

# Synthesis of Flaxseeds Aqueous Extract and Study it Effective on the Behavior Diabetic Mice

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## ABSTRACT

Diabetes mellitus is a group of metabolic diseases involving carbohydrate, lipid, and protein metabolism. The present study was carried out to investigate the Flaxseeds Aqueous Extract effective composition on the Diabetic Mice. Flaxseeds (*Linum Usitatissimum L.*) is a rich source of different types of phenolic such as lignin, phenolic acids, flavonoids, henylpropanoids and tannins. The Flaxseeds Aqueous Extract composition was determined by UV-vis, FT-IR and LC-Mass techniques. FTIR is confirm present Phenols, lignin, the plant fatty acid alpha-linoleic acid (ALA) and Glycosides Polyphenols which their act Diabetes mellitus reduced, flaxseeds water extract components were identified using Gas Chromatography- Mass Spectrometry (LC/MS). The aqueous extract also showed strong antioxidant capability, which may help explain its antidiabetic action by lowering oxidative stress in diabetes tissues. Overall, the results point to the presence of bioactive chemicals in flaxseed extract that may have therapeutic benefits against diabetes mellitus.

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## 1. INTRODUCTION

Diabetes Mellitus is a group of metabolic diseases that involve the metabolism of carbohydrates, lipids, adipose tissue, and proteins. It is characterized by persistent hyperglycemia resulting from defects in insulin secretion, action, or both [1,2]. In Iraq, 1.4 million people suffer from diabetes, and the prevalence of type 2 diabetes ranges from 8.5 to 13.9%. Diabetes causes serious and multiple complications if left untreated, including serious health problems affecting both small and large blood vessels. Diabetes has been associated with severe and life-threatening complications [3]. Vascular disorders are associated with diabetes mellitus, which is due to poor digestion of sugars and proteins. Since chemotherapy causes side effects, alternatives to drugs have been sought, and medicinal plants have been used. Medicinal plants play an important role in the treatment of diabetes, as medicinal plants have significant anti-diabetic properties without harmful side effects, as they are rich sources of anti-diabetic compounds such as flavonoids, alkaloids, phenols, and tannins, which improve the efficiency of pancreatic tissue by increasing insulin secretion or reducing glucose absorption in the intestine [4]. Among these plants are the seeds of the flax plant, whose scientific name is *Linum usitatissimum*. It is an annual, biennial, or perennial plant that reaches a height of about a meter. It has a slender stem, lanceolate leaves, and blue flowers. Its seeds are brown and oily. It is an oil crop belonging to the flax family Malpighiales and the Linaceae family. The origin of flax (*Linum usitatissimum*) is uncertain, as it is one of the oldest cultivated plants. However, it is accepted that the original homeland of flax extends from the eastern Mediterranean to India. It is believed that its use began in the Tigris and Euphrates River basin and the coastal part of the Levant [5]. It is currently cultivated all over the world for its fibers, seeds, and oil. Recent studies have demonstrated that the seed powder drink is a laxative and diuretic [6]. Flaxseed oil, fiber, and lignin have potential health benefits, including reducing cardiovascular disease, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune disorders, and neurological disorders, which has led to the diversification of the uses of the flaxseed plant [7].

Recent studies have demonstrated that flaxseed lowers cholesterol due to the very high content of oxalinolenic acid (C18:3n-3, an omega-3 fatty acid). Based on the benefits of the omega-3 fatty acid in fish oil against cardiovascular disease, flaxseed oil is marketed as a health food, noting that the omega-3 fatty acid in flaxseed oil is different from the omega-3 fatty acid found in fish oil [8]. The present study aimed to the aqueous extract preparation of flax seeds and investigate it effect on behavioral changes in mice with diabetes.

