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Therapeutic Role of Trigonella Foenum- graecum L. Seeds Alone or Combined with Glimepiride in Mitigating Alloxan-Induced Hepatopathy in Diabetic Male Rats.

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ABSTRACT

High blood sugar indicates diabetes. These problems result from insulin synthesis, action, or both failing. Insulin insensitivity or beta cell malfunction may cause it. Ginger, garlic, and Fenugreek are used to treat diabetes. Diabetes, obesity, and other diseases are treated with it in the study, diabetic rats had considerable liver histology changes and combination therapy groups had mild changes. Giving Fenugreek, insulin, and Glimepiride improved liver health in diabetic rats, and using them together had a good impact on liver function. Fifty male Wistar rats were divided into five groups and type 2 diabetes was induced by feeding a high-fat unsaturated diet followed by intraperitoneal injection of Alloxan (80 mg/kg) B.W. After one month of feeding on a high-fat diet induced diabetes mellitus (DM). The aim of this study to examine the effect of water extracted of Trigonella Foeneum graecum L. seeds on glucose metabolism and liver function in diabetic rats. Results indicated that the oral administration of water extracted of Trigonella Foeneum graecum L. seeds not only improved insulin sensitivity but also contributed to the overall restoration of liver health in the affected subjects. Seeds in the reduction of blood glucose and the reduction of the pathological effect on liver and body weight as an experimental animal model. In conclusion, the current study shows increase in the body weight of rats during feeding on high fat diet for one month except group one which feeding on normal diet. To induced type 2 diabetes and we notice the rat's weight loss and high blood sugar over the last three weeks after injection intraperitoneal of rats by Alloxan to induced the DM. some potential benefits of Fenugreek use. Oral Fenugreek showed improvements in blood glucose and liver functions (AST, ALP and ALT levels) were reduced following Fenugreek treatment. Despite a significant decrease in Triglyceride levels, Fenugreek use did not significantly alter Cholesterol levels. Higher doses and longer Fenugreek treatment duration are recommended for the optimum protection of the liver.

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INTRODUCTION

Diabetes is a group of conditions marked by increased blood glucose levels resulting from abnormalities in insulin production, insulin function, or a combination of both. The principal classifications of diabetes are type 1 diabetes and type 2 diabetes [1]. Gestational

diabetes mellitus (GDM) is another type of diabetes [2,3]. Type 2 diabetes has experienced a significant increase in recent years, becoming a leading cause of mortality and morbidity. Insulin-dependent diabetes mellitus (IDDM), a prevalent condition in youngsters, results from the autoimmune death of pancreatic beta cells, leading to a cessation of insulin production [4,5]. This kind of diabetes mellitus is prevalent in youngsters and accounts for 5–10% of the overall diabetes patient population [3].

Type 2 diabetes mellitus (T2DM) is primarily characterized by inadequate insulin secretion or the desensitization of insulin receptors, which obstructs glucose absorption into cells. This type is primarily observed in 90–95% of instances Type III diabetes is commonly associated with Alzheimer's disease, with variations based on ethnicity and geographic locations [3,5].

Various therapies are being used to mitigate the complications of diabetes [6]. Alloxan-induced diabetes is a form of insulin-dependent diabetes mellitus that occurs as a result of Alloxan injection to animals [7,8]. Alloxan has been administered in single or multiple doses through different routes (intraperitoneal, intravenous, and subcutaneous), with single intraperitoneal administration apparently the most employed mode. The dosage of the drug also varies across studies, ranging between 90 and 200 mg/kg of body weight (BW), with 150 mg/kg BW being the most frequently used dosage. Animal species, route of administration, and nutritional status have been considered to play a role in determining the dose of Alloxan appropriate for induction of diabetes [9]. Many preparations are made from the leaves, twigs, and seeds of this plant, and powders from the seeds are widely used in the treatment of diabetes [10]. Chronic hyperglycemia resulting from uncontrolled diabetes leads to severe distress [11]. As a result, researchers are increasingly interested in natural plants as alternative treatments for diabetes and diabetes-associated complications [12]. This is due to the intrinsic biological activities and minor side effects of the compounds present in natural plants, which are often safer and more effective than synthetic anti diabetes compounds [13]. Despite the efficacy of insulin treatment, its original cost was prohibitively high, rendering it inaccessible to numerous individuals with diabetes [14]. Recent interest in the use of herbal extracts with anti-diabetic properties such as Trigonella Foenum-graecum, a plant widely distributed all over the world, has stimulated the investigation of the hypoglycemic potential of this plant in both rats and humans [15]. The hypoglycemic activity of Trigonella Foenum-graecum seeds was first discovered in diabetes induced by the action of Alloxan in rats and improved glycemic control in patients with type 2 diabetes [16].

Trigonella Foenum-graecum L. seeds have been shown to reduce the symptoms of experimental diabetes without bringing hypoglycemia below the normal blood glucose levels in alloxan model rats [17]. It has been suggested that Fenugreek seeds have a beneficial effect on several systems of the body, including cholesterol levels in humans [18].

Mechanism of Action of Sulfonylurea via Sulfonylurea (Glimepiride) Receptors Diabetes mellitus is defined by hyperglycemia and its related consequences, resulting from insufficient insulin secretion and/or impaired insulin activity in the body [19]. Sulfonylurea (Glimepiride) are insulin secretagogues utilized in the management of type 2 diabetes [20]. The primary mechanism of action of these medications is the enhancement of insulin production from pancreatic β-cells [21]. Consequently, they significantly reduce hyperglycemia in individuals with type 2 diabetes, and sulfonylureas, in conjunction

with biguanides, are recommended by worldwide guidelines when metformin alone fails to adequately drop blood glucose levels [22]. Glimepiride is a Sulfonylurea compound with molecular weight 490.62 and molecular formula $C_{24}H_{34}N_4O_5S$. The hypoglycemic activity of glimepiride is relied on its ability to enhance insulin release from β- cells of the pancreas and acts via an extra-pancreatic mechanism [23]. In individuals with type 2 diabetes, elevated circulation of free fatty acids and non-esterified free fatty acid concentrations, coupled with enhanced gluconeogenesis in the liver, contribute to hyperglycemia [24].

Hyperglycemia, exacerbated by insulin resistance, leads to heightened lipolysis in peripheral adipocytes, resulting in elevated circulating free fatty acids [25]. However, elevated gluconeogenesis rates and glucose intolerance have been demonstrated prior to the beginning of type 2 diabetes [26]. Non-alcoholic fatty liver disease is now regarded as the hepatic manifestation of metabolic syndrome, characterized by insulin resistance and compensatory hyperinsulinemia [27]. Fatty liver disease that isn't caused by alcohol is more prevalent in patients with type 2 diabetes and may be a complicating factor of this condition [28]. Insulin resistance leads to heightened lipolysis in adipose tissue and reduced inhibition of lipolysis by insulin during the fed state [29]. Hyperinsulinemia occurs with an elevated release of fatty acids into the liver [30].

The progression from simple steatosis to hepatocellular injury and the more severe condition, non-alcoholic steatohepatitis, is driven by oxidative stress, lipid peroxidation, elevated production of pro-inflammatory cytokines, and factors derived from adipose tissue. [31]. In diabetes, liver gluconeogenesis surpasses its inhibitory impact despite hyperglycemia, accompanied by heightened glucose synthesis from non-carbohydrate precursors, including amino acids and glycerol [32]. Both effects facilitate the persistence of hyperglycemia [33]. In type 2 diabetes, the liver demonstrates histological alterations and significant long-term consequences [34]. The advancement of hepatic alterations is gradual and initial, but once established, it might lead to worse diabetic consequences [35].

