Evaluation of Aqueous Flaxseed Extract on Liver Histological Structure in Diabetic-Induced White Mice

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Abstract: This study aimed to evaluate the effectiveness of an aqueous extract of flaxseed on liver histology in diabetic rats. The results of the study showed that the liver of animals induced with diabetes had many histological changes, including the appearance of congestion in the central vein surrounded by hepatocytes that appeared irregular in shape. It was also noted that there was a loss of the radiographic appearance, irregularity in the venous sinusoids, expansion of the liver sinusoids, necrosis in the wall of the central vein, and the appearance of active transport vesicles. Nuclear death was also observed in some hepatocytes, and the cytoplasm of the hepatocytes was necrotic. The study also showed hypertrophy and thickening of the nuclei. Fatty degeneration was also observed with necrosis of the hepatocytes, indicating the occurrence of hepatocyte degeneration and focal destruction. Infiltration of inflammatory cells was also observed. The two groups of animals treated with the aqueous extract of flax seeds at a concentration of 0.2 showed that the central vein was surrounded by more regular cells than in the two previous groups, and that there was less blood congestion in the central vein of the liver. The shape of the venous sinusoids was more regular in most sections, and there was less accumulation of Kupffer cells in them, as well as the disappearance or less decomposition of the nuclei in some liver cells. It was also noted that there was an improvement in the level of radial organization of cells in the liver parenchyma.

1 INTRODUCTION

Diabetes is one of the most common chronic diseases in the world, with the number of people affected increase significantly due to various factors, including unhealthy lifestyle, obesity, and genetic factors [1]. This disease is characterized by high blood glucose levels due to insufficient insulin production or tissue resistance to it, leading to disturbances in the metabolism of carbohydrates, fats, and proteins [2] . Diabetes can be classified into two main types: Type 1 diabetes is an autoimmune disease that leads to the destruction of insulin-producing beta cells in the pancreas, making patients need lifelong insulin therapy [3]. Type 2 diabetes, the most common form, occurs as a result of tissue resistance to insulin, leading to an abnormal increase in blood glucose levels [4]. Since the discovery of this disease, humans have developed many pharmaceutical treatments used to treat diabetes. These drugs and medications rely on different mechanisms to stimulate insulin secretion, reduce insulin resistance, or slow down glucose absorption [5]. Among these well-known treatments is metformin, which is the first-choice treatment for type 2 diabetes and belongs to the biguanide group. This drug works by reducing glucose production in the liver, improving insulin sensitivity in muscles and peripheral tissues, and finally reducing glucose absorption from the intestine [6]. Other treatments used to treat diabetes are sulfonylureas. This class includes medications such as glibenclamide and gliclazide, which stimulate the beta cells in the pancreas to secrete more insulin, and also effectively lower blood glucose levels [7]. However, these medications may lead to some side effects, such as severe hypoglycemia and weight gain, or gastrointestinal disorders including nausea, vomiting, diarrhea, bloating, and abdominal pain, which makes their use limited in some cases [8]. Despite the significant developments in the field of drug treatments for diabetes, the search for highly effective natural alternatives with limited side effects is still of interest to researchers around the world [9]. Physicians, pharmacists, and drug manufacturers

