

# Effect of Flaxseed Extract on the Liver Histological Structure in Streptozotocin Induced Diabetic Rats

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## ABSTRACT

**Introduction:** Diabetes mellitus has become a serious warning to mankind health all over the world. The management goal of diabetes is to keep blood glucose levels as close as possible to healthy individuals. Medications used to treat diabetes are usually associated with complications and may cause different side effects. Many traditional anti-diabetic plants have become popular in the management of diabetes mellitus. Flaxseed has been used as traditional medicine for centuries. **Objective:** This study aimed to evaluate the hepatoprotective effects of flaxseed extract in streptozotocin (STZ)-induced diabetic rats. **Methods:** Diabetes mellitus was induced in Sprague-Dawley rats using a single injection of streptozotocin (60 mg/kg i.p.). The rats were divided into five groups of 8 rats each. Group NC, normal control rats; Group NF, normal rats treated with flaxseed extract (400 mg/kg); Group DC, diabetic control rats; Group DG, diabetic rats treated with glibenclamide (0.6 mg/kg); Group DF, diabetic rats treated with flaxseed extract (400 mg/kg); for 4 weeks. **Results:** There were significant increase in relative liver weight, blood glucose levels in DC group comparing to NC group ( $p < 0.05$ ). The disturbance of these parameters was ameliorated in DF and DG groups. Histological observation revealed congestion of central veins, degeneration of hepatocytes, and reduced glycogen granules in DC group. These pathological changes were ameliorated in the flaxseed extract and glibenclamide treated rats. **Conclusion:** Flaxseed extract may represent a candidate alternative treatment to control diabetes mellitus and its related hepatopathy.

**KEYWORDS:** Diabetes mellitus, glibenclamide, flaxseed, liver, rats, streptozotocin

## INTRODUCTION

Diabetes is one of the most common chronic diseases worldwide that affects individuals worldwide and is one of the major causes of death. Diabetes is a major threat to global public health, and the numbers of diabetic patients are rapidly increasing world-wide.<sup>1</sup> In 2013, the international diabetes federation approximated that 382 million adults had diabetes and 5.1 million deaths occur annually due to diabetes; the prevalence of diabetes has doubled in the last three decades and is projected to continue rising to 592 million cases by 2035.<sup>2</sup> Diabetes is characterized by hyperglycaemia,

glucose intolerance, insulin insensitivity or absolute absence of insulin due to damage of the pancreatic  $\beta$ -cells.<sup>3</sup> This frequently results in severe metabolic imbalances and pathological changes in many tissues.<sup>4</sup>

Diabetes mellitus is associated with a number of common symptoms, such as polyurea and polydipsia, and long term complications, including retinopathy, neuropathy, nephropathy, and atherosclerosis. Recent studies has reported that patients with diabetes have a high susceptibility for different liver diseases, including non-alcoholic fatty liver disease, abnormal liver enzymes, acute liver failure, cirrhosis, and hepatocellular carcinoma.<sup>5</sup>

Experimental studies have demonstrated many histopathological changes in the liver; such as hepatocytic necrosis, vacuolations of the cytoplasm and the nucleus, fragmentation of hepatocytes and sinusoids, fatty degeneration and vascular congestion of the central vein.<sup>6,7</sup>

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Several therapeutic agents have been developed to manage diabetes and its associated complications. However, the current conventional therapies may cause many side effects, the patients may develop resistance, and they do not adequately inhibit the development of the associated complications.<sup>8,9</sup> This necessitates discovery of more potent therapies. Certain plant extracts have been used traditionally to manage diabetes and several more are under study for their potential in managing hyperglycaemia and its complications. The herbal therapies have potential to adequately manage the disease while eliminating the adverse effects associated with conventional therapies.<sup>10,11</sup>

Flaxseed is a crop that is grown worldwide due to its associated health benefits. It contains many biologically active components including ALA, fibre, protein and phenolic compounds such as lignans.<sup>12</sup> Linolenic acid is helpful in controlling the level of circulating bad cholesterol, and hence prevents the development of cardiac diseases. Flaxseed is one of the richest sources of lignans, which have been studied extensively due to their therapeutic potential.<sup>13</sup> Lignans have been shown to prevent the development of various human cancers.<sup>14</sup> Progress is being made in its use in the management of diabetes mellitus and its complications. The underlying mechanisms of the anti-diabetic effect of lignans involve enhancing glycemic control, increasing the insulin sensitivity, and inhibition of hepatic gluconeogenesis.<sup>3,15</sup> The present study has been designed to study the protective effect of flaxseed extract on the histological changes of liver

in STZ- induced diabetic rats.