In the present study, we investigated the ameliorative effects of Trigonella Foenum-graecum L. seeds on blood glucose levels and their potential in mitigating diabetes induced hepatopathy and nephropathy.

1. Investigate the pathological effects of type 2 diabetes on some vital organs such as the liver, pancreas, and kidneys.

2. Diagnose the effects of hyperglycemia on body weight.

3. Examine the effects of Trigonella Foenum-Graecum L. Seeds in the reduction of blood glucose and in reducing the pathological effects on the liver, pancreas, and kidney.

MATERIAL AND METHODS:

Samples

A total of 50 rats were used and splited into five groups for this study. Male albino Wistar rats of the subjects being between the ages of (10) weeks, each weighing (200-250) g, were totally divided into five groups of ten rats in each.

- **Group (1):** used as negative control (C-), rats were inoculated with normal saline daily.
- **Group (2):** used as positive control (C+), rats were administered alloxan injection (80mg/kg) B.W.
- **Group(3):** treated by sulfonylurea(glimepiride) orally administered (4mg/kg) B.W. daily.
- **Group (4):** treated with water extract of

Trigonella Foenum- Graecum L. Seeds orally (1) g/kg B.W. daily.

- **Group (5):** combination orally administered (4mg/kg) B.W. from sulfonylurea(glimepiride) and 1g/kg of seeds daily.

Accommodated in sanitized polymeric cages (15 * 30 * 45) cm.ten rats per cage within a controlled environment maintained at 21 ± 1 °C, with regulated humidity and lighting conditions (12-hour light/dark cycle). The rats were supplied with ad libitum access to balanced standard pellets and clean water, facilitating a detailed investigation into the treatment of type 2 diabetes.

A rodent animal model was best and widely accepted for evaluating blood glucose, insulin resistance, obesity, dyslipidaemia, and non-alcoholic fatty liver disorders, which were generated by the overfeeding of a resection diet. By daily oral intake of a high-fat diet for four weeks, the tested rats were induced to earlier stages of metabolic syndrome and type 2 diabetes in humans along with the following measures.

Induction of diabetes after a 24-hour fast, diabetes was induced via intraperitoneal injection of freshly produced alloxan (80 mg/kg body weight) in 0.9% distill water. Rats were permitted to consume a 10% glucose solution for the initial 24 hours to mitigate hypoglycemia, but their initial access to food extended beyond 24 hours. At the conclusion of the 3-day period following Alloxan injection, rats exhibiting glucose levels over 505 mg/dL (glucometer).

Were designated to the hyper glycaemia group. After one day from induced the DM Fenugreek and Glimepiride were given for three months to the treated groups of rats.

Ethical Approval.

The institutional animal care and use committee at the University of Kufa approved the study, as documented in official request no. 2107 on 23 of January 2025.

Histopathological Examination

All invasive treatments on animals were done while they were under general anesthesia, which was made up of (50 mg/kg of body weight of ketamine and 50 mg/kg) of xylazine (Rhobifarma Ind. Farmacêutica Ltda, Brazil). From the follow-up heart puncture, blood samples were taken.

Following the post mortem examination, we collected the entire liver. Other organ samples were collected while the chest was open during the sacrifice procedure. After exsanguination, we took out the whole liver, cleaned it with PBS and then soaked in 10% formalin (Chemanol, KSA) for 24 hours. Later, the formalin was replaced, and the tissues were left for another 24 hours for more study. Later, the formalin was changed and kept for another 24 hours for further investigation and used a scientific balance to weigh it. For histological studies, sections of the whole organ were randomly cut from 40 rats in each experimental group (10 rats were sacrificed from each subgroup).

The sections were then fixed in buffered 10% formalin, treated in paraffin blocks, and sectioned at 3–5 µ m. From each rat, two blocks of liver tissue were made. There was one slide stained with red picrosirius and two slides stained with hematoxylin and eosin (HE), these slides came from different blocks. A rat subgroup looked at 37 slides, each with two parts of liver. This made a total of 90 slides for each experimental group, with 60 slides being HE and 30 slides being red picrosirius. Two different researchers, who were not part of the project, used light microscopy to look at all the slides.

Thorough histopathology examination

After the tissue samples were fixed in formaldehyde, they were encased in paraffin and cut into 5 µm slices. Hematoxylin and eosin were then used to color the slices. A BX51 light microscope and an Olympus digital camera (DP20) were used to look at and take pictures of the slides.

Statistical examination

GraphPad Prism software, version 7.04 (GraphPad, Inc.), was used to do the statistical tests. The numbers are shown as mean ± standard deviation (SD). The Tukey's post hoc test and one-way ANOVA were used. A difference that was statistically important was defined as having a P-value of less than 0.05. the error bars show mean ± SD, N= 40 rats. ANOVA three ways Tukey's multiple compared was used to calculate the significancy between and within groups at P≤0.05RESULTS

RESULTS

Effect of High fat diet on the body weight.

The results demonstrated increase in the body weight of rats during feeding on high fat diet for 30 days except the negative control (group one) which was fed on normal diet (figure 4.1).