resort to using and preferring herbal medicines and drugs over chemical medicines in some cases for several main reasons, including the high degree of safety from side effects, the acceptability of these treatments to the majority of patients, their low cost, their low resistance to bacteria unlike chemical medicines, the growing medical and pharmaceutical interest in them, their availability, accessibility, and sustainability, which makes them a more popular option, especially with their complementary use with other treatments[10]. Certain plants have shown scientifically proven effects in lowering blood sugar levels and improving the body's response to insulin. Prominent among these plants are fenugreek [11], cinnamon [12], Aloe vera [13], Moringa oleifera [14], Curcuma[15], garlic [16], mulberry leaves [17] and others. These plants contain soluble fiber that slows the absorption of carbohydrates, improves insulin sensitivity, and reduces glucose levels, thus potentially lowering blood sugar levels in people with type 2 diabetes [18]. Despite these medicinal uses, these plants and their wastes have many other uses through which they can preserve human health and the environment. Rice husk [19], watermelon [20], orange peels [21], buckthorn leaves[22], and waste tea leaves [23] can be used to treat various toxic pollutants such as heavy metals [24], water hardness [25], organic dyes [26] and other plants from water and soil. Among these plants that have many beneficial medical and environmental uses are flax leaves [18]. Flax has been used as a food and medicine for centuries due to its numerous health benefits and nutrient-rich ingredients. Flax is high in fiber, omega-3 fatty acids, and proteins, making it an important nutritional component that promotes digestion, supports heart health, and helps control blood sugar levels [27]. Medicinally, flax has been used since ancient times to treat digestive disorders, reduce inflammation, and support skin health [28]. Some recent medical studies have shown that flaxseeds may help lower blood pressure [29], reduce harmful cholesterol levels [30], and improve insulin sensitivity [31], making them beneficial for people with diabetes and heart disease. Flaxseeds also possess antioxidant and anti-inflammatory properties, making them a natural choice for promoting overall health and preventing certain chronic diseases [28]. Despite these many therapeutic benefits, the use of flax seeds in treating diabetes is not at the desired level, as many people in many countries of the world are still ignorant of the complementary health benefits of consuming these seeds and their potential applications as useful ingredients in foods and pharmaceutical products [32]. Therefore, the current study aimed to investigate the effect of aqueous flaxseed extract on the histological composition of the liver in diabetic mice.

2 MATERIALS AND METHODS

2.1 Induce of Diabetes Mellitus in the Mice by Alloxan

Diabetes was induced in mice weighing 23–32 g after overnight fasting by a single intraperitoneal injection of freshly prepared alloxan monohydrate ($C_4H_2N_2O_4 \cdot H_2O$) at a dose of 150 mg/kg body weight, according to the method described by [33] .

2.2 Synthesis of Aqueous Linum Usitatissimum Extract

Flax seeds (Linum usitatissimum) were obtained from local markets and washed several times by tap water and then by deionized water to get rid of suspended impurities. The clean seeds were dried and crashed in an electric grinder more than once, until fine powder obtained. After that, the aqueous extract of flax seeds was prepared using the method mentioned in the study of [34].

The required dose of flax seed aqueous extract was prepared based on the lethal dose (LD50) of 1.5 mg/g body weight in mice. Two concentrations (doses) of the extract were selected to test its effect, namely (0.1 and 0.2) mg/g. The calculated doses of the aqueous solution of flax seed extract were calculated and injected into the experimental mice according to their weights according to the following equation:

$$\text{Dose of Linum usitatissimum extract} = \text{LD50} \times \frac{\text{weight of mice}}{1000\ \text{g}}.$$

2.3 Preparation of Experimental Mice

Laboratory white male mice (24 mice) weighing 23-32 g and aged 7-8 weeks obtained from the College of Veterinary Medicine, Tikrit University, were used in this study. These mice were randomly divided into four groups, the details of which were as follows: the first group was the control group with six mice, and the second group was the experimental group with 18 mice. The mice were injected with alloxan to induce diabetes, then the experimental group was

divided into three groups: the first group was injected with alloxan with six mice, the second group was diabetic and injected with flax seed aqueous extract at a concentration of 0.1 mg/g, and the third group was diabetic and injected with the extract at a concentration of 0.2 mg/g of body weight) daily for 30 days. On the last day, the mice were anesthetized with chloroform, then the animals were dissected and the livers were removed from their site. Then the samples were fixed in formalin solution 10% for 24 hours and then washed by tap water and transferred to 70% alcohol for preservation. The tissue sections were prepared according to the method describe in [35] then stained using Hars hematoxylin and eosin (H&E) stain according to the method followed in [36]

3 RESULTS AND DISCUSSION

3.1 Histological Changes in MICE Live

The results of the current study showed that the induction of diabetes in male mice led to several histological changes in the liver of the treated animals. These changes include the appearance of congestion in the central vein surrounded by hepatocytes that appear irregular in shape. The loss of the radiographic appearance, irregularity of the venous sinusoids, bleeding within the sinusoids, expansion of the liver sinusoids, necrosis of the central vein wall, and the appearance of active transport vesicles are also observed, as shown in Figure 1.