## MATERIALS AND METHODS

### Flaxseed

Flaxseeds were obtained from the Department of Botany, Faculty of Science; Philadelphia University, Jordan. Two hundred grams of the defatted powder was macerated with 1.2L of 70% ethanol solvent for 24 hours at 30°C. The extract then was filtered by a sand core funnel and concentrated at 40°C and 90 rpm using a rotary evaporator, followed by freeze drying for 4 days at -80°C. The obtained crude extract was diluted with distilled water to yield light yellow syrup which was administered to the experimental animals at a dose of 400 mg/kg body weight.

### Animals and experimental design

Forty healthy adult male Sprague Dawley rats aged from 10 to 12 weeks old (weighing 200-250g) were used in this study. The animals were housed in polypropylene cages under the standard laboratory conditions (adequate cross ventilation; temperature: 24 ± 2°C; 12:12 hrs light: dark cycle; relative humidity: 46-79%), and were allowed two weeks period to acclimatize prior to the experiment. The rats were maintained on standard commercial dry pellet diet and water ad libitum. The animals were randomly divided into five groups of 8 animals in each group as described in Table 1.

**Table 1:** Animal grouping

No.	Group initial	Group definition	Treatment
1	NC	Normal control rats	- Pellets and water
2	NF	Normal rats treated with flaxseed	- Pellets and water - Flaxseed 400 mg/kg
3	DC	Diabetic control rats	- Pellets and water - STZ 60mg/kg
4	DG	Diabetic rats treated with glibenclamide	- Pellets and water - STZ 60mg/kg - Glibenclamide 0.6 mg/kg
5	DF	Diabetic rats treated with flaxseed	- Pellets and water - STZ 60mg/kg - Flaxseed 400 mg/kg

The rats were fasted overnight (12-14 hours). The animals were given a single intraperitoneal STZ injection of 60 mg/kg of body weight dissolved in citrate buffer PH 5.4. In order to prevent hypoglycemia, STZ-treated animals received a solution of 5% glucose instead of normal drinking water over 24 hours following the treatment. Normal control rats received an equivalent volume of distilled water. On the fifth day of post-induction, Accu-Chek Performa glucometer was used to measure the level of fasting blood glucose.

Only rats with high level of fasting blood glucose (more than 14 mmol/L) were considered as diabetic rats. The sixth day of diabetes induction was considered as the first day of treatment. Syringe and special designed metal ball-ended needle were used to feed the rats daily by gavage. NF and DF groups received 400 mg/kg flaxseed extraction diluted with distilled water. DG group were fed with glibenclamide 0.6 mg/kg, and the remaining animals (NC and DC groups) were untreated with flaxseed or glibenclamide.

The measurement of body weight (g) for all rats was recorded on the 1<sup>st</sup>, 5<sup>th</sup>, 10<sup>th</sup>, 20<sup>th</sup>, and 30<sup>th</sup> day of the experiment using Mettler Toledo SB16000 Balance (Painesville, OH, USA). At sacrifice, the measurement of liver relative to weight was determined for all rats according to the equation:

$$\text{The relative percentage of liver weight} = \frac{\text{liver weight}}{\text{body weight}} \times 100$$

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC), International Islamic University Malaysia (IIUM). The experiment was conducted according to the Guidelines for the Care and Use of Laboratory Animals of the Kulliyah of Medicine, IIUM.

### Histological Studies

At the end of the experimental period, the tested animals were sacrificed using Ketamine as an aesthetic. Liver specimens were rapidly removed, fixed in 10% formal saline for 72 hours, dehydrated through graded alcohols and cleared using two changes of xylene and embedded in paraffin wax. Serial transverse sections of 4-5 micron thickness were prepared using the microtome, stained Haematoxylin and eosin (H & E), others stained by periodic Acid-Schiff stain (PAS) with salivary amylase application.