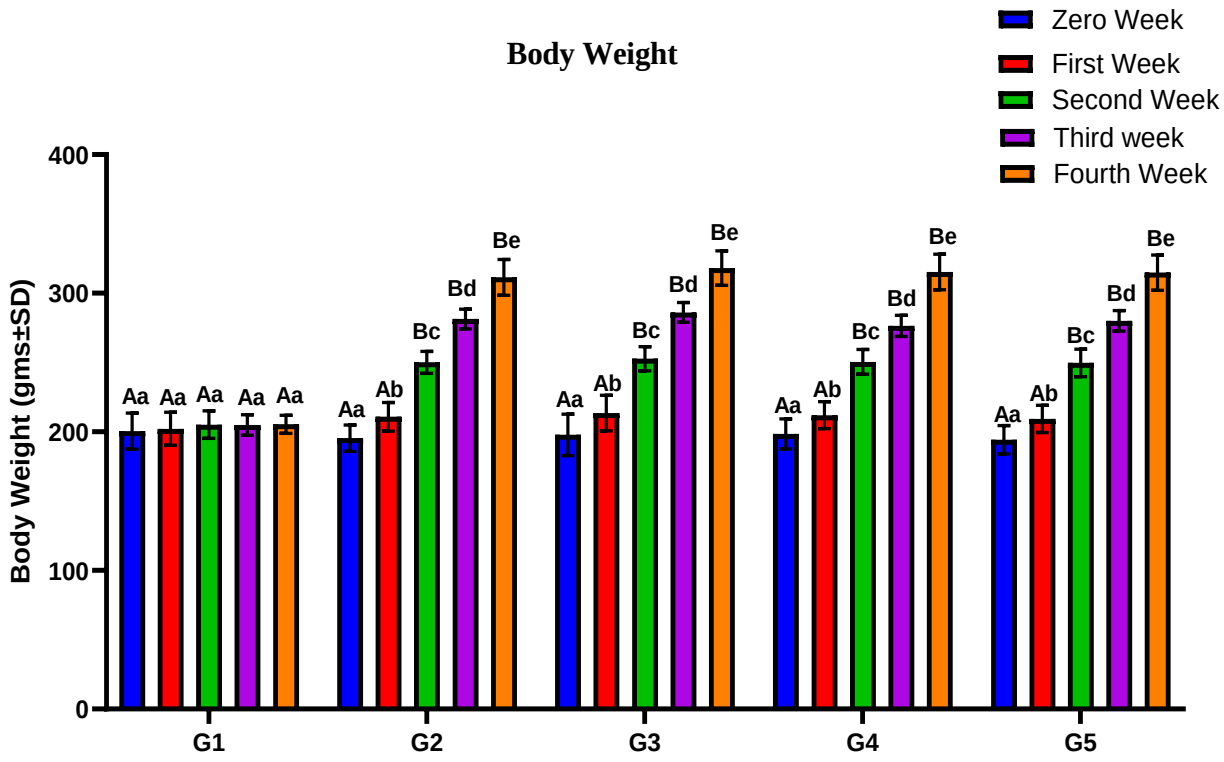

Fig. 1. The effect of high fat diet on body weight in rat during four weeks of feeding. The bar graph shows the body weight of rat during feeding on high fat diet for four weeks except group one which was fed on normal diet. The different small letters explain the comparism within group between weeks, while the different uppercase letters show the comparism of body weight between groups within same weeks. The error bars show mean ± SD, N= 7 rats. ANOVA two ways Tukey's multiple comparism was used to calculate the significancy between and within groups at P≤0.05.
blue- negative control, red- Alloxan, green- glimepiride, purple- Trigonella, orange- combination

The induction of diabetes type2 in rat.

The results show the effect of glucose

after feeding on high fat diet for 30 days except group one (negative control). The animal's weight reached (300-350) g, glucose was induced by intraperitoneal injection of alloxan at a dose of 80 mg /kg of body weight (20 mg per rat). Glucose was induced 3 days after injection to 505 mg /dL. We show the rat's weight loss and lipid profile. show in figure (4.2).

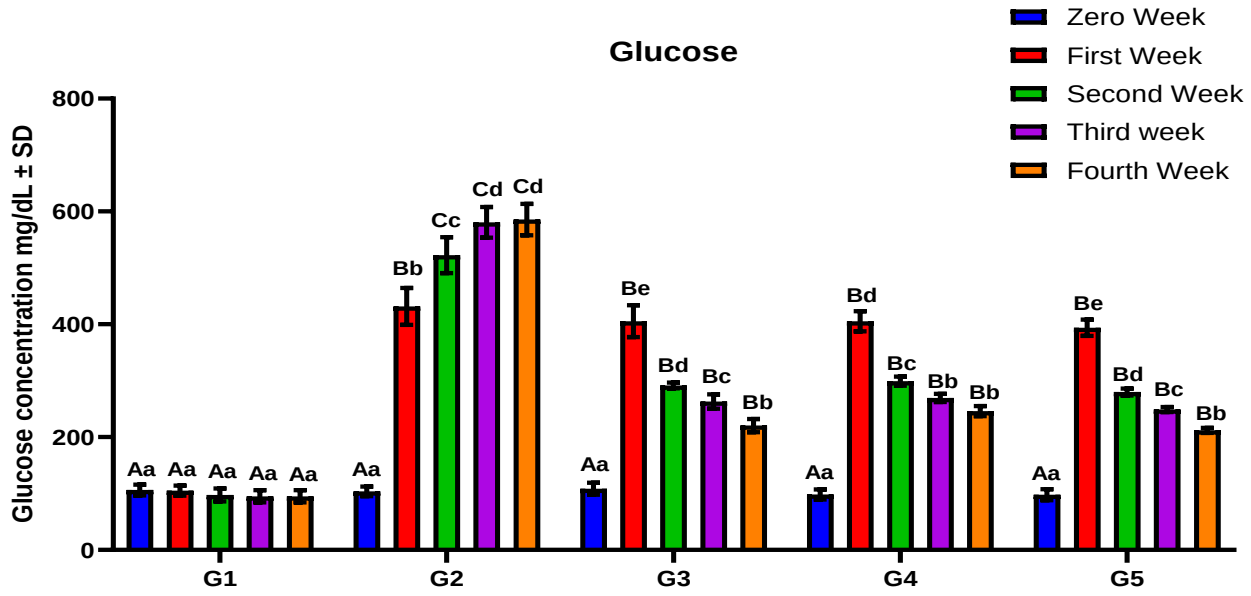

Fig. 2. The effect of glucose levels on body weight in rat after four weeks of feeding. The bar graph shows the effect of glucose in rat after feeding on high fat diet for month except group one (control negative). The different small letters explain the comparism within group between weeks, while the different uppercase letters show the comparism of glucose levels between groups within same weeks We notice the rat's weight loss and high blood sugar over the last three weeks. The error bars show mean ± SD, N= 7 rats. ANOVA three ways Tukey's multiple comparism was used to calculate the significancy between and within groups at P≤0.05. Impact of Fenugreek intervention on glycemic levels across various cohorts. All groups exhibited a notable reduction in glucose levels relative to diabetic rats. blue- control negative, red- alloxan , green- glimepiride, purple- trigonella, orange- co.

Liver functions and lipid profile.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels increased in diabetic rats. Fenugreek treatment in the oral daily groups significantly decreased in AST, ALT and ALP levels compared to the diabetic control group.

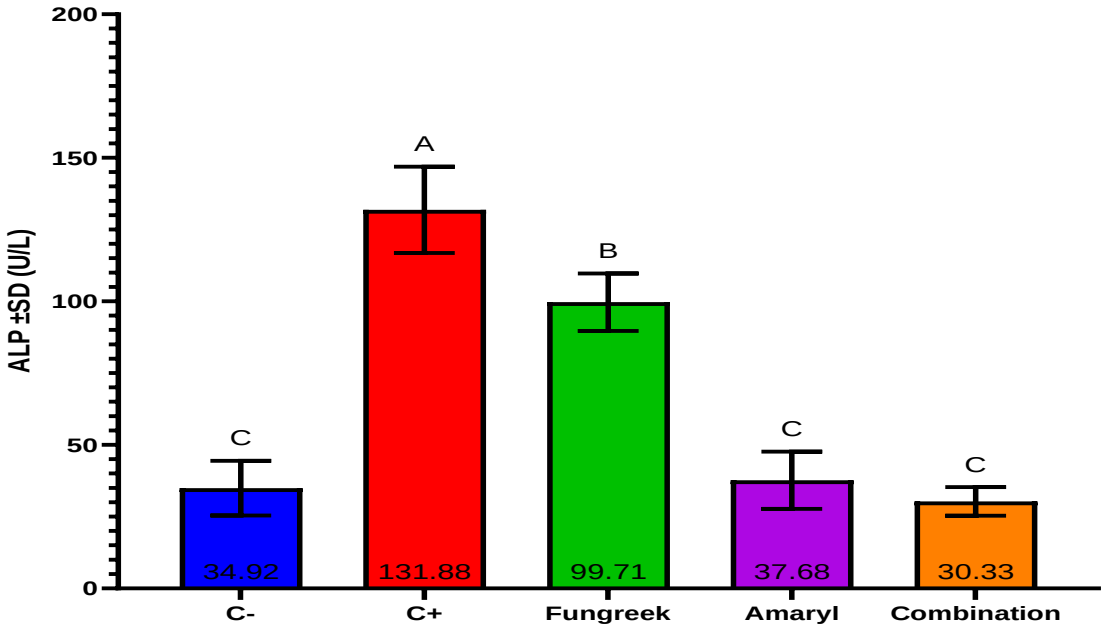

Fig. 3. Effect of treatment on the level of Alkaline phosphatase (ALP) in the serum of experimental animals. The data show variations in the Alkaline phosphatase levels in this marker in the rat serum. The combination group explains a significant reduction in the Alkaline phosphatase comparing to control positive group (Alloxan group), while the treatment by Trigonella shows significant reduction in the Alkaline phosphatase level comparing to control positive group but still more than both Glimepiride and combination groups. All error bars represent m± S.D. and One-way ANOVA (ANalysis of VAriance) with post-hoc Tukey multiple comparism was used to calculate the significance of variations at $p \leq 0.05$. The different uppercase letters explain the significant differences between groups, while the similar uppercase letters show no significancy between groups.