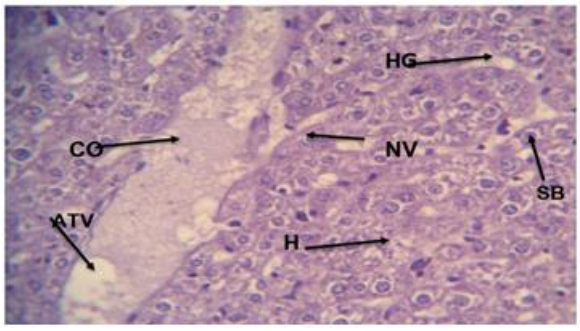

Figure 1: A cross-section of the liver tissue of male rats with alloxan-induced diabetes for 30 days shows central vein congestion (CO), central vein wall necrosis (NV), hepatocyte scattering (H), sinusoidal dilatation (SB), active transport vesicles (ATV), and sinusoidal hemorrhage (HG) (H&E 40X).

Nuclear death was also observed in some hepatocytes, and the cytoplasm of the hepatocytes was necrotic. The study also showed hypertrophy and thickening of the nuclei. Fatty degeneration was also observed with necrosis of the hepatocytes, indicating hepatocyte degeneration and focal destruction. Infiltration of inflammatory cells was also observed, as shown in the Figure 2.

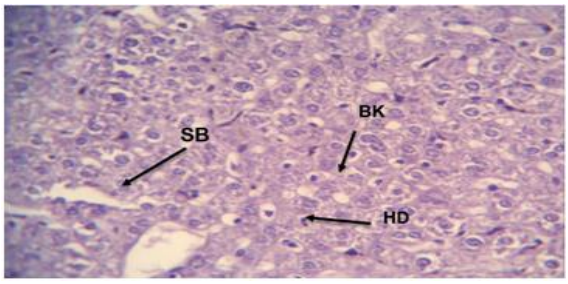

Figure 2: A cross-section of the liver tissue of male rats with alloxan-induced diabetes for 30 days shows the occurrence of severe necrosis and degeneration of hepatocytes (NC), degeneration of the nuclei of most hepatocytes (KA), expansion of the sinusoids (SB), enlargement of the hepatocytes (HT), thickening of the nuclei (BK), and degeneration of the hepatocytes (HD) (H&E 40X).

The current results also showed that the histological changes of the liver in the experimental group with diabetes were irregular in the hepatic parenchyma and around the central vein in the form of ropes, but rather irregular with the appearance of soft granules spread in the hepatic cells and the accumulation of glycogen granules inside the hepatic cells, the presence of a large number of dividing cells and cytoplasmic fatty explosion inside the hepatic cell, as shown in the Figure 3.

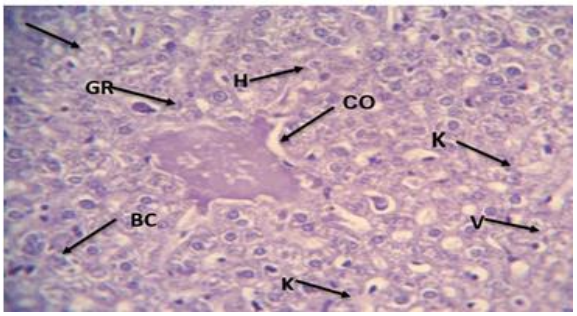

Figure 3: A section showing the irregularity of hepatocytes and expansion of blood sinusoids in the liver tissue of male mice with induced diabetes and the appearance of fine granules in the hepatocytes GR, the dissolution of most of the nuclei of the cells KA, Kupffer cells K. The explosion of dividing cells V BC, the scattering of hepatocytes H, the

occurrence of congestion of the central vein CO (H&E 40X).