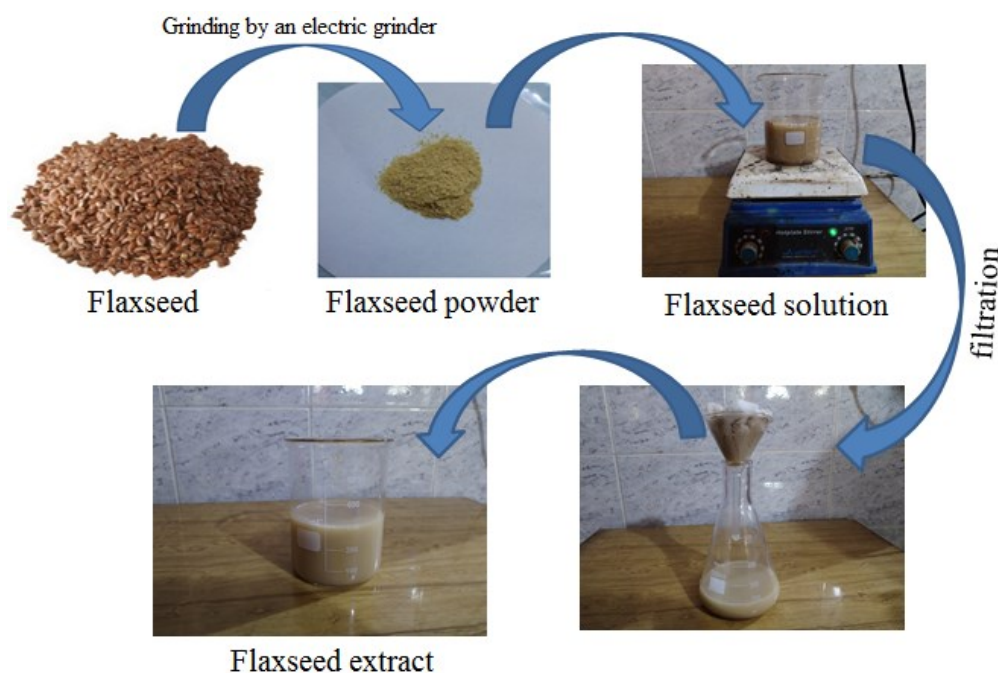
## 2. METHOD

### 2.1. MATERIALS

Flaxseeds were purchased from the local market at Diyala governorate in Iraq. All chemicals involved in the experiments are reagent grade such as chloroform ( $\text{CHCl}_3$ ), formalin was purchased from Sigma Aldrich and BDH, and Deionized water was used for the experiment. these seeds were ground into fine powder using an electric and characterization them by UV-VIS spectrometer (Shimadzu 1900, Japan), Fourier-transform infrared spectroscopy (FTIR, Shimadzu1800, Jaban) and Liquid chromatography–mass spectroscopy (Shimadzu LCMS 2010 A).

### 2.2. Preparation of the Flaxseed extract

The extraction was carried out using Deionized water as solvents; 50 g of Flaxseed grain were put in 500 mL deionized water at room temperature with stirring After 24 hours, the water turned yellowish, indicating Flaxseed extract formation in the water. The filtered extract was by funnel and cut gauze to remove all contamination; this extract would be used as a treating agent for mice Diabetes Mellitus, [Figure 1](#), [9].



[Figure 1](#). Synthesis of Flaxseed extract.

## 3. RESULTS AND DISCUSSION

### 3.1. Characterization of Flaxseed extract by UV-Vis Spectra

The raw flaxseed powder and its prepared aqueous extract were characterized using UV-Vis spectroscopy, scanning the wavelength from 200 nm to 400 nm. [Figure 2](#) shows the UV-Vis spectra of the raw flaxseed powder and its prepared aqueous extract, which were obtained using 500 mL of distilled water at room temperature for 72 hours. The visible absorption bands between 190-220 nm and 246-288 nm for the raw flaxseed powder and its aqueous extract confirm the presence of phenolic acids and flavonoids. The absorption band also indicates the presence of lignin and its derivatives in the extract prepared at a wavelength of 280 nm [10]. In addition, the UV spectrum clearly demonstrates the similarity between the raw flaxseed powder and its aqueous extract, as they contain the same chemical compounds that absorb the UV spectrum.

