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Hepatoprotective Effects of Trigonella foenum-graecum Seed Extract Against Streptozotocin-Induced Diabetic Liver Damage in Albino Rats

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ABSTRACT

Diabetes mellitus is a metabolic disorder that causes severe liver dysfunction due to oxidative stress and abnormal glucose metabolism. The present study aimed to evaluate the protective effect of Trigonella frenum-graecum (fenugreek) seed extracts prepared using ethanol, methanol, and acetone on liver damage induced by streptozotocin (STZ) in albino rats. Although many synthetic antidiabetic drugs are available, their long-term use leads to side effects, highlighting the need for safer herbal alternatives — this forms the research gap of the present study. Diabetes was induced in rats by STZ (50 mg/kg i.e.), and the animals were treated orally with different solvent extracts of fenugreek seeds for 24 days. The parameters studied included blood glucose, liver function markers (ALT, AST, ALP, and bilirubin), and oxidative stress indicators (MDA, SOD, CAT, and GSH). Treatment with fenugreek extracts significantly reduced blood glucose and liver enzyme levels while improving antioxidant status, with the ethanolic extract showing the most pronounced effect. Histopathological examination confirmed restoration of normal liver architecture. The study concludes that fenugreek seed extract possesses hepatoprotective and antidiabetic potential. Prospects include isolation of active compounds, dose optimization, and molecular studies to understand the mechanism of action

Keywords: *Trigonella foenum-graecum, Diabetic liver damage, Oxidative stress, histopathology, Diabetes.*

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1. INTRODUCTION

Diabetes is a prevalent global condition, affecting approximately 463 million individuals globally and 34.2million Americans. The number of people living with diabetes is estimated to rise by approximately 25% by2030. Diabetes is a chronic metabolic disease characterized by persistently elevated blood glucose levels. Over time, persistently high blood glucose levels can damage multiple organs and tissues, leading to significant health complications. Multiple health hazards are associated with diabetes, including an increased risk of cardiovascular disease, neurological damage, kidney disease, ocular damage, poor circulation, and immunological system impairment. Furthermore, diabetes impairs the body's wound healing ability, resulting in delayed healing and an increased risk of infection. Even small, minor wounds or injuries can become serious health risks for individuals with diabetes, with approximately 19%–34%potentially developing complications such as chronic wounds. These wounds, commonly referred to as diabetic foot ulcers, can lead to critical

complications such as amputation if not adequately treated. Consequently, it is imperative to intervene in the progression of chronic wounds in patients with diabetes to promote healing. [1] A multicentre epidemiological study performed by the Indian Council of Medical Research (ICMR) in the early 1970s reported that the prevalence of diabetes in the urban and rural populations ≥14 years of age was 2.3% and 1.5%, respectively.6 Since then, over the period 1971–2000, studies from different parts of India have reported a 10-fold increase in the incidence of diabetes in the urban area (from 1.2% in 1971 to 12.1% in 2000).6–24 Although WHO criteria for the diagnosis and classification of diabetes25 had been in use since 1980, many of these reports are not strictly comparable owing to variations in sample selection, diagnostic criteria, and differences in the age of the people screened.[2]Diabetes mellitus (DM) is a group of metabolic disorders in which the blood sugar is higher than the normal level, either because the production of insulin is not enough (type 1 DM) or the cells do not properly respond to the insulin (type 2 DM). [3]

Diabetes is a chronic metabolic disorder that continues to present a major worldwide health problem. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycaemia and disturbances of carbohydrate, lipid, and protein metabolism. As a consequence of the metabolic derangements in diabetes, various complications develop, including both macro- and micro-vascular dysfunctions [4]. Diabetes is a chronic illness characterized by elevated levels of blood glucose, accompanied by disturbed metabolism of fats and proteins. Blood glucose rises because it cannot be metabolized in the cells, due to a lack of insulin production by the pancreas or the inability of the cells to effectively use the insulin that is being produced. There are three major types of diabetes: (a)Type 1, in which the pancreas does not produce insulin; (b) type 2 in which the body cells are resistant to the action of insulin that is being produced and over time the production of insulin progressively decreases; and (c) gestational diabetes which occurs in pregnancy and can cause some complications during the pregnancy, and at birth and increases the risk of type 2 diabetes in the mother and obesity in the offspring. In addition, there are two other categories of glucose intolerance - impaired fasting glucose (IFG) and impaired fasting glycemia (IGT) that are intermediate conditions between normal and diabetic blood glucose levels, although the transition is not inevitable. People with IFG and IGT are at increased risk of CVD than people with normal blood glucose values.[5]

2. CLASSIFICATION OF DIABETES MELLITUS:

The classification of various forms of diabetes is schematically presented, based on the recommendation of the American Diabetes Association (ADA), which broadly classifies patients with DM into the following three categories.

Type 1 Diabetes Mellitus (T1DM):

It is an autoimmune disorder in which the β-cells of the pancreas are destroyed by the antibodies produced by the host, resulting in absolute insulin deficiency with undetectable plasma C-peptide concentrations. T1DM is popularly known as “Juvenile Onset Diabetes Mellitus” or “Insulin Dependent Diabetes Mellitus.” It is predominantly observed in individuals with genetic defects who require an external source of insulin. Islet cell autoantibodies, autoantibodies to glutamic acid decarboxylase (GAD), insulin, tyrosine phosphatases IA-2 and IA-2b, and zinc transporter ZnT8 are the serum autoimmune biomarkers in.

Type 2 Diabetes Mellitus (T2DM):

It is a metabolic disorder primarily caused by a combination of two factors, such as impaired insulin secretion by pancreatic β-cells and progressive failure of insulin-sensitive tissues to respond to insulin. T2DM is also popularly known as “Maturity onset DM” or “Non-Insulin Dependent Diabetes Mellitus.”

Gestational Diabetes Mellitus (GDM):

It is a multifactorial condition generally observed around 20–24weeks of pregnancy in ~10% of women due to glucose intolerance, in which ~50% of women have a higher risk of developing T2DM later due to the insulin resistance triggered by the placental hormones, such as placental lactogen and placental growth hormone (Sweeting et al. 2024).[6]

Diagnosis and Epidemiology of T1DM:

Although type 1 diabetes can be diagnosed at any age, it is one of the most common chronic diseases of childhood. Peaks in presentation occur between the years of age and at or near puberty. Whereas most autoimmune disorders disproportionately affect women, type 1 diabetes is slightly more common in boys and men. The incidence of type 1 diabetes varies with seasonal changes and birth month. More cases are diagnosed in autumn and winter, and being born in the spring is associated with a higher chance of having type 1 diabetes. Development of type 1 diabetes-associated autoimmunity (i.e., formation of islet autoantibodies) in the months or years before onset of symptomatic type 1 diabetes also shows some seasonal synchronisation. These concepts support the theoretical role for an environmental agent initiating or driving the pathogenic processes in type 1 diabetes.[7] The DAISY (diabetes autoimmunity study in the young study in Denver, Colorado, followed newborns from birth and to date has found no evidence that bovine milk ingestion, enteroviral infection, or vaccination contributes to the risk of diabetes; nevertheless, reports about the first two environmental factors have been conflicting.11 Recent reports (including from the DAISY study) that suggest that early ingestion of cereal or