3.2 Histological Changes in the Liver of Diabetic Rats Treated with Linum Usitatissimum

The results of the study showed congestion of the central vein in the diabetic group treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, as well as containing a blood clot, The results also showed irregularity in the rows of hepatic cells around the central vein in a radial manner, with the presence of a cluster of inflammatory cells and the appearance of active transport vesicles within the blood vessels, as shown in Figure 4.

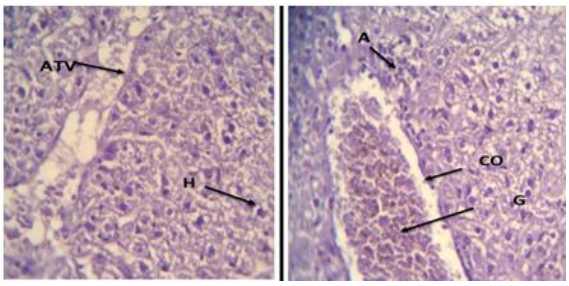

Figure 4: shows a cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, showing congestion of the central vein (CO) and a blood clot (G), infiltration of inflammatory cells (A), irregularity of the rows of hepatocytes around the central vein (H), and active transport vesicles (ATV) (H&E stain 40X).

It was also noted that some hepatic cells contained more than one nucleus, and there was an enlargement of the hepatic cells and dilation of the sinusoids containing Kupffer cells and the occurrence of bleeding in them. Necrosis, rupture and infiltration of inflammatory cells were also found in the liver parenchyma, as shown in the Figure 5.

The tissue sections of the liver of the diabetic group treated with flaxseed extract 0.1 mg/ml of body weight showed nuclei disintegration, cell enlargement, and nuclei thickening in most sections, as shown in the Figure 6.

It is also noted that the basement membrane (inner) of the central vein was peeled off and there was clear damage to the liver tissue in the diabetic group treated with flaxseed extract 0.1 mg/ml, and there was degeneration and necrosis of the liver cells, as well as the rupture and thickening of the nuclei and the appearance of edema, as shown in the Figure 7.

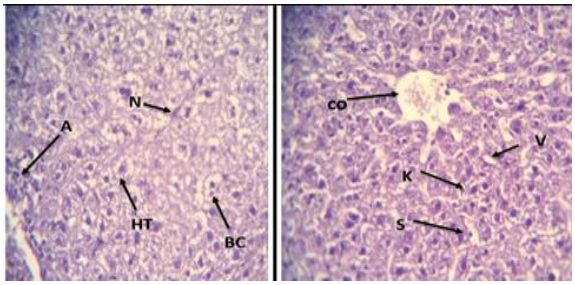

Figure 5: A cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, showing: dilation of the blood sinusoids containing Kupffer cells (S), infiltration of inflammatory cells (A), necrosis of hepatocytes, (N) binuclear hepatocytes, (BC) hyperplasia of hepatocytes (HT), Kupffer cells (K), vacuolization (V), (H&E stain 40X.

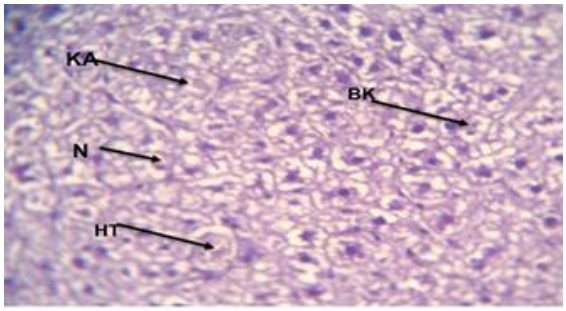

Figure 6: A cross-section of the liver tissue of male mice with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, hepatic cell hypertrophy (HT), degeneration of some nuclei (KA), necrosis of hepatic cells (N), and thickening of nuclei (BK) stained (H&E 40X).