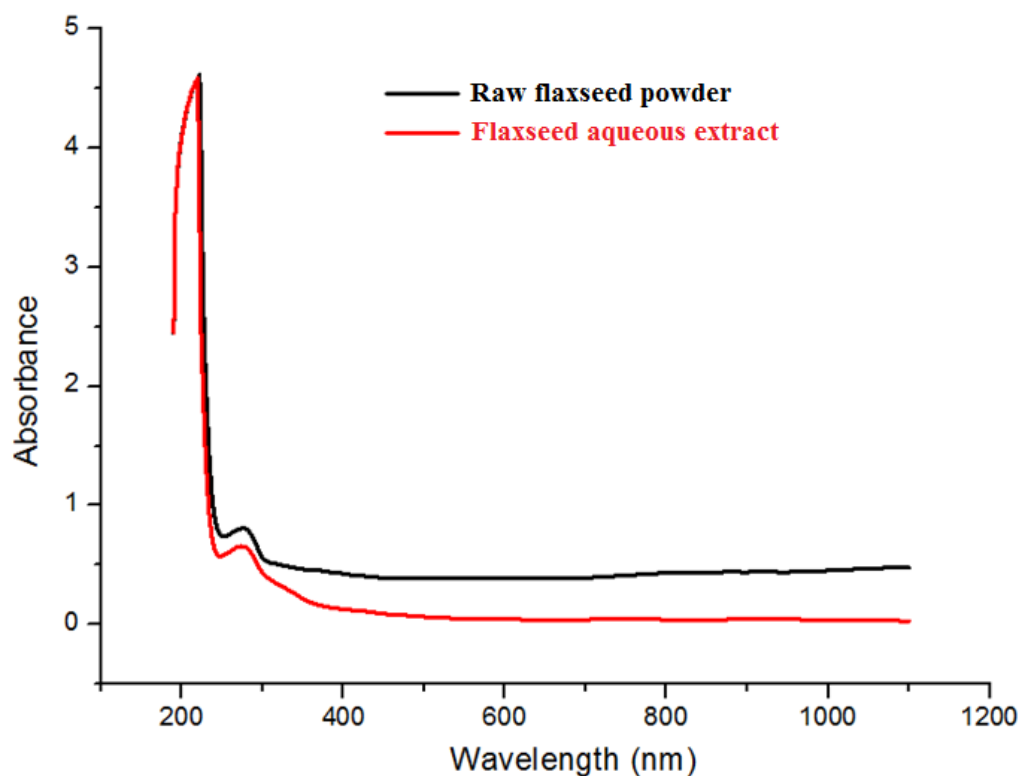


Figure 2. UV-vis spectrum of aqueous extract of flaxseed and flaxseed powder.

### 3.2. FT-IR Analysis of Flaxseed extract

An infrared spectroscopic study was conducted using the Fourier transform technique to identify the chemical compounds potentially present in the raw flaxseed powder and its aqueous extract based on the active groups involved in the composition of these chemical compounds. Figures 3 and 4 show the Fourier transform infrared spectra of the raw flaxseed powder and its aqueous extract, respectively. We note the similarity of the identified compounds through the peaks present in the FT-IR spectra of raw flaxseed powder and its aqueous extract, as both spectra confirm the presence of phenols, lignin, and the plant fatty acid alpha-linolenic acid (ALA) through the appearance of the wide (O-H) group stretch frequency at  $3307\text{ cm}^{-1}$  and  $3356\text{ cm}^{-1}$ . The noticeable presence of the hydroxyl group (O-H) can confirm the presence of phenols and phenolic glycosides Polyphenols, whose antioxidant properties help protect the body from diabetes [11], and lignin, which have anti-diabetic and antioxidant properties by directly scavenging free radicals and preventing lipid peroxidation [12]. Flavonoids, which are considered blood sugar-lowering substances, have a general chemical structure in the form of a carbon skeleton called a phenyl benzopyran nucleus. Depending on the position of the aromatic ring (phenyl) attachment to the benzopyran moiety, the type of flavonoid is determined. Flax seeds are a rich source of lignin, as the FT-IR spectrum of flax seed powder and its aqueous extract confirmed that they are rich in lignin and flavonoids by identifying the active groups that indicate the presence of flavonoids, such as the frequency of the carbonyl group stretching ( $\text{-C=O}$ ) of the benzopyran ring at  $1656\text{ cm}^{-1}$  and at  $1655\text{ cm}^{-1}$ , which also goes back to the frequency of the  $\text{C}_2=\text{C}_3$  bond stretching of the aromatic ring carbon [13,14] and the frequency of the ( $\text{C=C}$ ) group of the aromatic ring at  $1546\text{ cm}^{-1}$ ,  $1456\text{ cm}^{-1}$  and  $1454\text{ cm}^{-1}$ , in addition to the aliphatic ( $\text{-C-H}$ ) stretching frequencies which are within the carbon skeleton of flavonoids, lignin and plant fatty acid alpha-linolenic acid (ALA) at  $3012\text{ cm}^{-1}$  and  $2927\text{ cm}^{-1}$ , while the bending frequency of the same group appeared at  $1392\text{ cm}^{-1}$  and  $1318\text{ cm}^{-1}$ . While the intermediate peaks at  $1160\text{ cm}^{-1}$  represent the in-plane bending vibration frequency of the (O-H) group of phenolic and at  $1122\text{ cm}^{-1}$  the ether group (O-C-O) stretching frequency present in the structure of lignin and flavonoids within the benzopyrane ring. The frequencies at  $720\text{ cm}^{-1}$ ,  $753\text{ cm}^{-1}$ , and  $774\text{ cm}^{-1}$  are particularly associated with the bending vibration of the C-O and C-H groups in the aromatic rings and the characteristic C-O vibration of glycosides at  $1094\text{ cm}^{-1}$ ,  $1097\text{ cm}^{-1}$ , and  $1042\text{ cm}^{-1}$ , in addition to the out-of-plane bending of the C-H groups at  $861\text{ cm}^{-1}$  [15] for both spectra. It is worth noting that further studies are needed to investigate the effects of alpha-linolenic acid, lignin, and antioxidants from flaxseed on liver morphology in diabetic animals in experiments [16].