gluten increases risk of type 1 diabetes need to be confirmed (see fig A on bmj.com). The reason why the risk of islet autoimmunity is increased by exposure to cereal or gluten is not entirely clear and may result from a mechanism involving an aberrant immune response to cereal antigens in an immature gut immune system in susceptible individuals. Interestingly, several case reports exist of patients that induce interferon can generate insulitis (selective cell destruction) and diabetes in animal models, strengthening the link between the induction of diabetes and interferon. Interferon has therefore been implicated as an important cytokine linking viruses and the initiation of type 1 diabetes, and neutralising this cytokine may potentially prevent the disease.[8] A diagnosis of diabetes is based on a fasting blood glucose concentration above 7·0 mmol/L (126 mg/dL), a random blood glucose concentration above 11·1 mmol/L (200 mg/dL) with symptoms, or an abnormal result from an oral glucose tolerance test.5 In the absence of symptoms, abnormal glycaemia must be present on two different occasions. A diagnosis of diabetes can also be made based on a glycated haemoglobin (HbA1c) concentration above 48 mmol/mol (6·5%). However, since dysglycaemia progression can be rapid in patients with type 1 diabetes, HbA1c is less sensitive for diagnosis than fasting or stimulated blood glucose measurements. [9] The hemoglobin A1c test—also known as glycated hemoglobin, glycosylated hemoglobin, HbA1c, or simply A1c—is used to measure an individual's glucose control levels. The test shows average blood sugar levels over the past 90 days, expressed as a percentage. In addition, it can be used to diagnose diabetes mellitus. Haemoglobin is a protein found exclusively in red blood cells, giving blood its bright red colour. The primary role of haemoglobin is to carry oxygen from the lungs to all the cells in the body. Haemoglobin becomes glycated or coated with glucose from the bloodstream. As blood glucose levels increase, more glucose attaches to the haemoglobin protein, resulting in a higher A1c value. Since red blood cells have an average lifespan of about 3 months, the A1c test measures haemoglobin levels in the bloodstream over this period, making it a reliable indicator of blood sugar control. [10]

Diagnosis and Epidemiology of T2DM:

The global rising tide of obesity, physical inactivity, and energy-dense diets has resulted in an unprecedented increase in the number of patients with type 2 diabetes. In 2015, 415 million people were estimated to have diabetes, more than 90% of whom had type 2 diabetes, with a projected increase to 642 million by 2040.15Incidence and prevalence of type 2 diabetes vary according to geographical region, with more than 80% of patients living in low-to-middle-income countries, but the overall trend is an increase in diabetes prevalence in every country since 1980.1 An additional 318 million people have a preclinical state of impaired glucose regulation,15 but intensive lifestyle modify cation, pharmacotherapy, or both can reverse or delay development of type 2 diabetes. [11].The diagnosis of type 2 DM is made when the patient meets one of the following criteria: glycated haemoglobin (HbA1C) ≥ 6.5%, fasting blood glucose ≥ 126 mg/dL, or 2-h post-prandial glucose ≥ 200 mg/dL. Diabetes-related morbidity and complications can be substantially reduced with tight glycaemic control, aiming for an HbA1c of less than 7%. According to the International Diabetes Federation (IDF), 415 million adults aged 20–79 years were diagnosed with DM in 2015, and the number of people suffering from diabetes in that group rose to about 573 million adults in 2021. [12] While the need for screening programs for T2D is clear, there is ongoing debate about the optimal sequence of diagnostic tests for this purpose, who should be screened, and the frequency of tests. Fasting plasma glucose (FPG), 2-h oral glucose tolerance test (OGTT), and haemoglobin A1c (HbA1c) have been the most common tests used in screening programs. However, recently, the IDF issued a Position Statement recommending the incorporation of the 1-h OGTT (1 OGTT) in the diagnostic criteria for T2D and intermediate hyperglycaemia [4]. The 21st IDF Atlas reports that Brazil ranks as the 6th country globally in the number of patients with diabetes between 20 and 79 years of age and the 8th in the number of undiagnosed diabetes cases. In addition, Brazil's screening strategies is crucial in our population as a significant public health measure. In this Position Statement, the Brazilian Diabetes Society (Sociedade1 Brasileira de Diabetes [SBD]) suggests a screening algorithm for T2D[13]

Diagnosis and Epidemiology of GDM:

Gestational diabetes mellitus (GDM) is now one of the most common pregnancy complications and is defined as glucose intolerance of variable degrees that is first detected during pregnancy and gradually returns to normal blood glucose concentrations after delivery. The International Diabetes Federation in 2021 reported that 21.1 million women (20–49 years) developed hyperglycaemia in pregnancy, 80.3% of which were due to GDM. GDM is associated with an increased risk of perinatal mortality and morbidity, including unexpected complications and outcomes for both mothers and neonates during pregnancy, childbirth, and postpartum [14] The documented prevalence of consideration of variations due to these factors, a review of the global prevalence of GDM was carried out on the basis of studies between 2005 and 2015. Using the same methods, we have expanded this review by including studies published between August 2015 and December 2018. The overall updated GDM prevalence map by WHO regions and country-specific estimates of GDM prevalence are illustrated in Figs and 2. The prevalence of GDM is highest in the Middle East and some North African countries, with a median of 15.2% (interquartile range 8.8–20.0%), followed by South-East Asia (median 15.0%; range 9.6–18.3%), the Western Pacific (median 10.3%; range 4.5–20.3%), South and Central America (median 11.2%; range 7.1–16.6%), sub-Saharan Africa (median 10.8%; range 8.5–13.1%) and North America and the Caribbean (median 7.0%; range 6.5–11.9%). The lowest GDM prevalence and widest variation in prevalence is observed in Europe (median 6.1%; range 1.8–31.0%). . By contrast, among countries in North America, GDM prevalence is relatively consistent. As few studies are available to estimate GDM prevalence in Africa and South and Central America, more studies are clearly warranted in these regions.

[15] Gestational diabetes mellitus (GDM) is defined as glucose intolerance of any degree with first recognition during pregnancy. The oral glucose tolerance test (OGTT) is the gold standard test for diagnosing GDM. Different criteria used different values for the OGTT for diagnosing GDM. OGTT, though the gold standard, is a cumbersome procedure for participants as well as health care providers. It requires the participant to be in a fasting state, requires at least 2 h for sample collections, and a minimum of two blood samples are taken. The time required and samples collected can be higher depending on the criteria followed. The World Health Organisation (WHO) in 2011, as well as the American diabetic association (ADA) has accepted HbA1c as a diagnostic tool for diagnosing diabetes mellitus. However, there are no recommendations available for the use of HbA1c as a diagnostic tool for GDM. We carried out this study to evaluate the utility of HbA1c in diagnosing GDM.[16]

3. PLANTS:

Plants have always been an excellent source of drugs, and many of the currently available drugs have been derived from them. The ethnobotanical surveys suggest that about 800 plants may possess anti-diabetic potential. Some of these herbs might reduce blood glucose levels or might be useful for the management of the disease complications. Several reports explored the anti-diabetic activity as herbs contain different types of biological components. Among these alkaloids, glycosides, galactomannan gum, polysaccharides, peptidoglycans, homoglycan, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids, and inorganic ions have demonstrated activity, including treatment of diabetes[17]

Unani traditional medicine was founded by Hippocrates (460–377 BC) and further developed by Arabian and Persian scientists in the Middle Ages; hence, it is also called “Greco-Arabian” and “Persian” medicine. Later introduced to India, it is now widely practiced in many Arabic and Asian countries and is a traditional medical practice recognized by the WHO. Medicinal plants in Unani traditional medicine include Acacia arabica (bark), Allium sativum (roots), Azedarach indica (leaves), Centella asiatica (leaves), Cinnamomum verum (leaves, bark), Curcuma longa (roots), Lantana camara (leaves), Musa paradisiaca (leaves, fruits), Trigonella foenum-graecum (leaves, seeds), Withania somnifera (roots), Zingiber officinale (roots)[18]

Trigonella foenum-graecum:

Fig1. Trigonella foenum-graecum plant.