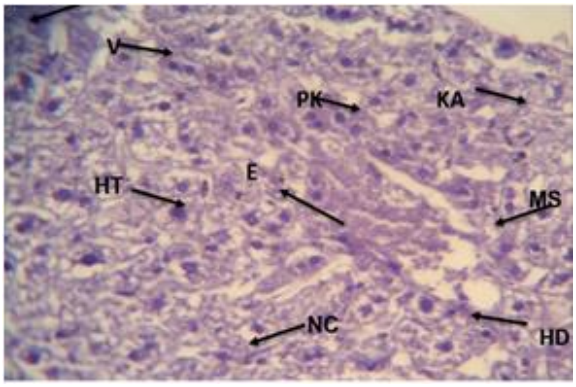

Figure 7: A cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight, sloughing of the central vein's inner membrane (MS), thickening of the

nuclei (PK), nucleolysis (KA), edema (E), necrosis of hepatocytes (NC), vacuolization (V), cell degeneration (HD), cell hyperplasia (HT) H&E 40X).

It was also noted in the sections that there was an accumulation of glycogen granules inside the liver cells, with the presence of some fluid accumulation (edema) inside the cells. It was also noted that there was a fatty cytoplasmic explosion inside the liver cell, as shown in the Figure 8.

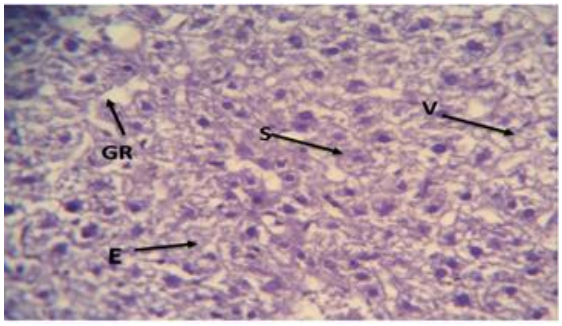

Figure 8: A cross-section of the liver tissue of male rats with induced diabetes and treated with flaxseed extract at a concentration of 0.1 mg/ml of body weight. Glycogen granules accumulated inside the hepatocytes (GR), (edema) E, cytoplasmic lipid vacuolation inside the hepatocyte (V), dilatation of the sinusoids (S H&E 40X).

3.3 Histological Changes in the Liver of Diabetic Rats Treated with Linum Usitatissimum

The results of the current study showed that the liver parenchyma of diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml of body weight for 30 days showed a central vein surrounded by more regular cells than in the two previous groups, as well as less blood congestion in the central vein of the liver, and the shape of the venous sinusoids was more regular in most sections, and there was less accumulation of Kupffer cells in them, as well as the disappearance or less death of nuclei in some liver cells, as shown in the Figure 9.

It was also noted in the diabetic group treated with flax extract that there was an improvement in the level of radial organization of cells in the liver parenchyma, which appeared almost normal, and the return of the organization of the liver cells in the form of hepatic cords. It was also noted that the division of the nuclei and liver cells occurred in a manner similar to the liver parenchyma in the control group, as shown in the Figure 10.

While some sections showed enlarged hepatic cells, degeneration of some cells and less nuclei degeneration were observed than in the two previous groups (Fig. 11).

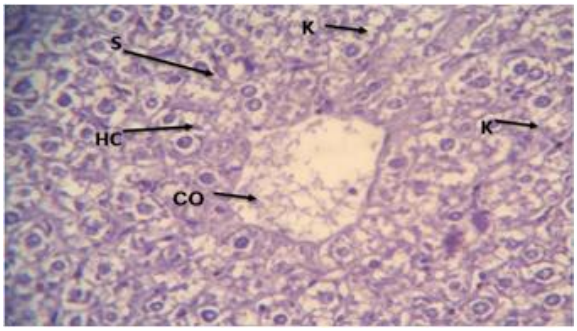

Figure 9: A cross-section of liver tissue in diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml for the central vein (CO), sinusoids, Kupffer cells (K), hepatocytes (HC), nuclei (K stained (H&E 40X).