### Statistical analysis

Data analyses were performed using statistic software IBM SPSS Statistics 21 (IBM Corporation, NY, USA). Data were expressed as mean  $\pm$  standard deviation (SD). The results were considered as

significant at P value less than 0.05. Student *t*-test were used to compare the data within the group. Additionally, one way analysis of variance (ANOVA) was used to compare the data between experimental groups, followed by Tukey's post hoc test for multiple groups' comparison.

## RESULTS

### *Effects of flaxseed extract on body weight and liver weight*

In normal groups, NC and NF, the rats maintained a steady increase in body weight and reached to about  $290 \pm 9.78$  g on day 30 compared to  $227 \pm 7.97$  g on the first day of the study (Figure 1. A). No significant difference ( $p > 0.05$ ) in body gained weight was observed between NC and NF groups during the whole study period. In DC group, the rats exhibited a slight increase of mean body weight from  $227 \pm 7.94$  g on day 1 to about  $229 \pm 6.51$  g on day 5, followed by a progressive decrease to reach  $196 \pm 12.04$  g on day 30. The reduction of mean body weight in DC group was significant when compared to NC group ( $p < 0.05$ ).

Treatment with glibenclamide and flaxseed extract seems to reduce the rate of weight loss that has been noticed in untreated STZ diabetic rats. The mean body weight in DG group progressively increased from  $229 \pm 6.76$  g on day 1 to  $260 \pm 9.05$  g on day 30. While in DF group, it gradually decreased from  $229 \pm 7.21$  g on day 1 to  $206 \pm 8.29$  g on day 30. The ameliorative effect of glibenclamide on body weight was statistically significant compared to DC group ( $p < 0.05$ ). However, the alteration of body weight in DF group didn't reach a significant level in comparison with DC group ( $p > 0.05$ ) (Figure 1. A).

The absolute liver weight was nearly similar in all groups. However, the relative weight of liver with respect to body weight was higher in diabetic groups than in normal groups (Table 2).

The relative weight of liver in both NC and NF groups was approximately  $2.94 \pm 0.22$  g. DC group was presented with a relative liver weight of  $4.40 \pm 0.28$  g, which is significantly higher than that in NC group ( $p < 0.05$ ). The increase in liver relative weight in diabetic rats was ameliorated in DF and DG groups to be  $4.31 \pm 0.45$  g and  $3.34 \pm 0.25$  g respectively, the latter was significantly less than in DC group ( $p < 0.05$ ) (Table 2).