Table1. Plant botanical Name

Kingdom	**Plantae**
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae

Genus	Trigonella
Species	T. foenum-graecum
Binomial name	Trigonella foenum-graecum L.

Plant-derived natural products have long-standing utility in treating degenerative diseases. It is estimated that about two-thirds of the world's population depends on traditional medicine for primary medical needs. Fenugreek (Trigonella foenum-graecum Linn.), a short-living annual medicinal plant belonging to the Fabaceae family, is used extensively in various parts of the world as an herb, food, spice, and traditional medicine. Fenugreek is considered as one of the oldest medicinal plants, and its health-promoting effects have been cited in Ayurveda and traditional Chinese medicine. The investigations into the chemical composition and pharmacological actions have seen a renaissance in recent years. Extensive preclinical and clinical research have outlined the pharmaceutical uses of fenugreek as antidiabetic, antihyperlipidemic, antiobesity, anticancer, anti-inflammatory, antioxidant, antifungal, antibacterial, galactogogue, and for miscellaneous pharmacological effects, including improving women's health. The pharmacological actions of fenugreek are attributed to a diverse array of phytoconstituents. The phytochemical analysis reveals the presence of steroids, alkaloids, saponins, polyphenols, flavonoids, lipids, carbohydrates, amino acids, and hydrocarbons..[19]

Merits of fenugreek seed consumption:

Fenugreek (Trigonella foenum-graecum). Soluble fibre in fenugreek seeds can slow down the absorption of glucose and lower a postprandial blood sugar spike. Furthermore, research indicated that fenugreek can stimulate insulin secretion, it also aids in glucose homeostasis in the body (Haxhiraj et al., 2024). Good glycaemic control also comes from the high fibre content, especially in type 2 diabetes.[20] These studies highlight fenugreek's antioxidant and beta-cell protective properties, which make fenugreek's potential as both an alternative to conventional therapies and a source for novel drug discovery, and both clinical and preclinical data strongly support fenugreek as an effective antidiabetic agent. Overall, strong evidence of the antidiabetic effect of fenugreek was found, but the underlying molecular mechanism of fenugreek extracts or isolated components needs more investigation. Furthermore, the optimal dose and treatment duration in clinical trials must be established in order to get a beneficial outcome from fenugreek.

Demerits of fenugreek seed consumption:

Side effects of fenugreek seed consumption. Fenugreek belongs to the Fabaceae family, which includes peanuts, chickpeas, soybeans, and green peas. Individuals with disinclinations to these shops may be at an increased risk of passing an antipathetic response to fenugreek. Symptoms may include itching, swelling, hives, and difficulty breathing. Due to its capability to lower blood sugar levels, fenugreek could interact with diabetes medications, similar to insulin or metformin, potentially causing hypoglycaemia (low blood sugar). People with diabetes should consult their healthcare provider before using fenugreek and carefully monitor their blood sugar levels. Fenugreek may also cause blood-thinning effects, which could raise the threat of bleeding, particularly in individuals with bleeding disorders or those taking anticoagulant medications like warfarin or aspirin. To minimize the threat of inordinate bleeding, it's advised to discontinue fenugreek use at least two weeks previous to any listed surgery. The safety of fenugreek supplements in children has not been adequately studied. While small quantities used in cuisine are generally considered safe, the goods of larger boluses set up in supplements remain unknown. It's stylish to consult with a paediatrician before administering fenugreek supplements to children. In addition to its implicit relations with diabetes specifics and blood thinners, fenugreek may interact with other medicines and supplements. It's essential to inform healthcare providers about all specifics and supplements being taken before starting fenugreek to help establish implicit relations.

4. METHODS AND MATERIALS:

Adult male albino rats (180–220 gm..) were obtained from NIMS University, Rajasthan, and housed under standard conditions. Diabetes was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg). Fenugreek seed extract, prepared by aqueous extraction, was administered orally (100–400 mg/kg) for 42 days. Blood glucose, liver enzymes, and oxidative stress markers were analysed, and liver tissues were examined histologically to assess the hepatoprotective effect of fenugreek against diabetic liver damage. Statistical analysis was performed using one-way ANOVA, with $p < 0.05$ considered significant.

Experimental design:

Twenty-four adult male albino rats weighing 180–220 g.)They were divided into four groups of 6 each. Group I represented control, group II diabetic rats, group III diabetic rats treated with 0.1mg of fenugreek seed aqueous extract, and group IV treated with 0.1mg of fenugreek extract, which dose equivalent to the Albino rat therapeutic dose daily for 6 weeks.

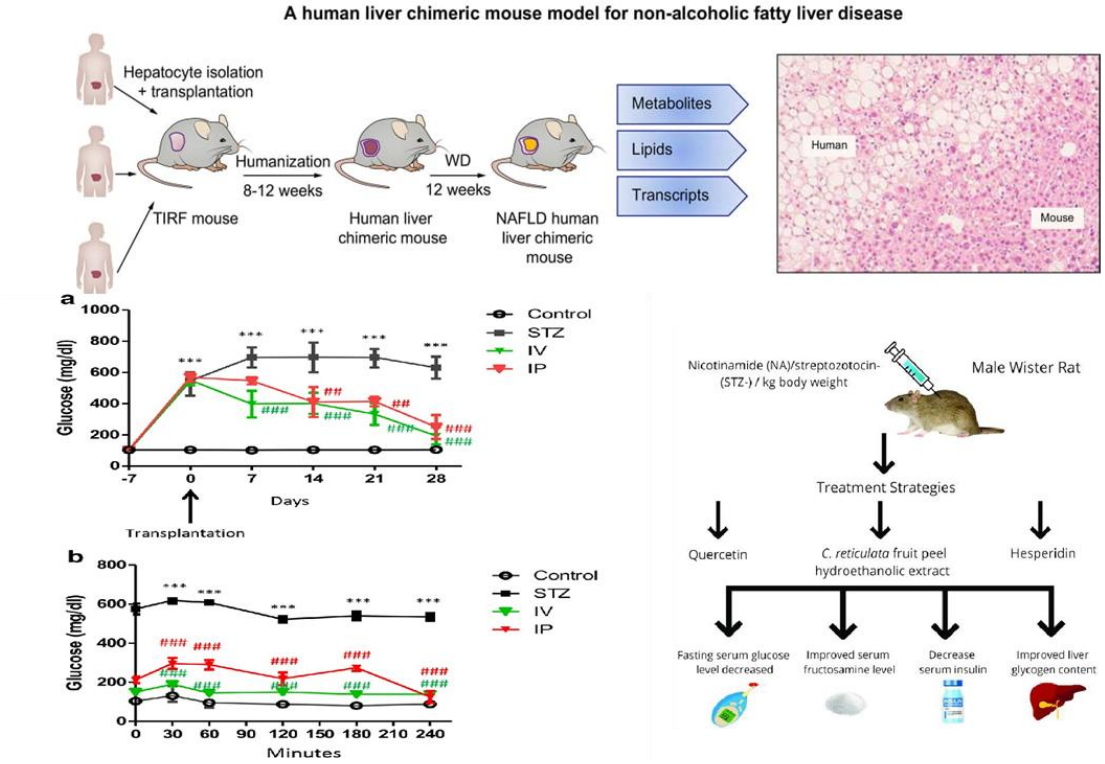

Fig2.A human liver chimeric mouse model for non-alcoholic fatty liver disease and Glucose Test