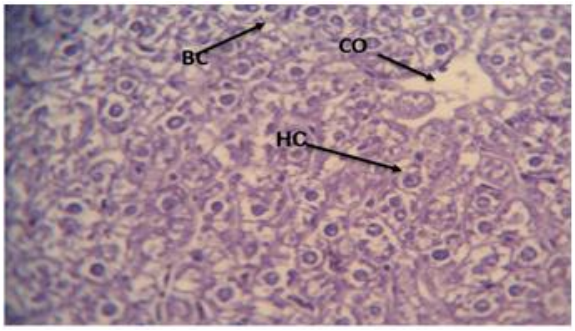

Figure 10: A cross-section of liver tissue in diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml. There was an improvement in the level of radial organization of cells in the liver parenchyma (HC), the central vein CO, and the dividing hepatocytes BC stained (H&E 40X).

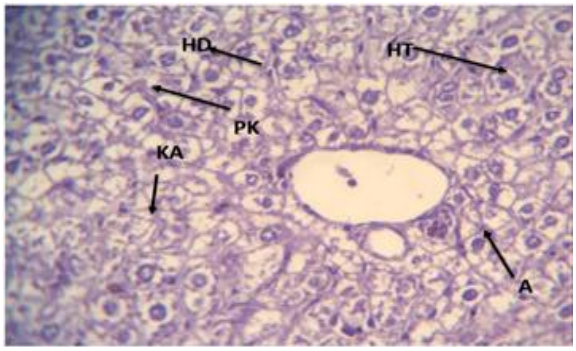

Figure 11: Transverse section of liver tissue in diabetic male rats treated with 0.2 mg/ml flaxseed extract. Note the presence of inflammatory cell infiltration and aggregation (A), nucleolar degeneration (KA), nucleolar thickening

(PK), cell hyperplasia (HT), and cell degeneration (HD). Stained (H&E 40X).

It was also noted in the results of the study in the group of diabetic male mice treated with flaxseed extract, infiltration of inflammatory cells and their accumulation around the central vein of the liver, as well as necrosis of the liver cells, and the enlargement of some liver cells, some of which were binucleated. This is illustrated in Figure 12.

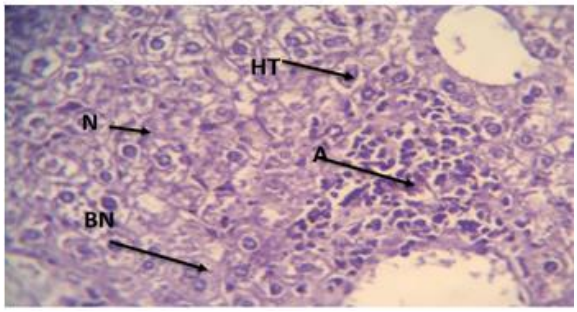

Figure 12: A cross-section of liver tissue in diabetic male rats treated with flaxseed extract at a concentration of 0.2 mg/ml Appearance of infiltration of inflammatory cells (A), hepatocyte necrosis (N), binucleated hepatocytes (BN), hepatocyte hyperplasia (HT), stained (H&E 40X).

3.4 Effect of Alloxan-Induced Diabetes on the Histological Structure of the Liver in Male Mice

The results showed that the induction of diabetes using alloxan led to changes in the liver tissue of diabetic mice compared to the liver of control mice. This is consistent with the study by [37], who attributed these changes to insulin deficiency and high blood glucose levels in mice.

It is known that alloxan is an analogue of glucose, which is what gives it the effectiveness in causing diabetes, as alloxan causes a deficiency in the body's supply of insulin after selective damage to the pancreatic beta cells (β-cells), and thus the cells will refrain from introducing glucose into the cell in the absence of insulin [38].

The results of the current study showed that the induction of diabetes with alloxan at a dose of 150 mg/ml in mice led to an increase in blood glucose levels during the experimental period compared to the glucose level in the control group animals.