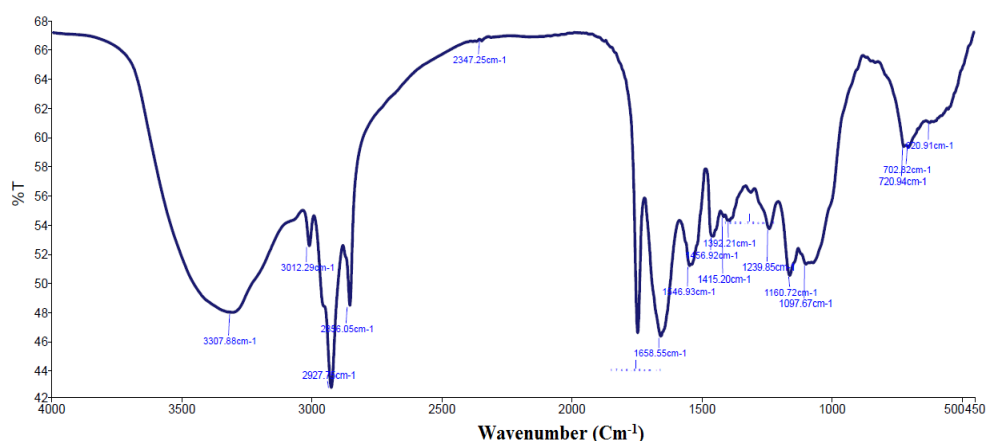


Figure 3. FT-IR spectrum of raw flaxseed powder.

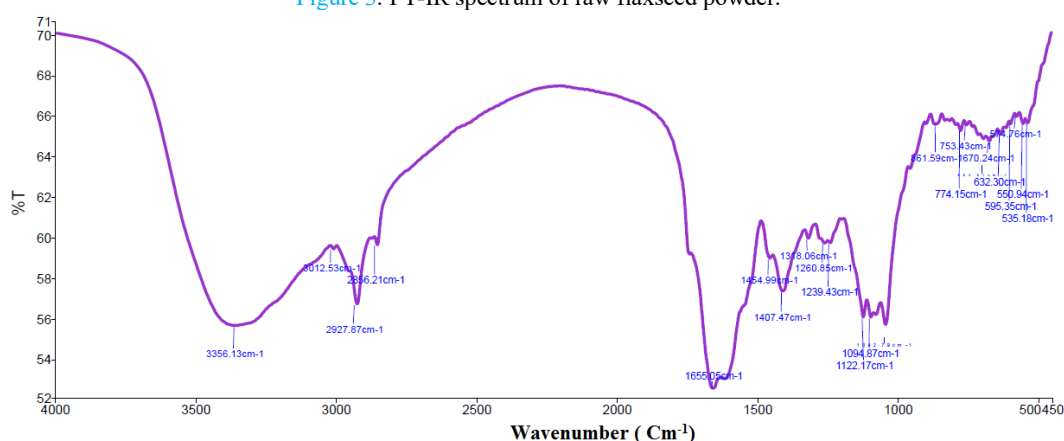


Figure 4. FT-IR spectrum of flaxseed extract.