Body weight analysis graph

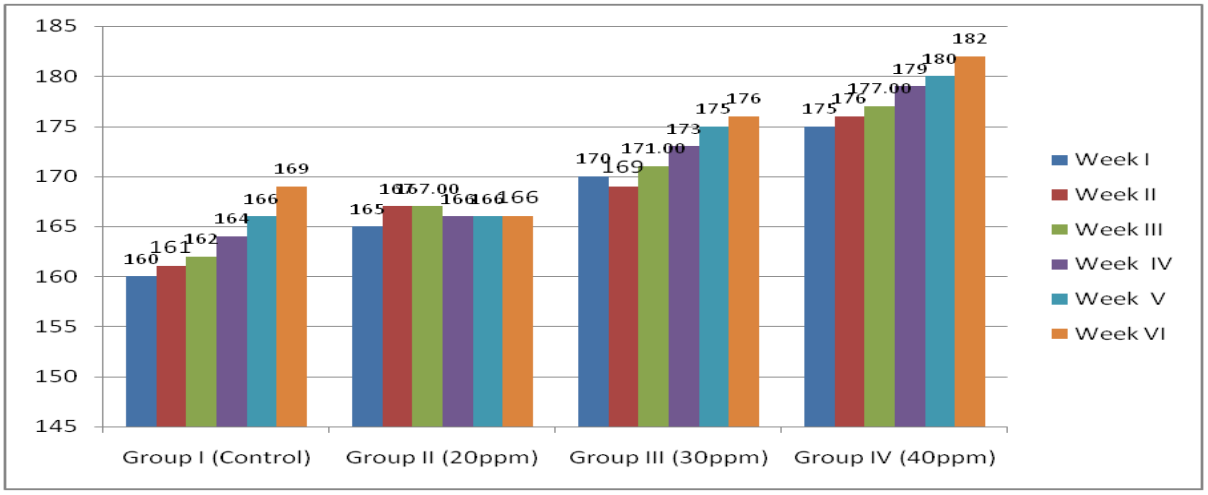

Fig3. VII Body weight analysis graph

Chemical Composition of Fenugreek Seed Extract (FE): The phenolic composition of FE originating from Egypt (Fayoum Government) has been investigated. Its FT-IR spectra showed strong absorption bands for alcoholic O-H, carbonyl groups, as well as for olefinic, CH-aliphatic and aromatic, and S-S bonds. Preparation of plant extracts. Dried fenugreek seeds were purchased from a supermarket—Nilgiris, Puducherry, India. The fenugreek seeds were made into powder and defatted using petroleum ether and then subjected to methanolic extraction. About 100 g of fenugreek seed powder (FSP) was mixed with 80 % methanol (1:5) and kept at room temperature for 5 days. After 5 days, it was filtered, and the residue was re-extracted twice under the same conditions to ensure complete extraction, and the combined solvent was evaporated by rotary evaporator to get the residue. The residue was lyophilized and stored at −70 °C, and later this was used for in vitro and in vivo studies.

Extract preparation for biological experiments:

Seeds of Fenugreek were cleaned and dried at 40°C, and mechanically dissolved in a ratio of ethanol 80% (1:10) in a Soxhlet for 72 h. Filtration was done on the mixture using a Büncher funnel with filter paper in and then concentrated until

it was dry using a rotary evaporator.. The experimental animal was then given the extract orally after it had been diluted in distilled water to treat hyperglycaemia.

Diabetes Induction:

Chemical used: Streptozotocin (STZ)

Dose: 50 mg/kg body weight

Route: Intraperitoneal injection (i.e.)

Preparation: Dissolve freshly in 0.1 M citrate buffer (pH 4.5)

Fasting: Rats fast overnight before injection

Confirmation: After 72 hours, check fasting blood glucose — rats with glucose >200 mg/dL are considered diabetic.

Grouping and Treatment (6 Weeks)

Table2.Grouping and Treatment (6 Weeks)

Group	Treatment	Description	Dose and Route	Duration
Group I	Control	Normal rats, given distilled water only	Oral 2 ml/kg	6 weeks
Group II	Diabetic + 20 ppm extract	Diabetic rat treated with fenugreek seed extract (20 ppm)	Oral (gavage) daily	6 weeks
Group III	Diabetic + 30 ppm extract	Diabetic rat treated with fenugreek seed extract (30 ppm)	Oral (gavage) daily	6 weeks
Group IV	Diabetic + 40 ppm extract	Diabetic rat treated with fenugreek seed extract (40 ppm)	Oral (gavage) daily	6 weeks

Group I (Control): Normal rats (no STZ, no extract)

Group II (20 ppm): Diabetic rats treated with 20 ppm fenugreek extract

Group III (30 ppm): Diabetic rats treated with 30 ppm fenugreek extract

Group IV (40 ppm): Diabetic rats treated with 40 ppm fenugreek extract

Parameters to Measure at the end of 6 weeks

Blood glucose levels

Liver enzymes (ALT, AST, ALP)

Histopathological changes in liver tissue

Body weight changes

Antioxidant markers (SOD, CAT, GPx, MDA)

Groups:

Group I (Control): Normal rats (no STZ, no extract)

Group II (20 ppm): Diabetic rats treated with 20 ppm fenugreek extract for 60 days

Group III (30 ppm): Diabetic rats treated with 30 ppm fenugreek extract for 60 days

Group IV (40 ppm): Diabetic rats treated with 40 ppm fenugreek extract for 60 days

Table3.Grouping and Treatment albino rat

Group / Weeks	Week I	Week II	Week III	Week IV	Week V	Week VI
Control (Group I)	160	161	162	164	166	169
20 ppm (Group II)	165	167	167	166	166	166
30 ppm (Group III)	170	169	171	173	175	176
40 ppm (Group IV)	175	176	171	179	180	182

Phytochemical test

Fig4. Trigonella foenum graecum seed and seed powder

We conducted a phytochemical screening on both the seed extract and oil extract derived from fenugreek seeds. Wagner's test was performed to ascertain the presence of alkaloids. Separate seed and oil extract samples (2–3 mL) were introduced into individual test tubes. Subsequently, the seed tincture was supplemented with 1 mL of HCl and several drops of Wagner's reagent. Vigorous agitation of the test tube resulted in the appearance of a reddish-brown hue, which served as an indicative marker of the alkaloid presence. The foam test differentiated saponins within the seed and oil extract solution. Equal volumes of seed and oil extract (5 mL) were combined with purified water (5 mL) and subjected to vigorous shaking. A stable foam formation was observed, indicating

The presence of saponins.