This is because alloxan attacks the pancreatic beta cells, which inhibit the secretion of insulin, which leads to elevated blood glucose levels. Because insulin works to introduce glucose molecules into living cells, it balances and regulates blood glucose levels.

The results of the study are also consistent with what was stated by researchers [39], who concluded that rats with induced diabetes had an increase in blood sugar levels, which leads to severe, harmful changes in the liver, pancreas, and kidneys.

The results of our current study are also consistent with those of [40] in his study on rabbits, which found that alloxan injections lead to an increase in blood glucose levels and, consequently, dilation of the sinusoids. This may be due to weak venous flow at the hepatic vein or inferior vena cava level.

The results of the current study showed that induced diabetes in male albino mice led to the infiltration of inflammatory cells in the liver parenchyma, and the appearance of cytoplasmic rupture due to liver cell damage, which occurs for immune reasons or as a result of the toxic effect of alloxan, represented by oxidative stress resulting from the accumulation of free radicals that cause the destruction of liver cells, as well as the oxidation of lipids of the cell membrane or mitochondrial membranes, causing the appearance of an inflammatory and immune response, according to what was mentioned by [41].

The results showed the appearance of cytoplasmic fatty deposits within the liver cells, and that this accumulation of fatty materials reflects the activity of the enzyme (Insulin degrading enzyme) as a result of a decrease in the insulin hormone. Therefore, we notice the clarity of the cytoplasm and the clarity of the cell membrane [42].

While researchers [43] indicated that type 2 diabetes causes fatty degeneration within liver cells through the presence of fats in the liver parenchyma.

It was also noted through the tissue sections obtained in the current study that there was blood congestion in some areas. This is due to the weakness of blood drainage resulting from hepatic venous obstruction, which leads to a disturbance in blood flow through the hepatic parenchymal cells. This is what was indicated by [44], regarding the occurrence of blood congestion when diabetes occurs.

This is consistent with what the researcher [45] has concluded that diabetic rats suffer from hepatic cell necrosis due to poor blood supply to the liver as a result of arterial blockage and thrombosis in the hepatic artery, which leads to a lack of oxygen. This

causes the release of lysosomal enzymes and other secretory substances into the blood, which explains the occurrence of necrosis, and damage of hepatic cells.

The results of the current study also demonstrated a clear division in the parenchymal cells in this group, as demonstrated by the observation of a number of binucleated parenchymal cells. This division in hepatocytes may be attributed to an important adaptive response when the liver is afflicted with acute or chronic diseases, and when hepatocytes become inflamed or destroyed, resulting in cell division to replace these cells with new ones, thus maintaining the liver's adequate function [46].

It was also noted that some hepatic cells were enlarged and the hepatic cells were irregularly arranged in bands or cords around the central vein. This is consistent with what was stated by [47] (Farokhi et al., 2011), which confirmed the enlargement of hepatic cells, the lack of radial arrangement of hepatic cells, and the accumulation and infiltration of inflammatory cells.

An increase in the average diameter of hepatocytes was also observed. This increase is attributed to hepatocyte hypertrophy due to the accumulation of glycogen when blood glucose levels are high, coupled with a lack of insulin. This hypertrophy is then converted into glycogen by the liver cells, leading to decreased glycogenolysis due to metabolic imbalances associated with hyperglycemia, as indicated by [48].

Changes were also observed in the nuclei of some hepatocytes in the livers of male mice, such as karyomes, which occupied most of the cell volume. This is consistent with what [49] confirmed, who demonstrated in their study of the chronic toxic effects of lead on the livers of mice, that changes in the nuclei of hepatocytes occur due to increased cellular and nuclear activity in the detoxification process.

In some sections, nuclei were observed to be disintegrating, and in others, pyknosis of the nucleus occurred when compared to the control group. This result is consistent with the findings of Kumar et al., 2013, who confirmed that the cell undergoes necrosis after a toxic injury, which is characterized by degeneration, after which the cell condenses and thickens its chromatin.