### 3.3. Study of flaxseed extract by Liquid Chromatography – Mass spectrometry (LC-MS)

Liquid chromatography with mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with those of mass spectrometry (MS). Combining chromatography with mass spectrometry is common in chemical analysis because it enhances the individual capabilities of each technique. While liquid chromatography separates multiple components of mixtures, mass spectrometry provides the chemical structures of molecules with high resolution and sensitivity. This combination of techniques can be used to analyze biochemical, organic, and inorganic compounds typically found in complex samples of environmental and biological origin. The phenolic and flavonoid compounds in flaxseed may vary depending on the cultivar, the genetic makeup of the cultivar, and the climatic conditions of the growing regions. Therefore, [17], documented the presence of a group of acids such as Gallic acid, hydroxybenzoic acid, p-coumaric acid, ferulic acid, caffeic acid, cinnamic acid, and benzoic acid in several flaxseed cultivars via high-performance liquid chromatography (HPLC), but at varying levels depending on the cultivar. In the current decade, there has been growing interest in flavonoids and phenolic molecules due to their antioxidant capacity and potential benefits in pharmaceutical applications, particularly in the fight against diabetes [18]. Flaxseed powder and its aqueous extract were characterized, and the chemical compounds were identified and quantified by liquid chromatography–mass spectrometry (LC-MS). Chromatographic separation was performed on a reversed phase C8 column and the column was washed with a gradient consisting of (1) methanol: acetonitrile (50:50) and (2) 0.1% acetic acid. X-ray chromatograms were obtained from the analysis of a mixture of compounds as references, indicating that some compounds were eluted together. In contrast, each compound in the chromatogram can be easily identified by its specific ion trap fragmentation behavior and according to its retention times with the help of liquid chromatography technique. This method allows the structure of unknown molecules to be determined through fragmentation. Mass spectrometry (MS) measures the mass-to-charge ratio ( $m/z$ ) of the chemical fragments, and liquid chromatography (LC) spectra show the retention time (RT) of the compounds, as shown in Table 1 and Figures 5, 6, 7 and 8, which show the difference between the content of raw flaxseeds compared to their aqueous extract. LC-Mass spectrometry confirms the presence of chemical compounds with antidiabetic and antioxidant properties.

The phenolic compounds of flaxseeds and their aqueous extract, listed in Table 1, mainly consist of two classes: phenolic acids, flavonoids and lignin. Phenolic acids are mainly ferulic acid, cinnamic acid, chlorogenic acid, gallic acid, and vanillin. Among the phenolic compounds in flaxseed, which comprise a large proportion of polyphenols, are lignin, which mainly consist of matarisinol, pinoresinol, diphyllin, and secoisolariciresinol. Some other bioactive components, such as flavonoids, have also been reported in flaxseed powder [19,20]. The LC-MS spectrum confirmed the presence of lignin, flavonoids, phenols, and phenolic acids, especially linolenic acid, which reinforced and confirmed the results obtained by the FTIR spectrum Figures 3 and 4. The main peak observed was the corresponding LC-MS chromatogram with maximum absorption at 280 nm, which was confirmed by the UV spectrum in Figure 2. These signals may be consistent with Figures 5 and 7 the MS mass spectra of the raw flaxseed powder and its aqueous extract, respectively. From the data of this spectrum, an ion at  $m/z$  542 can be observed at a retention time of 24 min, which corresponds to the deprotonated molecule of the different ligands. It can be shown that at least one lignin was detected at this  $m/z$ . Peaks were also observed at a retention time of approximately 21-24 min and 25 min Figure 6 and 8 By comparing the retention times and chromatograms of the extracted ions with those obtained from ions at  $m/z$  190, 264, and 553, it was clear that the peaks were due to lignin (polyphenols), which have anti-diabetic and antioxidant properties. On the other hand, the peaks observed at  $m/z$  355 and 476 with a retention time range of 3- 4.5 min are considered clear evidence of the presence of the active ingredient linolenic acid, which helps prevent or treat a variety of diabetic complications. Given these properties, this study was conducted to evaluate the hepatoprotective effect of flaxseed extract in a rat model of alloxan-induced diabetes.