Table4.Phytochemical analysis of Trigonella foenum-graecum in different solvents

No.	Phytochemicals	Distilled Water	Methanol	Acetone	Ethanol
1	Tannins	Positive	Positive	Positive	Positive
2	Anthraquinones	Negative	Positive	Positive	Positive
3	Flavonoids	Positive	Positive	Positive	Positive

4	Alkaloids	Positive	Positive	Positive	Positive
5	Terpenoids	Positive	Positive	Positive	Positive
6	Saponins	Positive	Positive	Positive	Positive
7	Cardiac glycosides	Positive	Positive	Positive	Positive
8	Glycosides	Positive	Negative	Positive	Positive
9	Reducing sugars	Positive	Positive	Positive	Positive
10	Plantains	Positive	Positive	Positive	Positive
11	Steroids	Positive	Positive	Positive	Positive
12	Phenolic	Positive	Positive	Positive	Positive
13	Amino acids	Positive	Positive	Positive	Positive
14	Proteins	Positive	Positive	Positive	Positive
15	Quinones	Positive	Positive	Positive	Positive

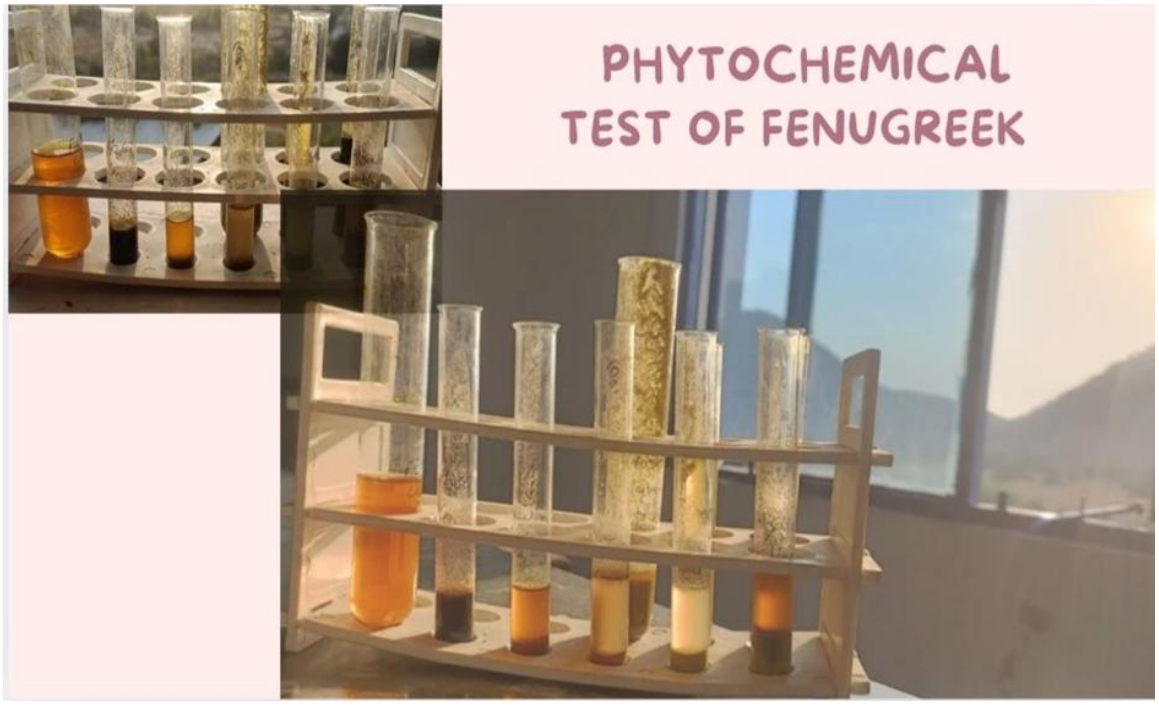

fig5. Different phytochemical tests of Trigonella foenum-graecum

Qualitative Phytochemical Analysis

The extracts were tested for the presence of bioactive compounds by using standard methods

Flavonoids: Flavonoid extract mixed with a few fragments of magnesium turnings. Concentrated HCl was added dropwise. The appearance of pink scarlet colour after a few minutes indicates the presence of flavonoids.

Phenols and Tannins: The sample was mixed with 2ml of 2% solution of FeCl3. A blue-green or black coloration indicates the presence of phenols and tannins.

Saponins: 5ml of distilled water is mixed with the extract in a test tube, shaken vigorously. The formation of stable foam is taken as an indication of the presence of saponins.

Alkaloids: Alkaloids 2ml of 1% HCl mixed with crude extract and heated gently. Mayer's and Wagner's reagents were added to the mixture. Turbidity of the resulting precipitate is taken as evidence for the presence of alkaloids.[

Steroids: A reddish brown ring at the interface was observed only with the extract of S. chirata out of six screened plants, indicating the presence of steroids only in this plant.

Phlobatannins: The Presence of a red precipitate. Sativus root juice only was taken as evidence for the presence of phlobatannins in this.

Carbohydrates: The red violet ring that appeared at the junction in most of the extracts was confirmed by the presence of carbohydrates except P. dactylifera and R. sativus.

Glycosides: Similarly, a colour change from violet to blue to green, confirming the presence of glycosides, was also observed in all other extracts except P. dactylifera and R. sativus.

Coumarins: Interestingly, the formation of yellow colour as an indication of coumarin presence was also found only in those four extracts, which showed the presence of carbohydrates and glycosides. The results were again negative for P. dactylifera and R. sativus, indicating thereby the absence of coumarins in their extracts.

Alkaloids: A yellow precipitate was observed in three extracts, thereby confirming the presence of alkaloids. Surprisingly, this time, F. religiosa was also devoid of alkaloids in addition to P. dactylifera and R. sativus.

Proteins: White precipitate formation, which turns yellow on boiling, was only observed in the extract of S. chirata and F. religiosa, thereby showing the presence of proteins and confirming the absence of proteins in the rest of the extracts.

Emodin's: Absence of red colour indicated the absence of emodin in all six extracts.

Anthraquinones: Absence of a pink, violet, or red coloration in the ammoniacal layer indicated the absence of free anthraquinones in all six extracts.

Anthocyanins: The absence of pink-red to blue-violet coloration indicated the absence of anthocyanins in all six extracts.

Leucoanthocyanins: Absence of red colour in the organic layer indicated the absence of leucoanthocyanins in all six extracts.

Phytochemical Analysis

Crude ethanol extract of T. foenum-graecum seeds was subjected to analyse for checking the occurrence and existence of alkaloid, steroid, flavonoid, carbohydrate, glycoside and glucosides in it.

Biochemical Analysis

Glucose levels were measured as described by Trinder (1969). Insulin was determined according to. Glycated haemoglobin (HbA1c) was determined according to Sudhakar and Pattabiraman. Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL-C) were measured using methods. respectively. Low-density lipoprotein (LDL-C) and very low-density lipoprotein (VLDL-C) were calculated as mentioned by Friedewald et al. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assessed as explained. Serum albumin was calorimetrically determined by the method of Doumas et al. Serum uric acid was measured according to Fassati et al. (1980). Creatinine was detected as described by Young (2001). The antioxidant enzymes Superoxide Dismutase (SOD), Catalase (CAT), and Glutathione Peroxidase (GPx) were estimated using the methods outlined, respectively. Serum tumour necrosis factor-alpha (TNF-α) was measured using the method described. The serum interleukin-1 beta (IL-1β) level was assessed with the ELISA Kit. The determination of C-reactive protein (CRP)concentration in a serum sample.