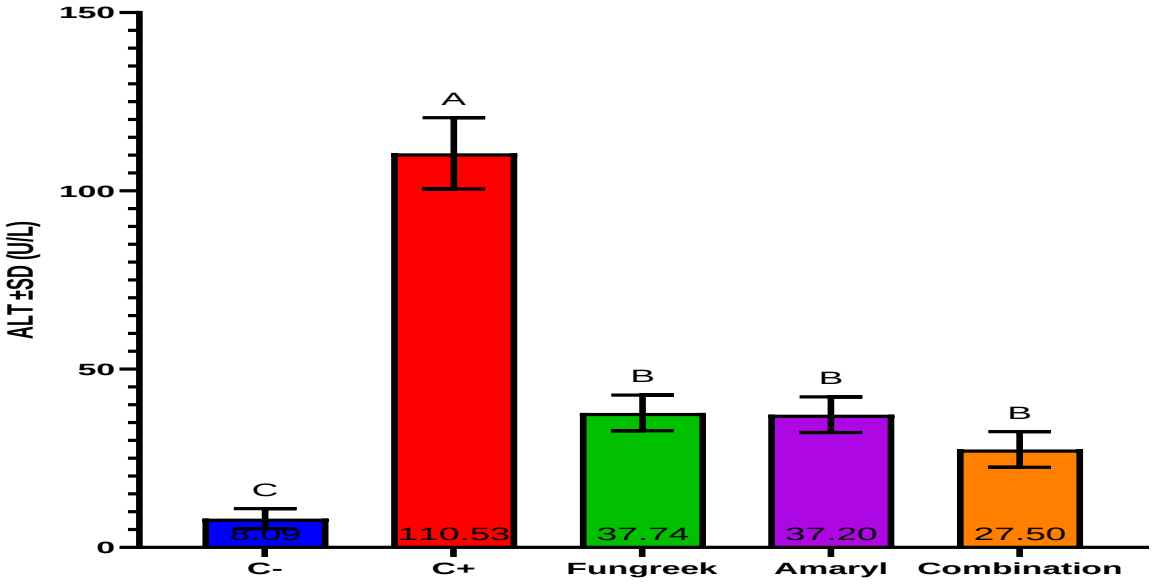

Fig. 4. Effect of treatment on the level of Alanine aminotransferase (ALT) in the serum of experimental animals. The data show variations in the Alanine aminotransferase levels in this marker in the rat serum. The combination group explains a significant reduction in the Alanine aminotransferase comparing to control positive group (Alloxan group), while the treatment by Trigonella shows significant reduction in the Alanine aminotransferase level comparing to control positive group but still more than both Glimepiride and combination groups. All error bars represent m± S.D. and One-way ANOVA (ANalysis of VAriance) with post-hoc Tukey multiple comparism was used to calculate the significance of variations at $p \leq 0.05$. The different uppercase letters explain the significant differences between groups, while the similar uppercase letters show no significancy between groups.

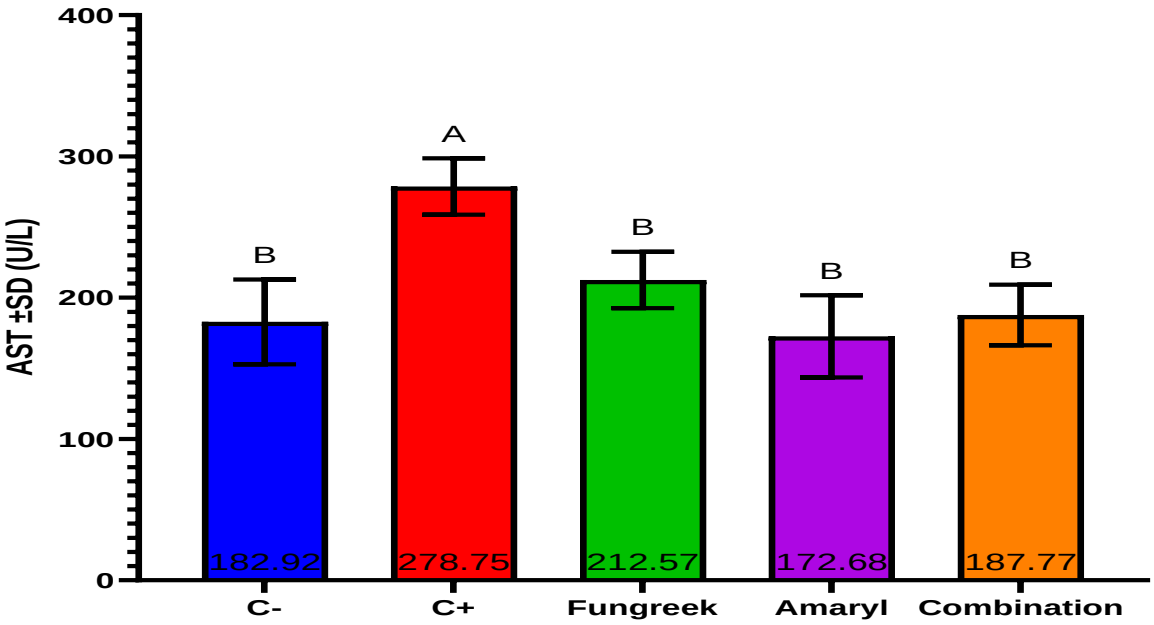

Fig. 5. Effect of treatment on the level of Aspartate aminotransferase (AST) in the serum of experimental animals. The data show variations in the Aspartate aminotransferase levels in this marker in the rat serum. The Glimepiride group explains a significant reduction in the Aspartate aminotransferase comparing to control positive group (Alloxan group), while the treatment by Trigonella shows significant reduction in the Aspartate aminotransferase level comparing to control positive group but still more than both Glimepiride and combination groups. All error bars represent m± S.D. and One-way ANOVA (ANalysis of VAriance) with post-hoc Tukey multiple comparism was used to calculate the significance of variations at $p \leq 0.05$. The different uppercase letters explain the significant differences between groups, while the similar uppercase letters show no significancy between groups.

Histopathological alterations in the liver.