Table 1. The main compounds identified in flaxseed powder and its aqueous extract by LC-MS. Peak number, Rt Retention time in minutes, M-H ( $m/z$ ) Mass divided by charge number

Active compound	Compound Family	Rt (min)		M-H $m/z$	
		Raw	Extract	Raw	Extract
Gallic acid	Acid	2.90	3.00	190	542
Chlorogenic acid	Acid	4.30	3.20	186	526
Linolenic acid	Acid	4-4.5	3-4	355	476
Catechin	Flavonoid	4.80	3.90	264	453
Methyl gallate	Phenol	5.40	6.50	330	609
Pyrocatechol	Phenol	0	7.00	0	683
Rutin	Flavonoid	0	8.10	0	564
Vanillin	Phenol	0	8.81	0	364
Cinnamic acid	Acid	14.40	13.50	441	0
Diadzyein	Flavonoid	0	12.30	485	318
Kaempferol	Flavonoid	15.40	0	352	0
Hesperetin	Flavanone glycoside	17.10	0	396	0
lignan	Polyphenols	21-24	24.00	190, 264 and 553	542

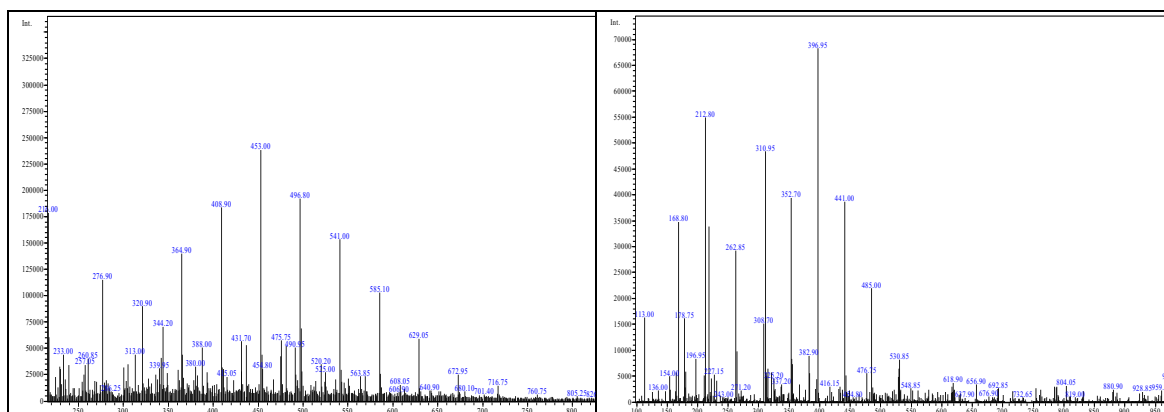


Figure 5. Mass spectrum (MS) of raw flaxseed powder.

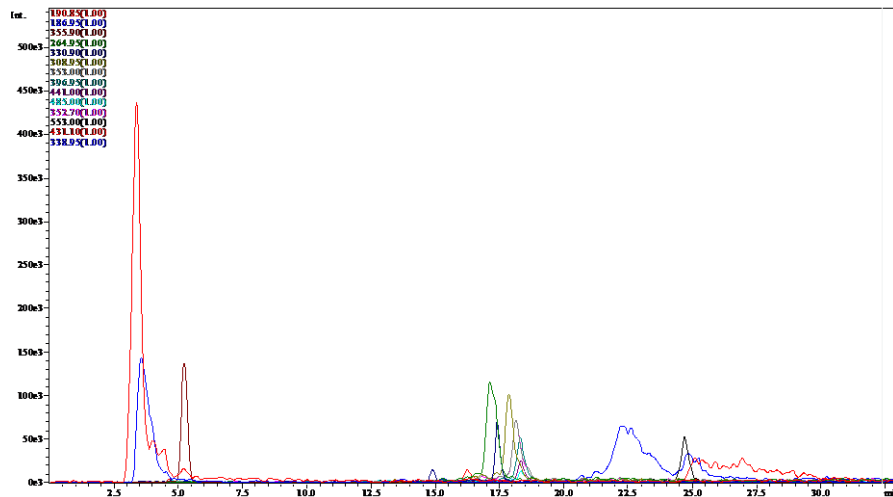


Figure 6. Liquid chromatography (LC) spectrum of raw flax seed powder.