5. BIOCHEMICAL ASSAYS

The glucose oxidase procedure was followed for fasting plasma glucose assay, and a formerly pronounced ELISA procedure was followed for plasma insulin estimation (Manita et al. 2023). Determination of HbA1c has followed a known procedure. The serum lipid profile, serum liver functions (AST, ALT, and albumin), blood urea, and serum creatinine were investigated following reported standard techniques.

6. DISCUSSION

Targeted organs

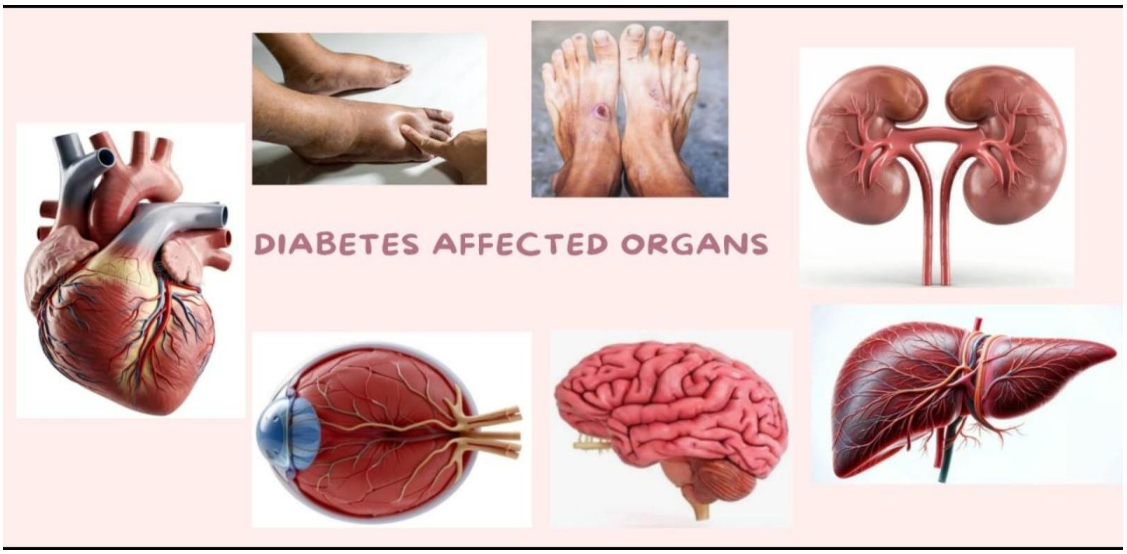

Fig6.Targeted organs in Diabetes Mellitus II

The presence of diabetes mellitus (DM) increases the risk of any form of cardiovascular disease (CVD) and of death in hypertensive patients. In the natural course of arterial hypertension (HT) it has been seen that the development of type 2 DM during treatment multiplies the risk of cardiovascular complications over the long term. In patients with dry eye accompanied by type 2 diabetes, lid margin abnormalities, orifice plugging, and tear film instability were more severe compared to those with dry eyes alone. Chronic inflammation and MGD play a crucial role in the pathogenesis of DED in DM patients. Alzheimer's disease (AD) is a progressive neurodegenerative disorder with multifaceted risk factors, including diet and metabolic dysfunction. The rising prevalence of AD and diabetes has drawn attention to their shared pathophysiological mechanisms. The "cafeteria diet," characterized by high-fat, high-sugar, and energy-dense foods, has emerged as a significant contributor to metabolic dysfunctions, including obesity and insulin resistance, which are risk factors for both diabetes and neurodegenerative diseases. This study explores the effects of the cafeteria diet on cognitive impairment, AD pathology, and its potential role in exacerbating diabetes-related neurological complications. Chronic kidney disease (CKD) is a prevalent and progressive condition affecting over 850 million individuals worldwide. In 2017, the global prevalence of CKD was estimated at 9.1%, and CKD accounts for approximately 1.2 million deaths and >2.5 million people receiving kidney replacement therapy each year. The incidence of CKD is rising faster than most other chronic diseases, which is expected to become the fifth-leading

cause of death globally by 2040.CVD is a leading cause of morbidity and mortality among people with a history of diabetes-related foot ulceration (DFU). Whilst diabetes mellitus is associated with elevated cardiovascular risk, studies have indicated that people with DFU face an even greater susceptibility to major adverse cardiovascular events (MACE). with type 2 diabetes mellitus, peripheral neuropathy, and prior left forefoot amputation secondary to osteomyelitis presented to the emergency department for right foot swelling. Lower limb enema may be related to liver dysfunction and hypoalbuminemia. A decline in serum albumin causes reduced oncotic pressure and facilitates fluid extravasation into the interstitial space, resulting in peripheral enema. This patient had mild hypoalbuminemia of 30 g/L at presentation, serving as a plausible contributing mechanism of symptoms. However, the absence of other clinical findings, such as ascites, abdominal mass, or peripheral signs of chronic liver disease, delayed the clinical suspicion of a hepatic cause. A wide spectrum of clinical presentations has made establishing an HCC diagnosis challenging. The liver and pancreas are known to secrete growth factors, exocrine and endocrine hormones resp. which is implicated in glucose metabolism. Numerous studies have shown the positive involvement of hepatocyte growth factor (HGF) in managing hyperglycaemia. Viral or transgenic overexpression of HGF in rat or murine beta cells leads to an increase in the quantity of engrafted beta cells and its proliferation. In vivo studies have confirmed that HGF upregulates GLUT-2, insulin, and glucokinase gene expression in beta cells. As a result, HGF-overexpressing islets identify glucose and secrete insulin in a manner superior to normal islets18. Moreover, SCID mouse and Edmonton rat models showed enhanced transplant performance and reduced requirement of the number of islets for successful islet transplantation with adenoviral delivery of HGF to their islets. Also, HGF showed decreased streptozotocin-induced beta cell death in RIP-HGF mice. There is improved graft survival with the adenoviral-mediated HGF transfer into normal mouse islets.