Histological examination of hepatic tissue revealed distinct morphological changes across experimental groups, as demonstrated in Figures 1–15. Liver sections from the healthy control group (Figures 1–2) exhibited normal hepatic architecture, with well-preserved hepatocytes, organized sinusoidal spaces, and intact portal triads in contrast, liver tissues from diabetic rats (positive control group; Figures 9–15) showed extensive pathological lesions, including severe hepatic vein congestion, marked fatty degeneration (fat droplet infiltration within hepatocyte cytoplasm), and pronounced hemorrhagic areas with sinusoidal red blood cell accumulation. Additional features included hepatocyte karyo pyknosis, dilation and congestion of sinusoids, fibrous tissue proliferation around the portal areas, hypertrophy of arterial walls, and necrotic lesions. as shown in Fig (9-10). Oral fenugreek extract treatment showed normal hepatocytes in shape and size with normal hepatic tissue texture (Black arrows) while the hepatic sinusoids show mild dilatation or space increasing (Red arrows). We stain the tissue with HandE. as shown in Fig (5). morphology with nearly normal hepatocytes, well-organized sinusoids, and portal structures, indicating a protective effect against alloxan-induced hepatic damage. Fenugreek-treated rats (Figures 5–6) showed normal hepatocyte size and shape with typical tissue texture. Mild sinusoidal dilation and occasional leukocytic perivascular cuffing were observed, reflecting partial hepatoprotection. The combination therapy group (Glimepiride + fenugreek). (Figures 7–8) exhibited the most significant improvement in hepatic structure. Hepatocytes appeared normal in morphology, with minimal

sinusoidal dilation. Some sections showed mild leukocytic infiltration around portal areas, but overall tissue integrity was well maintained. All liver sections were stained with hematoxylin and eosin (HandE) and examined under a BX51 light microscope. Digital imaging was performed using an Olympus DP20 camera at 10X and 20X magnification.

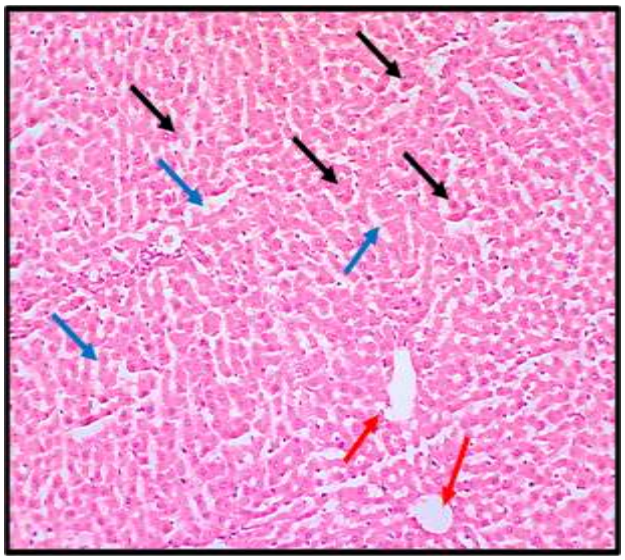

Fig. 6. The histological section of the liver in rat of control negative group. The section shows normal texture of hepatic tissue without any significant occupied lesion, the section shows normal hepatocytes (Black arrows) with normal portal section (Blue arrow) and normal sinusoidal space (red arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

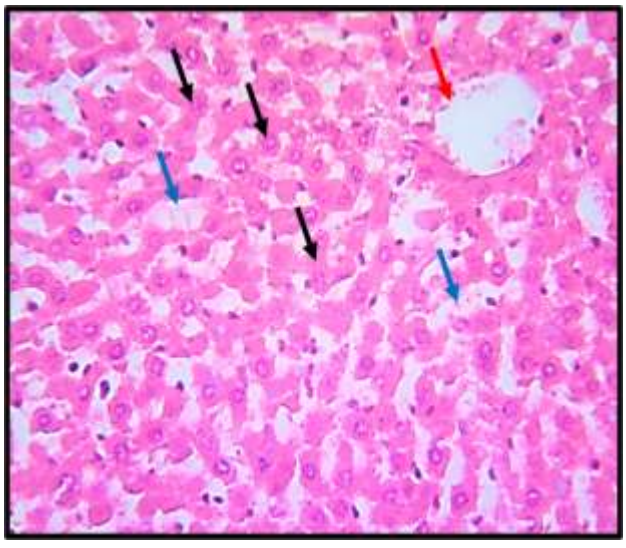

Fig. 7. The histological section of the liver in rat of control negative group. The section shows normal texture of hepatic tissue without any significant occupied lesion, the section shows normal hepatocytes (Black arrows) with normal portal section (Blue arrow) and normal sinusoidal space (red arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 20X magnifier scale.

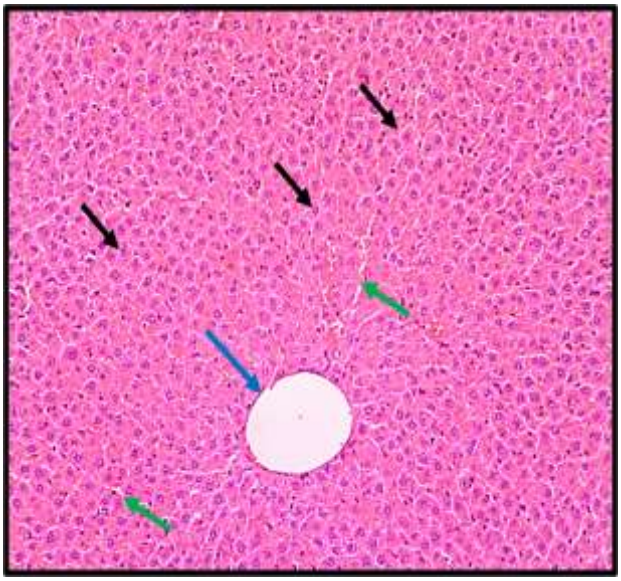

Fig. 8. The histopathological section of liver in rat for Glimepiride treatment group (4mg/kg B.W. orally administration as a single dose daily). The section shows normal hepatocytes in texture (Black arrows) and normal sinusoidal spaces (Blue arrows) with normal portal area (Green arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

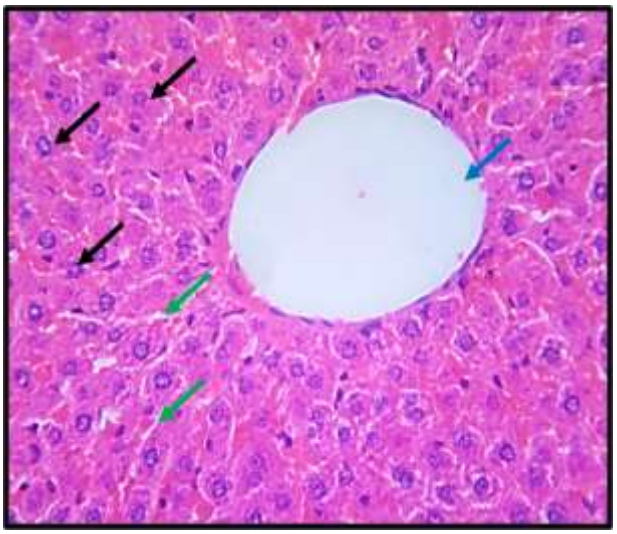

Fig. 9. The histopathological section of liver in rat for Glimepiride treatment group (4mg/kg B.W. orally administration as a single dose daily). The section shows normal hepatocytes in texture (Black arrows) and normal sinusoidal spaces (Blue arrows) with normal portal area (Green arrow). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 20X magnifier scale.