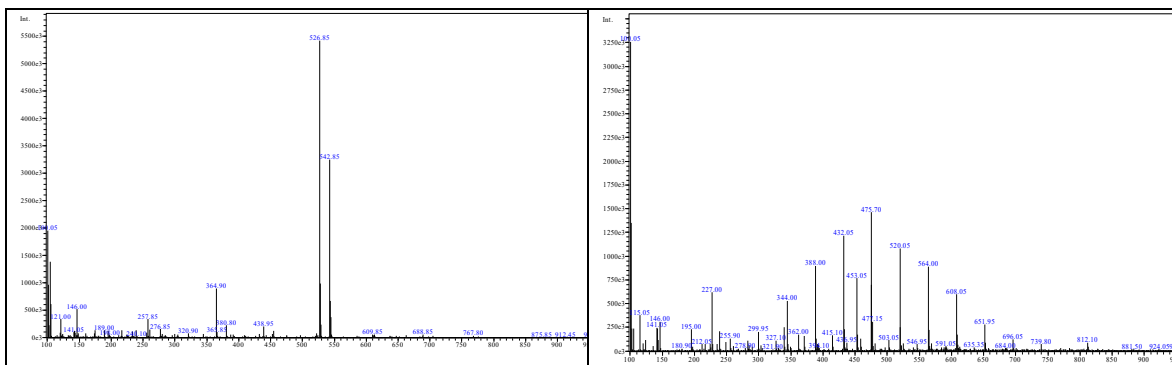


Figure 7. Mass spectrum (MS) of the flaxseeds aqueous extract.

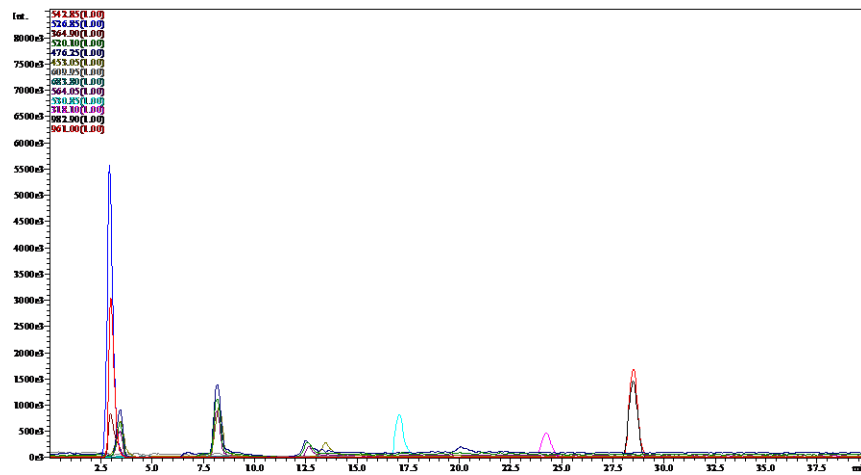


Figure 8. Liquid chromatography (LC) spectrum of the flaxseeds aqueous extract.

### 3.4. Behavioural Changes

The results of the current study showed that injecting experimental animals, namely male white mice, with alloxan to induce type 2 diabetes and treating them with flaxseed extract led to a variety of behavioral changes, including fatigue and general decreased activity, increased feed intake, lethargy, and resorting to sleep and introversion to one side of the cage, as shown in Figure 9.

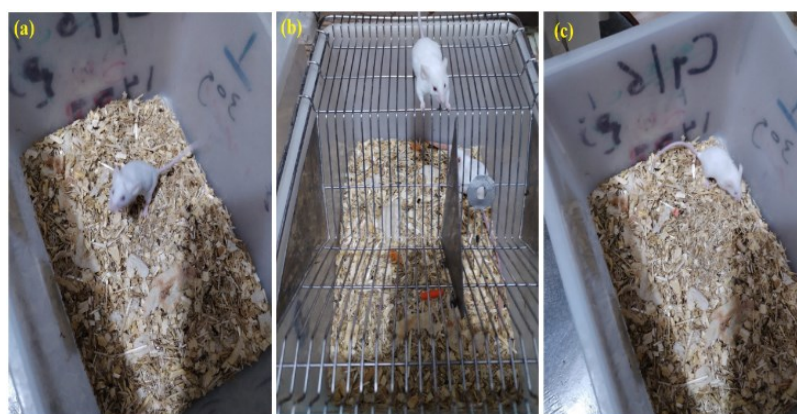


Figure 9. Shows (a) the control group of mice, (b) hyperactivity as a result of injection with alloxan solution, (c) withdrawal and lethargy as a result of injection with an aqueous solution of flaxseed extract.