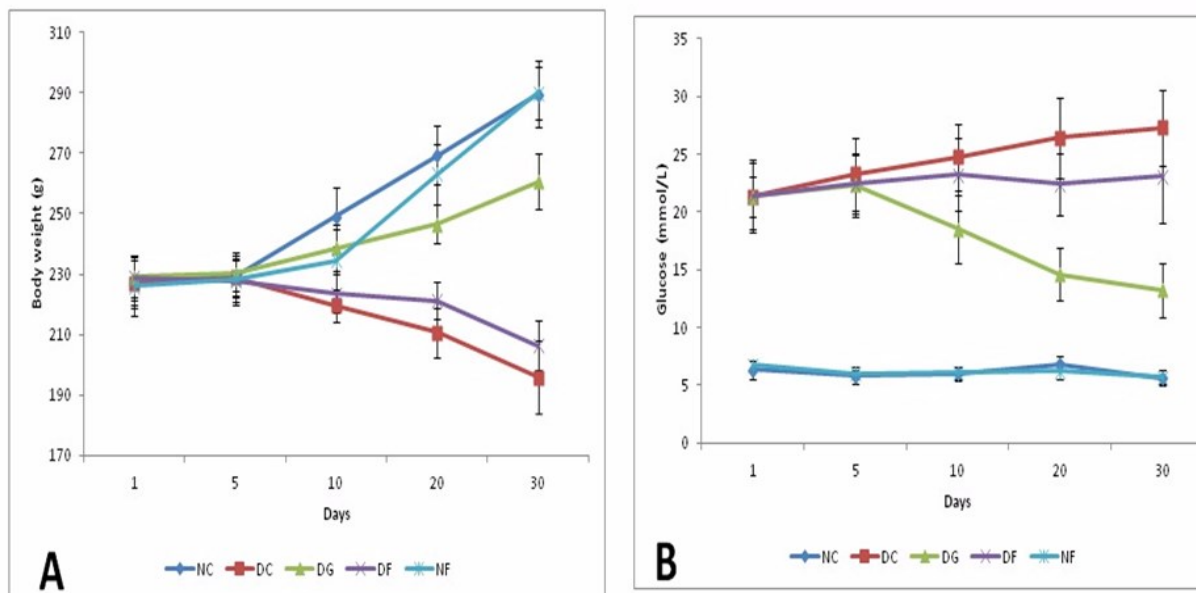


Figure 1. A. Mean  $\pm$ SD of body weight of all groups during different days. B. Mean  $\pm$  SD of fasting blood glucose level of all groups during different days.

Table 2 Mean body weight, absolute liver weight and relative liver weight of experimental groups\*

Group	Body	Absolute liver w	Relative liver weight
NC	289.6 $\pm$ 10.89	8.50 $\pm$ 0.41	2.94 $\pm$ 0.17
DC	195.8 $\pm$ 12.04	8.60 $\pm$ 0.26	4.40 $\pm$ 0.28
DG	260.7 $\pm$ 9.05	8.69 $\pm$ 0.63	3.34 $\pm$ 0.25
DF	206.3 $\pm$ 8.29	8.88 $\pm$ 0.65	4.31 $\pm$ 0.45
NF	290.1 $\pm$ 8.67	8.50 $\pm$ 0.59	2.94 $\pm$ 0.27

**Effects of flaxseed extract on fasting blood glucose level**

In normal groups, NC and NF, the rats have consistently maintained their fasting blood glucose levels close to 6  $\pm$  0.62 mmol/L. On the other hand, all diabetic groups had much higher levels of fasting blood glucose following administration of STZ (Figure 1. B).

The rats in the diabetic groups DC, DG and DF, had an initial mean fasting blood glucose level of approximately 21  $\pm$  2.59 mmol/L. Subsequently, in DC group, these levels were raised up to 27  $\pm$  3.30 mmol/L on day 30. The elevation of fasting glucose level in DC group was statistically significant as compared to NC group (p<0.05) (Figure 1. B).

Treatment of diabetic rats with glibenclamide and flaxseed extract has reduced the elevated levels of

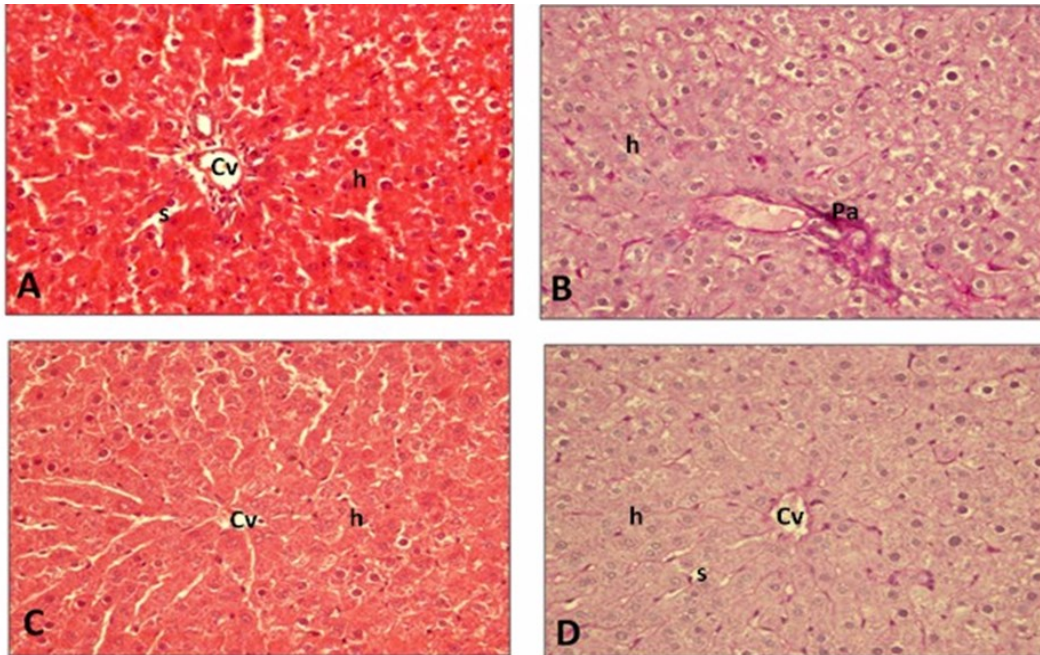
fasting blood glucose throughout the study period. In DG group, the mean fasting blood glucose slightly increased to 22  $\pm$  2.74 mmol/L on day 5, then dropped to 18  $\pm$  2.92 mmol/L on day 10, and continuously decreased to reach 15 $\pm$ 2.29 mmol/L and 13 $\pm$ 2.37 mmol/L on days 20 and 30, respectively. The reduction of fasting glucose level in DG group was statistically significant as compared to DC group (p<0.05).