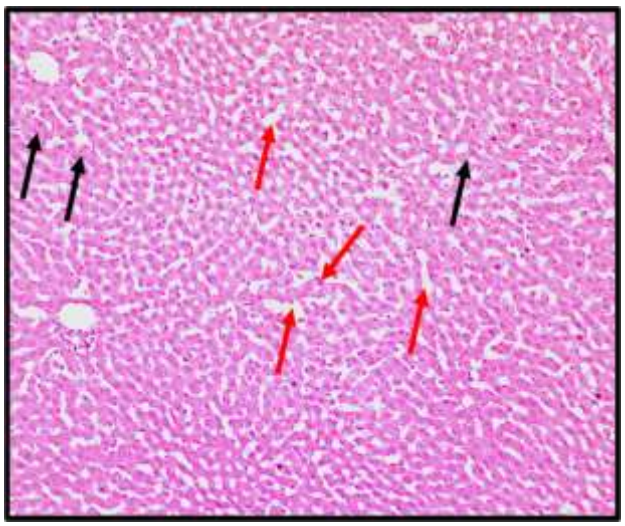

Fig. 10. The histopathological section of liver in rat for Fenugreek treatment group (the dosage of 1gm/kg body weight, administered orally as a single daily dose). The section shows normal hepatocytes in shape and size with normal hepatic tissue texture (Black arrows) while the hepatic sinusoids show mild dilatation or space increasing (Red arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

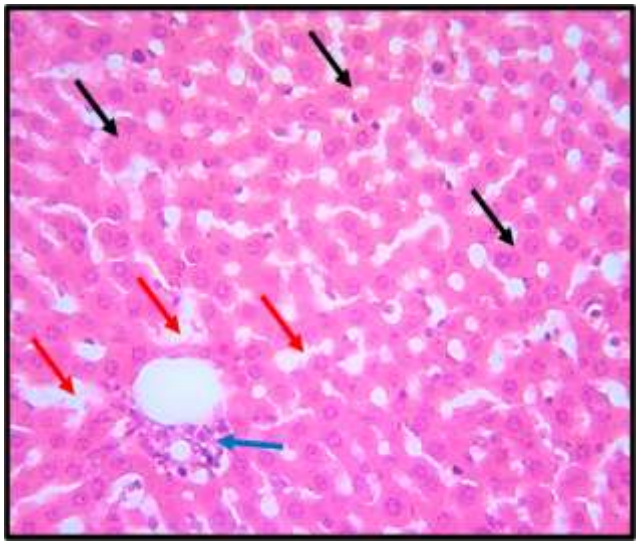

Fig. 11. The histopathological section of the liver in rat for Fenugreek treatment group (the dosage of 1gm/kg body weight, administered orally as a single daily dose). The section shows normal hepatocytes in shape and size with normal hepatic tissue texture (Black arrows) while the hepatic sinusoids show mild dilatation or space increasing (Red arrows). Leukocytes pre-vascular cuffing can be seen in this section (Blue arrow). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 20X magnifier scale.

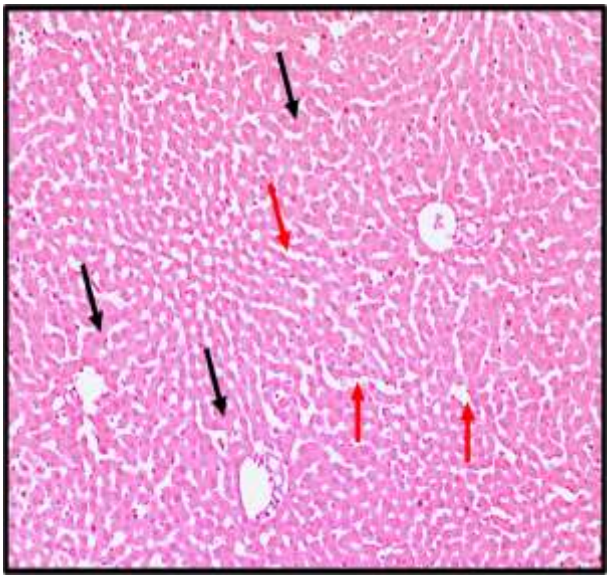

Fig. 12 The histopathological section of liver in rat for Combination treatment group. (Glimepiride + Fenugreek at a dosage of 4 mg/kg body weight administered orally and 1gm/kg body weight daily of seeds and sulfonylurea, respectively). The section shows normal hepatocytes in shape and size with normal hepatic tissue texture (Black arrows) while the hepatic sinusoids show mild dilatation or space increasing (Red arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

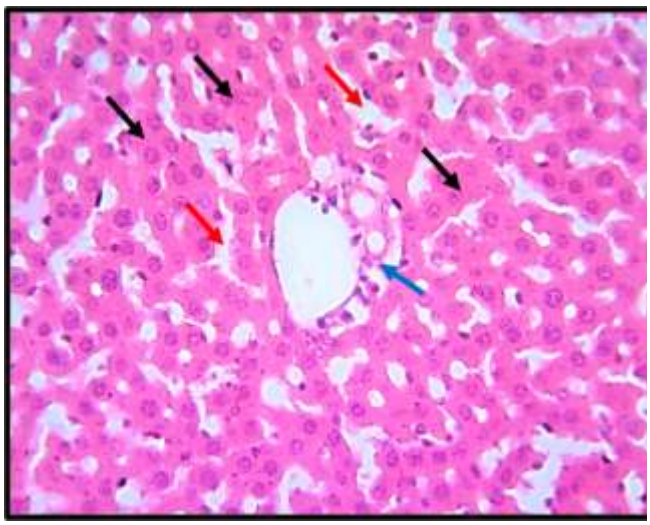

Fig. 13. The histopathological section of liver in rat for Combination treatment group. (Glimepiride + Fenugreek at a dosage of 4 mg/kg body weight administered orally and 1g/kg body weight daily of seeds and sulfonylurea, respectively). The section shows normal hepatocytes in shape and size with normal hepatic tissue texture (Black arrows) while the hepatic sinusoids show mild dilatation or space increasing (Red arrows). Leukocytes pre-vascular cuffing can be seen in this section (Blue arrow). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 20X magnifier scale.

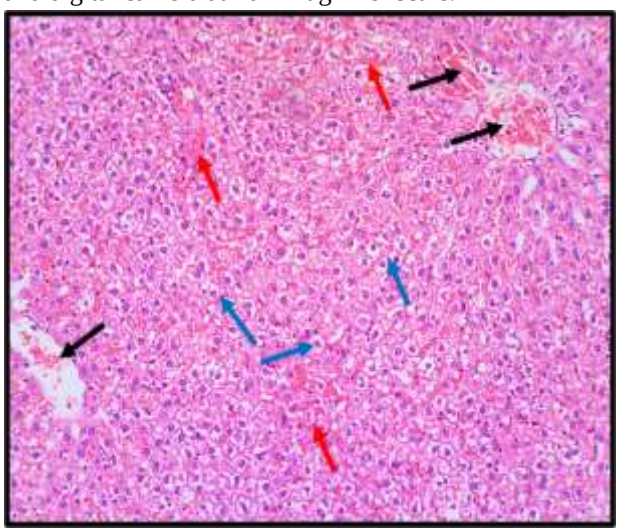

Fig. 14. The histopathological section of liver in rat of Control positive group (intraperitoneal injection of Alloxan at a dosage of 80 mg/kg body weight). The section shows sever hepatic vein congestion (Black arrows) with sever fatty degenerative changes (Fatty liver, fat droplet infiltration in the cytoplasm of hepatocytes, blue arrows). The section shows sever hemorrhagic lesion characterized by accumulation of RBCs in the sinusoidal space of hepatic tissue (Red arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