Liver

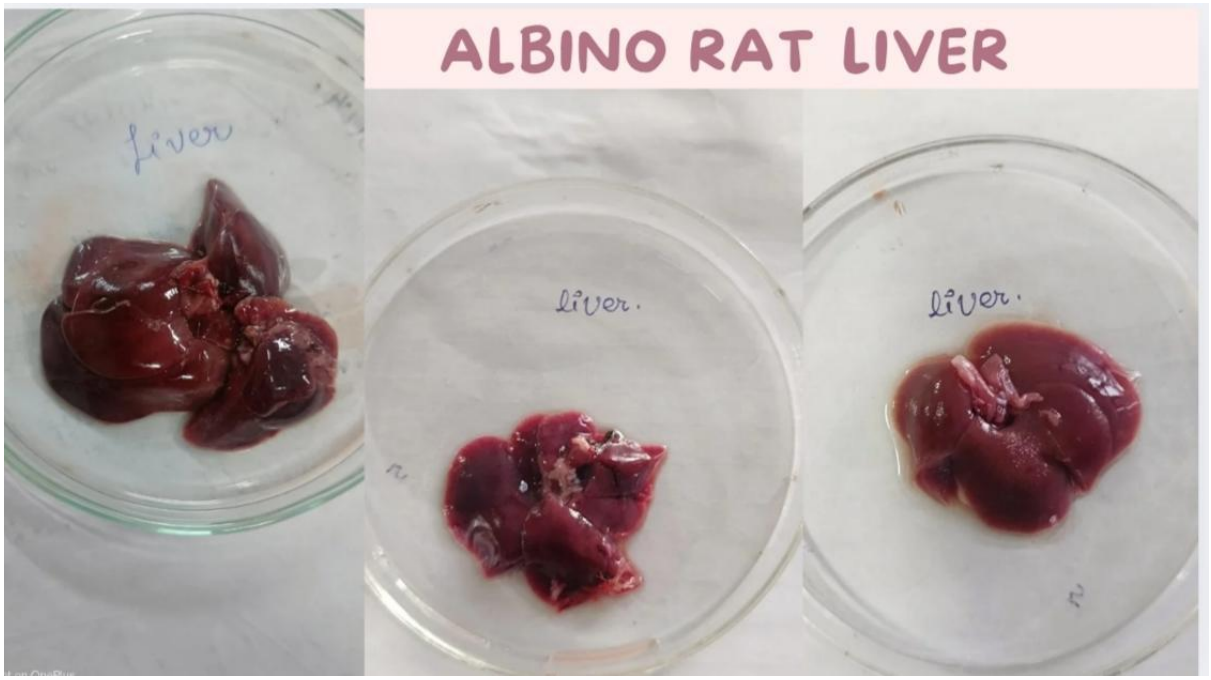

Fig7. Liver of Rattus Norvegicus

The liver is the largest internal organ, providing essential metabolic, exocrine, and endocrine functions. These include the production of bile, the metabolism of dietary compounds, detoxification, regulation of glucose levels through glycogen storage, and control of blood homeostasis by secretion of clotting factors and serum proteins such as Albumin.[40]disease progression is characterized by the development of lipotoxic effects leading to hepatocellular damage and lobular inflammation (features of MASH, which is the main driver of progressive liver disease and which induces faster progression of liver fibrosis. The severity of liver fibrosis is measured on a five-stage scale ranging from least to most severe: F0 (absence of fibrosis), F1(perisinusoidal or portal fibrosis), F2 (perisinusoidal and portal or periportal fibrosis), F3 (septal and bridging fibrosis), and F4 (cirrhosis). Clinically significant liver fibrosis (stage ≥F2) is a strong predictor of death from any cause and liver-related complications. Liver transplantation (LT) is the most effective treatment for end-stage liver disease, and ischemia-free liver transplantation (IFLT) is a technique that can improve post-transplant prognosis for patients 8 Post-transplant diabetes mellitus (PTDM) is a common complication after LT, with studies reporting an incidence as high as 40%. While insulin has become the primary treatment for both pre-transplant and post-transplant DM, it can lead to side effects such as hypoglycaemia, allergies, adverse cardiovascular outcomes, kidney failure, and reduced quality of life. What's more, the World Health Organization (WHO) has reported that insulin use may increase the risk of chronic liver disease and HCC.10,11 Many patients on insulin therapy, including intensive insulin therapy, insulin therapy alone may not be the most suitable treatment for patients with severe DM and HCC. Therefore, there is growing interest in transplantation as an alternative approach. Liver-pancreas transplantation or organ cluster transplantation was once a focus of treatment, offering a solution for both liver disease and DM in a single surgery.

Prepare a methanolic/ethanolic/acetone extract of fenugreek seeds.

Dissolve the required concentration in distilled water to make doses of 20, 30, and 40 ppm. Administer daily using an oral feeding needle.

Blood Collection:

Fluoride and Tulsi (10 ppm/1 ml/ 40mg 60days/rat) dissolved in distilled water. Blood samples were collected after fasting on day 0 and again after the experiment using a heart puncture technique with Chloroform and IP. We collected blood samples from rats and divided them into three vials. The first vial was an EDTA vial, the second was a plain vial, and the third was a fluoride vial. The EDTA vial was used for whole blood testing, the plain vial was used for insulin testing, the fluoride vial was utilized, and for liver function tests {LFT} and renal function tests {RFT}. After centrifuging the blood samples for 15 minutes at 3000 rpm to separate the serum, the following metrics were evaluated: Glycaemic index, which includes insulin resistance, serum insulin levels, and glucose levels; ALT and AST activity were measured using ALT and AST; and direct, indirect, and total bilirubin levels were also evaluated. To assess weight gain, the animals' body weights were recorded both at the beginning of the study and later measured weekly until the study concluded.

Fig8. Light Microscope, microtome, tissue, albino rat blood collection

Result / Interpretation:

Group I (Control) showed gradual and normal improvement, indicating stable liver function.

Group II (20 ppm) exhibited slight variation, showing minimal improvement in liver condition.

Group III (30 ppm) showed a steady increase in recovery parameters, suggesting moderate hepatoprotective activity.

Group IV (40 ppm) showed the highest improvement with significant elevation in values over six weeks, indicating strong hepatoprotective and restorative effects of fenugreek extract.

Histopathological organ :Liver affected by diabetes

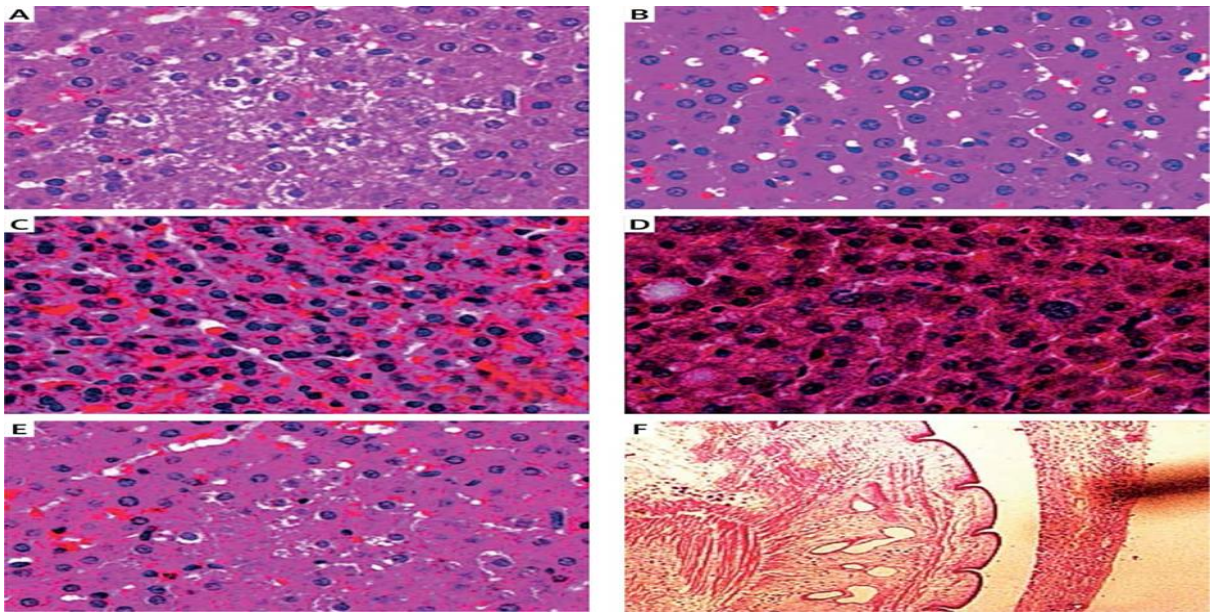

Image1. Normal liver tissue

Panel A — (medium power view of liver parenchyma)

At this magnification, look for lobular architecture and uniformity of hepatocyte plates — preserved in normal liver.