In DF group, the rats showed a slight elevation in the mean fasting glucose level up to 23  $\pm$  3.14 mmol/L on the 10<sup>th</sup> day of study period, followed by a stable glucose level until the end of the experiment. However, the hypoglycemic activity of flaxseed extract didn't reach to a significant level compared to the DC group (p>0.05) (Figure 1. B).

**Effects of flaxseed extract on liver histopathological changes**

Liver sections obtained from normal groups, NC and NF, revealed normal histological architecture; with regular hepatic lobules with central veins and peripheral portal areas were observed. Moreover, regular distinct hepatocytes with sinusoidal spaces were extending radially from the central veins to the boundaries of portal areas.

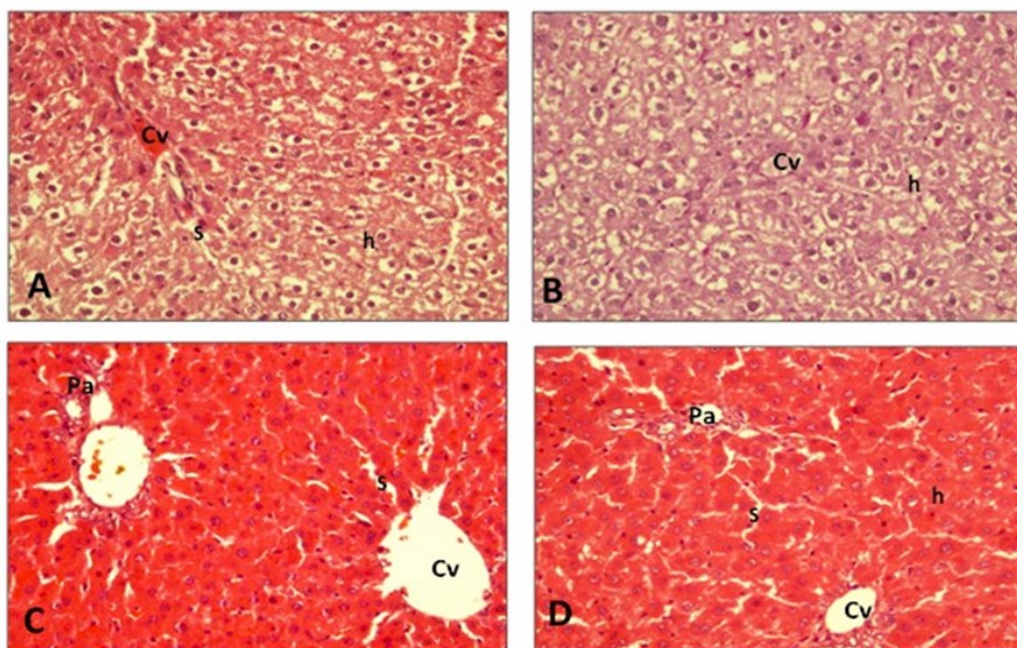
The sinusoids were lined by endothelial cells containing Kupffer cells (Figure 2, A &C). PAS stained sections of NC and NF groups showed abundant distribution of glycogen granules within the cytoplasm of hepatocytes (Figure 2, B &D).