In addition, karyolysis of some hepatocytes was observed in this result, as the cell appeared uniform in color due to the complete disappearance of the nucleus.

The results of this study also showed the appearance of granulomas or fine granules in the liver tissue in the form of a collection of inflammatory cells. This was indicated by [50], that granulation results from the collection of macrophages with other inflammatory cells, especially T-cells, as the appearance of these cells is evidence of a delayed immune reaction due to a toxic substance such as alloxan. The results of the current study showed that liver parenchyma in diabetic male rats treated with the extract provided protection to liver cells. Flaxseeds are one of the richest sources of the plant-based fatty acid alpha-linolenic acid (ALA), and consuming them may help prevent or treat a variety of diabetic complications, according to [51].

The results of the current study are consistent with experimental findings conducted on mice treated with flaxseed, which contains the antioxidant secoisolaricinole diglucoside (SDG). The study concluded that the development of type 1 diabetes can be prevented by approximately 71% and type 2 diabetes by 80% [52]. While a study [53] showed significant improvements in blood sugar control in type 2 diabetics who were treated for 12 weeks with flaxseed-derived lignin supplements.

Lignin may act as an antioxidant by directly scavenging free radicals and preventing lipid peroxidation, as indicated by [54].

One of the results reached by the current study is the radial rearrangement of liver cells and blood sinusoids, and this may be due to the flaxseed extract containing lignin and alpha-linolenic acid, as lignin works as an antioxidant by directly scavengers of free radicals and preventing lipid peroxidation, and this is what was reached [54].

This study is consistent with what Ventura et al., 2023 reported in their study on fish oil, eicosapentaenoic acid, and docosahexaenoic acid, where increased hepatic LDL receptor activity resulted in decreased plasma LDL levels.

As noted by Ahmed, 2011, peroxisome proliferator-activated receptor alpha (PPAR-α), a transcription factor that plays an important role in controlling carbohydrate and lipid levels, is significantly upregulated by a flaxseed oil diet [55].

This may be because alpha-linolenic acid is found in large quantities in flax seeds, so through a series of reactions, alpha-linolenic acid is metabolized into docosahexaenoic acid and eicosapentaenoic acid Zahid and Lamis, 2017.

The results showed the presence of some pathological histological changes, including small dilation of the sinuses. This is consistent with the findings of [56], who indicated that the reason for this is due to a decrease in venous blood flow and central vein occlusion, which may lead to an increase in venous pressure, which leads to dilation of the sinusoids. Or it may be due to chemicals that lead to a decrease in venous blood flow as a result of inflammation, which then leads to the expansion of the sinusoids [57], while [58] mentioned in their study that acidic liver infiltration with or without necrosis is due to a reaction to chemicals.

5 CONCLUSIONS

The results of this research clearly indicate that the daily intake of aqueous flaxseed Linum usitatissimum extract has a protective and healing impact on the liver tissue of diabetic mice induced by alloxan. Histological analyses indicated that untreated diabetic mice showed significant liver damage, characterized by congestion, necrosis, fatty degeneration, cytoplasmic rupture, and infiltration of inflammatory cells indicative of liver dysfunction associated with uncontrolled hyperglycemia and oxidative stress. Nevertheless, the diabetic groups receiving flaxseed extract, especially at the elevated concentration of 0.2 mg/ml, showed significant enhancements in liver structure.

These enhancements involved diminished central vein congestion, normalized sinusoidal structure, reduced Kupffer cell aggregation, and a reinstatement of radial hepatocellular organization. The extract's capacity to reduce liver damage could be linked to its abundant bioactive compounds like alpha-linolenic acid, lignans, and secoisolariciresinol diglucoside (SDG), recognized for their antioxidant, anti-inflammatory, and insulin-sensitizing effects. The noted hepatoprotective benefits indicate that flaxseed extract may act as a possible complementary treatment option in addressing diabetes and its liver-related complications. Its natural source, relative safety, and various mechanisms of action render it a strong contender for additional pharmacological advancement.

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