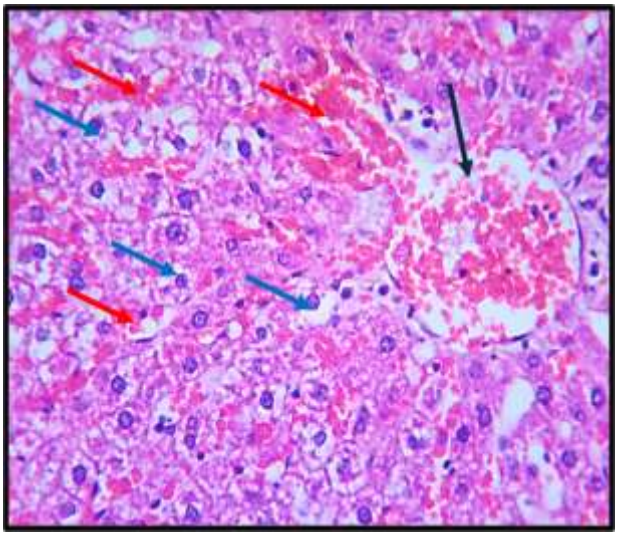

Fig. 15. The histopathological section of liver in rat of Control positive group (intraperitoneal injection of Alloxan at a dosage of 80 mg/kg body weight). The section shows sever hepatic vein congestion (Black arrow) with sever fatty degenerative changes (Fatty liver, fat droplet infiltration in the cytoplasm of hepatocytes, blue arrows). The section shows sever hemorrhagic lesion characterized by accumulation of RBCs in the sinusoidal space of hepatic tissue (Red arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 20X magnifier scale.

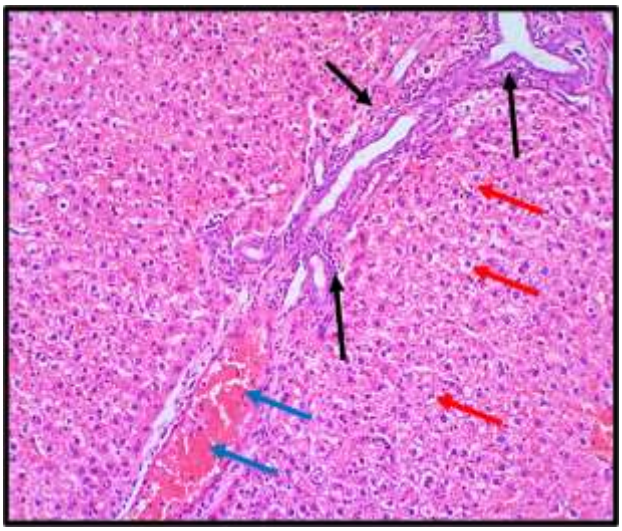

Fig. 16. The histopathological section of liver in rat of Control positive group (intraperitoneal injection of Alloxan at a dosage of 80 mg/kg body weight). The section shows sever fibrous connective tissue formation in the liver especially around portal area such as around bile duct and hepatic artery and venules (Liver fibrosis, Black arrows) with sever hepatic vein congestion (Blue arrows) and fatty degenerative changes of hepatocytes (Red arrows). The tissue is stained by H&E stain and the

section is captured using light microscope and digital camera at 10X magnifier scale.

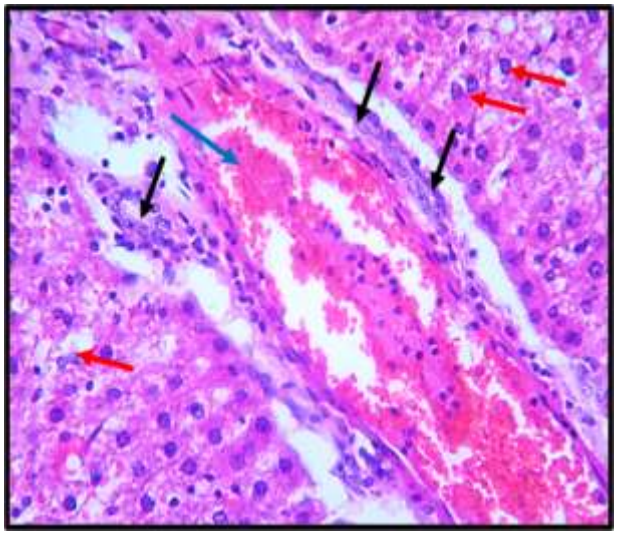

Fig. 17. The histopathological section of liver in rat of Control positive group (intraperitoneal injection of Alloxan at a dosage of 80 mg/kg body weight). The section shows sever fibrous connective tissue formation in the liver especially around portal area such as around bile duct and hepatic artery and venules (Liver fibrosis, Black arrows) with sever hepatic vein congestion (Blue arrows) and fatty degenerative changes of hepatocytes (Red arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 20X magnifier scale.

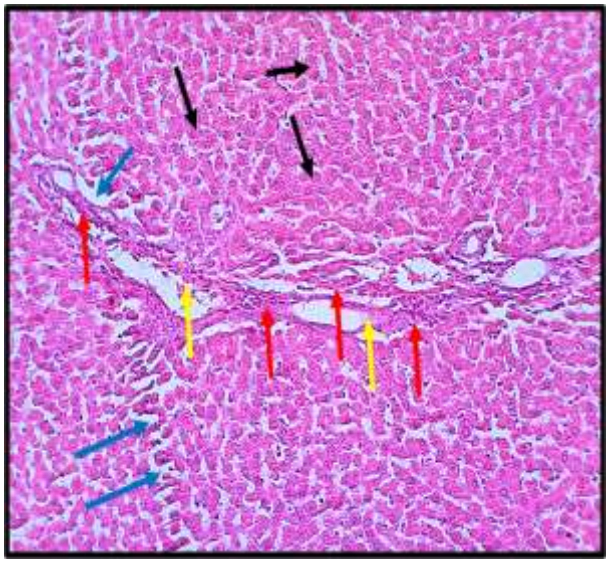

Fig. 18. The histopathological section of the liver in the control positive group (intraperitoneal injection of Alloxan at a dosage of 80 mg/kg body weight). The section shows severe degenerative changes in the hepatocytes (acute cellular swelling, Black arrows) with increasing sinusoidal spaces in the portal area (Blue arrows). The section shows fibrous connective tissue formation around hepatic vein and artery (Red arrows) with clear damage of blood vessels wall (Yellow arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