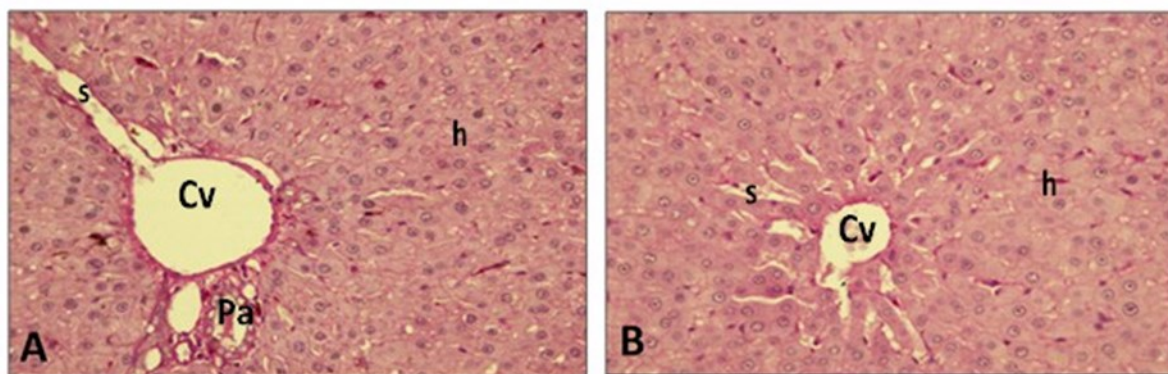
**Figure 2.** Liver sections of: **A.** normal control rats showing normal histological structure, regular distinct hepatocytes with sinusoidal spaces arranged radially around the central vein. **B.** normal control rats with normal histological structure and numerous glycogen granules distributed throughout the slide area and portal area. **C.** normal rats treated with flaxseed extract illustrating normal histological structure of hepatocyte, blood sinusoid. **D.** normal rats treated with flaxseed extract showing normal histological structure and PAS positivity granules in most of the hepatocytes. “Cv, central vein; h, hepatocyte; Pa, portal area; s, sinusoid”. (A and C, H&E; B and D, PAS, original magnification X40).

In diabetic (DC) group, liver sections stained with H&E and PAS showed noticeable pathological changes as compared to normal control group. These changes were indicated by the presence of congested central veins, degenerated and irregular-shaped hepatocytes, cloudy swelling and vacuolization of cytoplasm (Figure 3, A & B). Additionally, the hepatocytes of diabetic untreated rats were further presented with a marked reduction of glycogen granules (Figure 3, B).

However, treatment with flaxseed extract and glibenclamide has reduced the severity of these changes. Sections obtained from DF and DG groups showed normal hepatocytes with no signs of cytoplasmic cloudy swelling or vacuolization, less congestion of central veins, and normal contents of glycogen granules, indicating a restoration of normal liver architecture (Figure 3, C & D) (Figure 4. A & B).



**Figure 3.** Liver sections of: **A & B.** STZ diabetic rats showing degeneration of hepatocytes, vacuolization of cytoplasm, and cloudy swelling, congested central vein and loss of glycogen granule in B. H&E, X400. **C.** diabetic liver rats treated with flaxseed extract presented with less pathological changes and improved liver architecture. H&E, X400. **D.** diabetic rats treated with glibenclamide demonstrating restoration of the normal liver structure. “Cv, central vein; h, hepatocyte; Pa, portal area; s, sinusoid”. (A, C & D, H&E; B, PAS, original magnification X40).



**Figure 4.** Liver sections of diabetic rats treated with (A) flaxseed extract and (B) glibenclamide showing restoration of liver architecture and normal glycogen granule contents in the hepatocytes. “Cv, central vein; h, hepatocyte; Pa, portal area; s, sinusoid”. (PAS, original magnification X40).