Panel B — (higher power showing individual hepatocytes)

Differentiate small preparation/section artifacts (tiny clear spaces) from true vacuoles of steatosis (which displace the nucleus).

Panel C — (parenchyma with sinusoids & erythrocytes)

Kupffer cells are expected; increased numbers would suggest activation/inflammation.

Panel D — (very high-power field of hepatocytes)

Ballooning degeneration makes cytoplasm look swollen and rarified — not present here.

Panel E — (another parenchymal field; maybe periportal or mid-lobular)

Use Oil Red O (on frozen tissue) to confirm fat; PAS-diastase for glycogen.

Panel F — (low power view of the liver capsule / portal area)

The portal triad contains the portal vein branch, hepatic artery branch, and bile duct; in chronic disease you see portal expansion and fibrosis — not seen here.

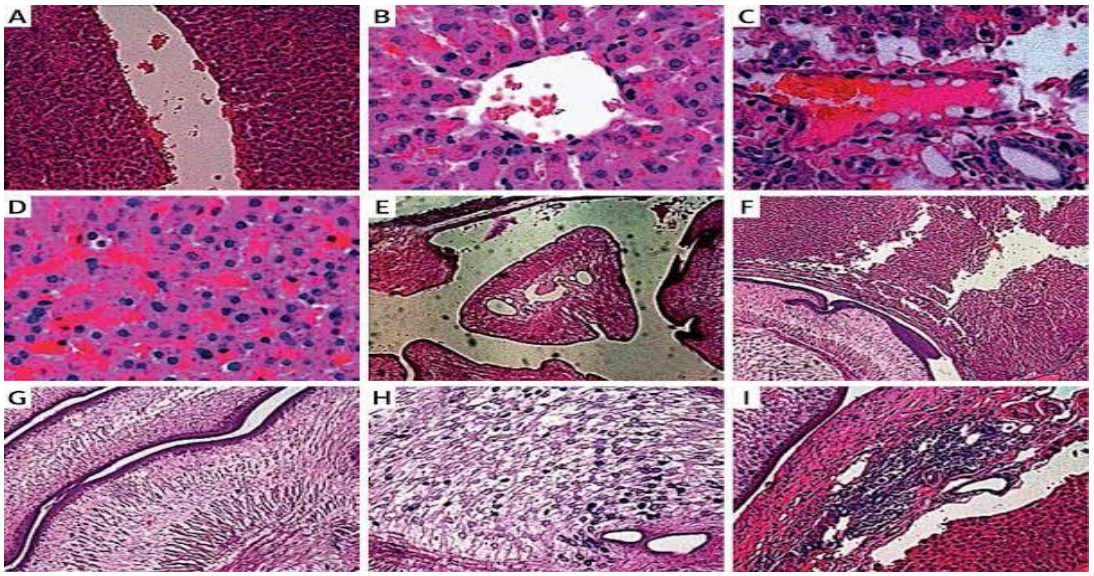

Image2.Diabetic liver tissue

Panel A

What I see: A large pale lumen structure consistent with a central vein (or large vessel); surrounding hepatocyte plates are relatively uniform, but there may be mild sinusoidal congestion (reddish material).

Meaning: Pericentral (zone 3) region — in metabolic injury/ischemia and steatosis changes often appear here first. Congestion can reflect impaired outflow or vascular congestion, often seen with fatty change and inflammation.

Panel B

What I see: A round white empty space (likely an artifact or fat vacuole or central venule) with surrounding hepatocytes showing enlarged cytoplasm and prominent nuclei. There are scattered red cells in the lumen.

Meaning: If the white space is an intracellular vacuole → macro vesicular steatosis (large fat vacuole displacing cytoplasm/nucleus). Fatty change is a hallmark of NAFLD associated with type 2 diabetes.

Panel C

What I see: A small vessel or portal tract with red blood and some surrounding inflammatory cells and perhaps congested capillaries. Possibly early haemorrhage or congested portal vessel.

Meaning: Vascular congestion and focal haemorrhage can occur with inflammation or severe sinusoidal injury. Look for portal inflammation which may indicate steatohepatitis.

Panel D

What I see: Hepatocytes with pink, swollen cytoplasm and scattered pyknotic/condensed nuclei; occasional small clear vacuoles. Numerous small dark nuclei (inflammatory cells) between hepatocytes.

Meaning: Hepatocyte ballooning (swollen pale hepatocytes) and lobular inflammation — two features of steatohepatitis (NASH). Ballooning + inflammation suggests active injury, not just simple steatosis.

Panel E

What I see: A section with different colour tones (greenish background) and islands of pink tissue separated by pale/green bands. This strongly resembles a special connective-tissue stain (ehowing fibrosis (green/blue) around portal areas or in pericellular/perisinusoidal distribution.

Meaning: Presence of fibrosis — if perisinusoidal/zone-3 it is typical for NASH-related fibrosis. Fibrosis indicates progression beyond simple steatosis.

Panel F

What I see: Lobular area showing disrupted architecture with possible fibrous bands extending into parenchyma (subcapsular/portal extension).

Meaning: More advanced fibrosis, perhaps early bridging — consistent with progression toward stage 2–3 fibrosis if confirmed.

Panel G

What I see: Concentric, organized fibrous tissue may be at the capsule or portal area with surrounding hepatocytic changes. The layered look suggests chronic reactive change.

Meaning: Chronicity — a chronic fibrotic reaction or thickening of the portal tract/capsule.

Panel H

What I see: Dense cellular infiltrate made of small, rounded nuclei (lymphocytes/macrophages) and reactive hepatocytes at the interface of portal tract and parenchyma — a ductular reaction-like or interface hepatitis pattern.

Meaning: Portal inflammation and ductular reaction are signs of chronic injury and are commonly seen with progressive NASH or other chronic liver diseases.

Panel I

What I see: Mixed picture of fibrous tissue, inflammatory cells and blood vessels — perhaps portal tract with expansion and periplasmic collagen deposition.

Meaning: Portal expansion with inflammation and fibrosis — again supports chronic progressive injury rather than simple fatty change.

7. CONCLUSION

The present investigation demonstrates that Trigonella frenum-graecum (fenugreek) seed extracts possess significant hepatoprotective and antidiabetic activity against streptozotocin-induced diabetes in albino rats. Among the three solvent extracts tested, the ethanolic extract exhibited the most potent effect in lowering blood glucose levels, restoring liver function enzymes, and enhancing antioxidant defences. The improvement in biochemical and histopathological parameters indicates that fenugreek's bioactive compounds help protect liver tissues from oxidative and metabolic damage caused by diabetes. Therefore, fenugreek seed extract may serve as a promising natural therapeutic agent for managing diabetes-induced hepatic complications and improving the liver in the early stage of liver damage, i.e., cirrhosis, but after liver fibrosis, i.e., the last stage of liver damage, transplantation is the only option.

Fenugreek seed extract exhibits a dose-dependent protective effect on the liver of STZ-induced diabetic albino rats. The methanolic, ethanolic, and acetonic extracts, particularly at 40 ppm, significantly improved liver function, indicating their potential role in diabetes management and liver protection

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