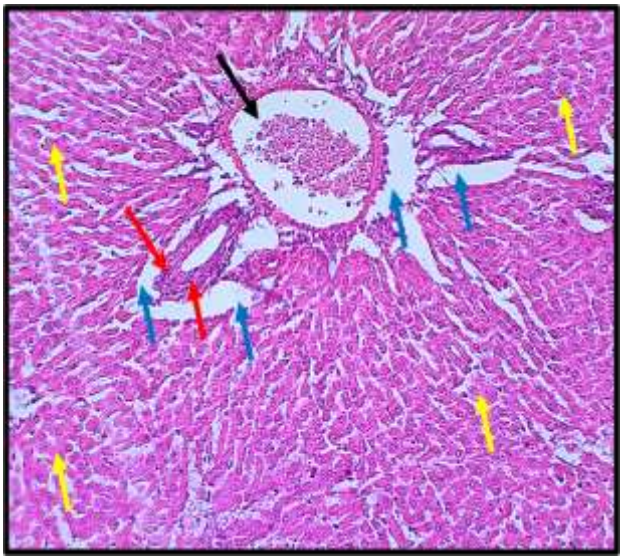

Fig. 19. The histopathological section of the liver in the control positive group (Alloxan intraperitoneal injection at a dosage of 80 mg/kg body weight).). The section shows sever blood vessels congestion (Hepatic vein, Black arrow) with increasing of artery muscular wall (Muscular layer hyperplasia, red arrows). The section shows multiple necrotic lesions in the portal area around hepatic blood vessels (Blue arrows) and the hepatocytes shows acute cellular swelling (Yellow arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

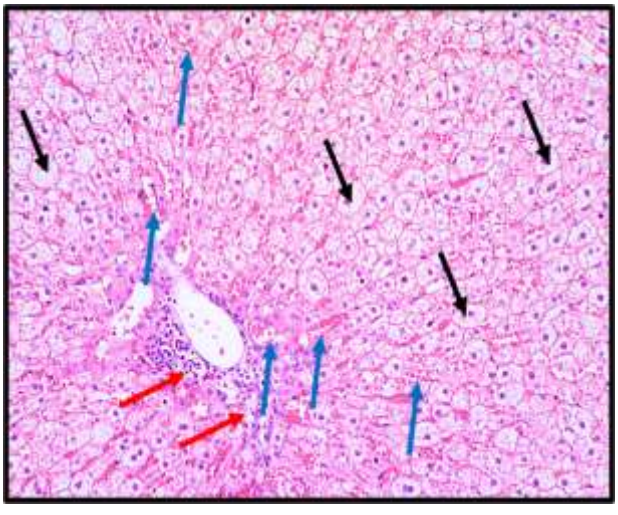

Fig. 20. The histopathological section of the liver in the control positive group (Alloxan intraperitoneal injection at a dosage of 80 mg/kg body weight). The section shows sever fat droplets infiltration in the hepatocytes (fatty degeneration, fatty liver, Black arrows) with sever pre vascular leukocytes cuffing

(inflammatory changes, red arrows) and sever RBCs accumulation in the hepatic interstitial spaces (hemorrhage, blue arrows). The tissue is stained by H&E stain and the section is captured using light microscope and digital camera at 10X magnifier scale.

DISCUSSION

Diabetes is a metabolic disorder that causes high blood sugar levels because insulin is not released or used properly. There is a big rise in the number of people around the world who have diabetes. In untreated diabetic rats, the levels of liver enzymes Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Aspartate aminotransferase (AST) in their blood were high. After treatment with fenugreek, these levels went down a lot. Damage to the liver cells is what caused the high serum level [36].

Fenugreek caused a big drop in liver enzyme levels, which suggests that it protects liver cells. These results are in line with earlier research that showed Fenugreek can help prevent problems when taken every day [37,38]. Before type 2 diabetes starts, the pathway that controls the expression of important genes that help with glucose and lipid metabolism is usually broken. This happens when insulin signaling decreases and fat builds up in the liver [39]. Insulin resistance is thought to be the main cause of the first fat deposit in the liver since insulin stops the important genes that make new fat and triglycerides [40].

Natural substances that can help with type 2 diabetes while protecting the liver and making insulin more sensitive may be helpful for treating this illness, which is toxic to lipids and can cause liver failure and other complications [41]. Fenugreek is a plant that is used all over the world. This paper gives a full evaluation of how fenugreek seed extract affects liver function. In terms of both biochemistry and histology. The purpose of this study was to look at how alloxan affects the liver. In our previous study, we found that rats who were given alloxan had smaller pancreatic islets and pyknosis of islet cells [42].

This study demonstrated that giving alloxan to the liver caused central venous congestion, as well as significant widening of sinusoidal gaps and pyknosis of hepatocyte nuclei. The glycogen level, which is the main way that glucose is stored inside cells in different organs, shows how well insulin works. This is because insulin activates glycogen synthetized and lowers glycogen phosphorylase, which helps cells store more glycogen [43].

The study shows that feeding rats a high-fat diet (HFD) for four weeks caused their blood glucose levels to rise and their weight to drop, which suggests that their metabolism was not working properly [44]. The result ANOVA and Tukey's post-hoc analysis ($p < 0.05$) showed that there were big differences between the groups. The Fenugreek intervention made hyperglycemia a lot less severe than the diabetic controls. This is in line with research that shows Fenugreek can lower blood sugar since it has fiber and bioactive substances [45]. The fact that the HFD-fed rats lost weight could be a sign that their glucose metabolism was messed up, like it is in diabetic animals [46].

Result error bars (mean ± SD) show that the data is reliable, which supports fenugreek's possible use in managing blood sugar levels. Untreated diabetic rats had far greater levels of blood alkaline phosphatase (ALP) than non-diabetic controls, which showed that their liver cells were damaged [47]. But fenugreek treatment brought ALP levels down a lot in diabetic rats, which suggests that it protects the liver in diabetes [48]. Fenugreek (Trigonella Foenum-graecum) has worked quite well to reduce liver enzymes. A study by [49] showed that giving diabetic rats fenugreek seed extract by mouth every day lowered their AST and

ALT levels dramatically. This is likely because the polyphenolic components in fenugreek seed extract protect the liver from oxidative damage. The histology results from the livers of rats that were given Glimepiride (4mg/kg/day orally) show that the liver's structure was conserved, as shown by normal hepatocytes, sinusoidal spaces, and portal tracts The tissue is stained with Hematoxylin and Eosin stain (HandE staining, 10× magnification). This means that Glimepiride at the dose investigated does not cause liver damage also found similar results, saying that diabetic rats given therapeutic doses of sulfonylureas did not have any substantial liver damage [50]. Said that Glimepiride's safety profile includes only small changes in the liver, perhaps because it only affects pancreatic β-cells [51]. Mild sinusoidal dilation could mean that blood flow is increasing or that the body is starting to adapt (for example, fenugreek-induced vasodilation or metabolic demand). There is no necrosis or inflammation, which means that the change is not pathological [52].

The liver damage seen here, which includes karyo pyknosis, vascular congestion, and fatty infiltration, is consistent with classic alloxan-induced hepatotoxicity. Alloxan (80 mg/kg) makes reactive oxygen species (ROS), which damage hepatocytes and cause steatosis (fatty liver) by messing with mitochondria and causing lipid peroxidation [53].

The black arrow shows sinusoidal congestion, while the red arrows show hemorrhagic lesions. These signs point to microvascular damage, which is consistent with alloxan's endothelial toxicity [53] the fatty degenerative alterations (blue arrows) show that lipid metabolism is not working right because alloxan stops insulin from being released, which makes hepatic lipotoxicity worse [42].

CONCLUSION.

The findings indicated that alloxan-induced diabetes affects the morphology of pancreatic islets. Fenugreek appears to be a promising antidiabetic botanical. Additional research is required to elucidate the mechanism of action and determine the appropriate dosage range. Extended durations are advised to attain histological enhancement.

CONFLICTS OF INTEREST

The authors assert that there are no conflicts of interest pertaining to this publication.

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