## DISCUSSION

Diabetes mellitus is one of the most common causes of liver damage (Manna et al., 2010). It has been correlated with the entire spectrum of liver diseases, including abnormal levels of liver enzymes, non-alcoholic fatty liver disease and liver cirrhosis and carcinoma.<sup>16</sup> There is clear evidence that oxidative stress during diabetes plays an important role in the induction of these diseases (Adeyemi et al., 2014). The conventional treatment of diabetes mainly involves a sustained reduction of blood glucose levels using different agents, such as sulfonylurea and thiazolidinediones.<sup>17</sup> However, these synthetic drugs do not adequately inhibit the development of the associated complications, and furthermore, they may cause a variety of side effects.<sup>18</sup> Hence, there is a demand for alternative therapies to manage diabetes mellitus and its associated complications.<sup>17</sup>

Certain herbal extracts have shown the ability to treat diabetes and prevent the development of its long-term complications without causing adverse effects. Flaxseed is a rich source of lignans, which has antidiabetic and antioxidant properties.<sup>19,20</sup> Considering these properties, this study was conducted to evaluate the hepatoprotective effect of flaxseed extract in a model of STZ-diabetic rats. Changes in different parameters, including body weight, liver relative weight, blood glucose level and histopathological picture were determined in the diabetic rats treated with flaxseed extract, and compared with those receiving a commercially available drug, glibenclamide.

The present study showed a significant increase of fasting blood glucose levels in the untreated diabetic rats. Oral administration of flaxseed extract reduced the levels of fasting blood glucose in diabetic rats; which is consistent with earlier reports that demonstrated antidiabetic activity of flaxseed and attributed it to its content of lignans that inhibit the hepatic gluconeogenesis.<sup>3,15</sup> Treatment with glibenclamide has also decreased the blood glucose levels in diabetic rats, and it was

found to be more effective in comparing with flaxseed extract.

In the present study, STZ-induced diabetic rats showed severe loss of body weight. This reduction is due to the degradation of structural proteins during diabetes mellitus.<sup>21,22</sup> However, there is an increase in liver weight in proportion to the body weight. Liver hypertrophy during diabetes mellitus might be the result of an increased hepatic accumulation of triglycerides.<sup>21</sup> These findings are in agreement with the previous studies which reported increased liver relative weight in STZ-induced diabetic rat.<sup>6,21,23</sup> Treatment with glibenclamide and to a lesser extent flaxseed extract has reversed the progressive changes of body weight and liver weight in diabetic rats. The capability of these treatments to protect the change of body weight and liver weight seems to be due to their ability to reduce hyperglycemia.

Evaluation of liver sections obtained from STZ-diabetic rats revealed the presence of several histopathological changes including congestion of central veins, degeneration of hepatocytes, reduction of glycogen granules, swelling and vacuolization of cytoplasm. Our findings are in good agreement with several previous studies, which reported similar histopathological changes following induction of diabetes using STZ injection.<sup>6,24,25</sup> However, less pathological changes and improved liver architecture were observed in flaxseed extract and glibenclamide treated diabetic rats, indicating protective effects of these treatments against the hepatic changes associated with diabetes.

The mechanism of the ameliorative effect of flaxseed extract on the histopathological changes in STZ-induced diabetic rats is still not clear. However, flaxseed is one of the richest sources of the plant-based  $\omega$ -3 fatty acid, alpha-linolenic acid (ALA), and its ingestion may help in preventing or treating a variety of diabetic complications.<sup>26</sup> Dietary intake of  $1.25 \pm 0.07$  g ALA among adults' diabetic patients with peripheral neuropathy reduced the odds of

peripheral neuropathy.<sup>27</sup> Experimental study in Zucker diabetic fatty Gmi-fa/fa female rats showed that Secoisolariciresinol diglucoside (SDG) antioxidant from flaxseed can prevent the development of type 1 diabetes by approximately 71% (90) and type 2 diabetes by 80%.<sup>28</sup> Significant improvements were observed in glycemic control in type 2 diabetic patients treated for 12 weeks with lignin supplementation derived from flaxseed.<sup>15</sup> Furthermore, lignans act as antioxidants through the direct scavenging of radicals and by preventing lipid peroxidation.<sup>3</sup> Further studies are needed to investigate the effects of ALA, lignin and antioxidants from flaxseed on liver morphology in experimental diabetic animals.

## CONCLUSION

It is concluded that daily treatment with flaxseed extract improves blood glucose level, and histopathological status of STZ-induced diabetes mellitus. The experimental evidence obtained from this study indicates that flaxseed extract represents a candidate alternative treatment to control diabetes mellitus and its related hepatopathy

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## Conflict of interest statement

The authors declare that they have no personal or financial conflict of interest.

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