH NORMAL APPEARANCE OF HEPATOCYTES, BLOOD VESSELS, AND SINUSOIDS. (B) DIABETIC RATS SHOWED DILATATED AND CONGESTED BLOOD VESSELS (STAR) AND SINUSOIDS WITH HIGH NUMBER OF DEGENERATING HEPATOCYTES WITH KARYO-PYKNOSIS (ARROW). (C) ORAL FENUGREEK SAMPLES SHOWED DIFFUSE VACUOLAR DEGENERATION OF HEPATOCYTES (ARROW) WITH NUMEROUS SHRUNKEN PYKNOTIC NUCLEI (DASHED ARROW). (D) EVERY OTHER DAY TREATMENT SHOWED HEPATOCYTES WITH MANY CELLS SHOWING PYKNOTIC NUCLEI (ARROW) AND MORE EXTENSIVE CONGESTION OF THE SINUSOIDS (STAR). (E) EVERY DAY FENUGREEK INJECTION DEMONSTRATED EXTENSIVE DEGENERATION OF HEPATOCYTES (ARROW) WITH SHRUNKEN PYKNOTIC NUCLEI (DASHED ARROW).

DISCUSSION: In the current study, we utilized an STZ model to compare different routes of fenugreek administration¹⁰. STZ has been extensively used in diabetes research, particularly for evaluating various therapeutic approaches, including plant extracts for diabetes management²¹⁻²³. Fenugreek is a widely consumed plant across different regions.

Our study provides a comprehensive evaluation of fenugreek seed extract's effects on the pancreas, liver, and kidneys, both biochemically and histologically. Diabetes induction was confirmed biochemically by elevated blood glucose levels and histologically by signs of pancreatic islet destruction. We attempted to measure glycosylated hemoglobin (HbA1c), but the results were unreliable (data not shown), possibly due to challenges in measuring HbA1c in Sprague-Dawley rats, as noted by other researchers^{24, 25}. Alternative methods, such as ELISA or high-performance liquid chromatography (HPLC), have been suggested for more accurate HbA1c

estimation²⁶. Fenugreek seed extract administration reduced blood glucose levels, likely due to its high content of alkaloid trigonelline and steroidal saponins, particularly 4-hydroxyisoleucine, which is known to be insulinotropic²⁷. Our findings confirm previous reports²⁸ indicating that a dose of 50 mg/kg of orally administered fenugreek for 4 weeks in STZ diabetic rabbits produced similar effects.

The architecture of pancreatic tissue correlates with the stage and severity of diabetes²⁹. In our study, STZ injection caused significant damage to pancreatic islet cells and exocrine and endocrine components, consistent with previous findings³⁰. Fenugreek treatment appeared to protect pancreatic tissues, likely due to diosgenin's antidiabetic effects, such as β -cell regeneration and enhanced insulin secretion^{1, 31}. Diosgenin has also been reported to improve blood glucose levels and preserve pancreatic, liver, and skeletal muscle tissues¹. Previous studies³² on fenugreek oil in an Alloxan-induced diabetic rat model demonstrated

partial reversal of pancreatic cell damage and improved renal function. Oral fenugreek treatment led to a significant decrease in creatinine levels, unlike EOD and ED injections. This finding aligns with previous reports³³, showing improved renal function with fenugreek administration. Histological improvements were better with oral treatment³³, likely due to the longer treatment period. Other studies have also reported improved creatinine levels and glomerular basement membrane integrity with oral fenugreek extract, supporting its potential as a treatment for diabetes and related renal complications^{34,35}.

Recent metabolomics studies on fenugreek flavonoids have highlighted their impact on the liver, kidneys, and pancreas^{36, 37}. Fenugreek flavonoids were found to lower insulin resistance, enhance glycolysis and gluconeogenesis, and protect kidney and pancreatic islet cells.

Serum levels of liver enzymes (ALT and AST) were elevated in untreated diabetic rats but significantly reduced following fenugreek treatment, indicating liver cell protection³⁸. Fenugreek's ability to reduce liver enzyme levels confirms its protective effect, consistent with previous findings of fenugreek's beneficial impact as a daily supplement³⁹⁻⁴². Although some studies reported a protective role of fenugreek aqueous seed extract⁴³, no histological liver protection was observed.

Our study showed a significant reduction in triglyceride levels with fenugreek treatment compared to diabetic rats, though HDL levels increased significantly only after daily injections, suggesting a better effect with the daily injection route. No substantial changes in total cholesterol levels were observed, which contrasts with previous reports (44) where a higher dose (500 mg/kg) improved lipid profiles. This discrepancy may be due to the lower dose used in our study (100 mg/kg). Catalase and glutathione-S-transferase (GST) enzymes, crucial for antioxidant defense in the liver, were significantly increased in the EOD and ED injection groups compared to diabetic rats, with oral treatment showing less significance. Elevated catalase levels in all groups indicate fenugreek's potential as an antioxidant booster due to its diosgenin content^{1,31}.

Previous studies have also supported fenugreek's antioxidant effects⁴⁵. Peroxidase activity was significantly increased in the liver of untreated diabetic rats, likely due to oxidative stress from STZ-induced diabetes⁴⁶. All treated groups showed a significant reduction in peroxidase activity compared to diabetic rats. Glutathione peroxidase levels remained unchanged except in the ED injection group, where a significant increase was observed, suggesting a more effective impact in this group compared to others (EOD injection and oral).

Our study also found that total protein levels, reduced in untreated diabetic rats, were restored with fenugreek treatment. This increase in protein levels could be attributed to reduced hyperglycemic effects and glycation of antioxidant enzymes, which cause structural protein and enzyme damage due to reactive oxygen species⁴⁶.

Fenugreek may improve glucose utilization, insulin release, and possibly reduce intestinal glucose absorption⁴⁷. Oral fenugreek intake is associated with liver protection and improved quality of life for diabetic patients⁴⁸, with additional benefits as a digestive stimulant and for its antibacterial and antioxidant properties⁴⁹. In conclusion, daily fenugreek injections demonstrated superior antidiabetic effects and better serum values compared to other groups. Although improvements in antioxidant enzymes and diabetic parameters were observed across all treatment groups, histological restoration of kidney, liver, and pancreas tissues was not fully achieved, likely due to the low dose and short duration of the study (one month). Fenugreek shows promise as an anti-diabetic agent, but further research is needed to elucidate its mechanism of action and optimal dose range. Longer studies are recommended to achieve histological improvements.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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How to cite this article:

Mane K, Patil R and Burungale S: Effect of fenugreek supplementation in diabetes. Int J Pharmacognosy 2024; 11(10): 525-35. doi link: [http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.11\(10\).525-35](http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.11(10).525-35).

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