This result is consistent with what was stated by [21] and that the reason for this is due to the emergence of a state of psychological disorders such as depression caused by diabetes, or it may be a result of increased risks of damage to various body organs and poor control of blood sugar levels, or it may be due to a disorder in the central nervous system (neuropathy), which leads to an increase in the secretion of the hormones epinephrine and norepinephrine, which are secreted from the adrenal gland medulla. This result is consistent with what was indicated by some studies such as [21] in their study on diabetes and its relationship to depression. The study also agrees with what was stated by [22] and also agrees with his study on depression and its relationship to diabetes in the elderly, as a study conducted in Turkey at the Faculty of Medicine (at Suleyman Demirel University) showed that depression is the most common side effect in diabetics who suffer from High blood sugar and poor control. Kalra and Sahay (2018) indicated that withdrawal and lethargy may be due to symptoms caused by diabetes, which cause animals to appear less active, such as cramps, fatigue, decreased reflexes, and dizziness. A state of imbalance was also observed, especially in the final days of the experiment. The loss of balance in the mice's movements may be due to the effects of alloxan or diabetes, which causes neuropathy [21]. In addition to imbalance in movement, which may be caused by neuropathy, their appetite for water increases, leading to frequent urination due to diabetes, or it may be due to the effect of diabetes on kidney function, leading to increased urine leakage. In addition, imbalance in movement may be because by neuropathy [23]. The results showed that the treatment with the aqueous extract of flax seeds in the diabetic groups resulted in a clear lack of appetite for food and water consumption, which led to clear weakness and thinness in the body of the mice in these groups. In contrast, [24] indicated that their lack of activity may be due to a decrease or weakness in the rate of both metabolic activity or energy metabolism.

### 3.5. Macroscopic Changes

The current study concluded that inducing diabetes with alloxan and treating animals with flaxseed extract at fixed concentrations (0.1, 0.2, 0.3) caused a number of macroscopic changes in mice with experimental diabetes when compared to control animals. These changes included differences in the average weights of the mice before and after developing diabetes. The average weight of the mice before developing diabetes was 23-32 g, but over the course of the days after developing diabetes and treatment, the average weight of the mice reached 26-35 g during the study period, as shown in Figure 10.

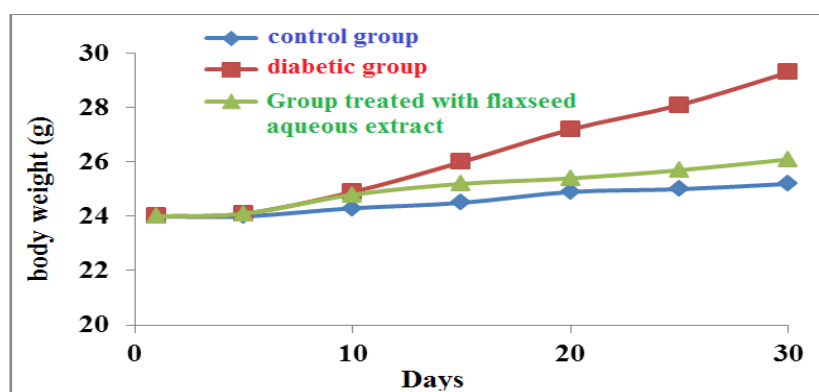


Figure 10. Shows the weight gain of the experimental mice over a period of 30 days.

The increase is likely due to the deposition of excess fat that has not been metabolized in the body. This result is consistent with a study on the effects of alloxan-induced diabetes. Alternatively, overeating may lead

to significant weight gain, which is a more common risk factor, and therefore may increase the incidence of obesity, which leads to the accumulation of fat in the liver [25]. Which found that diet causes significant weight gain. Therefore, the result obtained in the current study—weight gain—may be due to type 2 diabetes, in particular, which requires adherence to a healthy diet and regular exercise.

Treatment with flaxseed extract led to an increase in the average liver weights of diabetic mice. For 30 days after injection, the alloxan-infected group showed an increase in the average liver weight of mice from 2 g in the control mice to approximately 3 g on the thirtieth day of infection. A difference was observed in the weight gain of mice and liver between the alloxan-infected and aqueous flaxseed extract-treated groups over the entire study period, as shown in Figure 12, as shown in Figure 11.

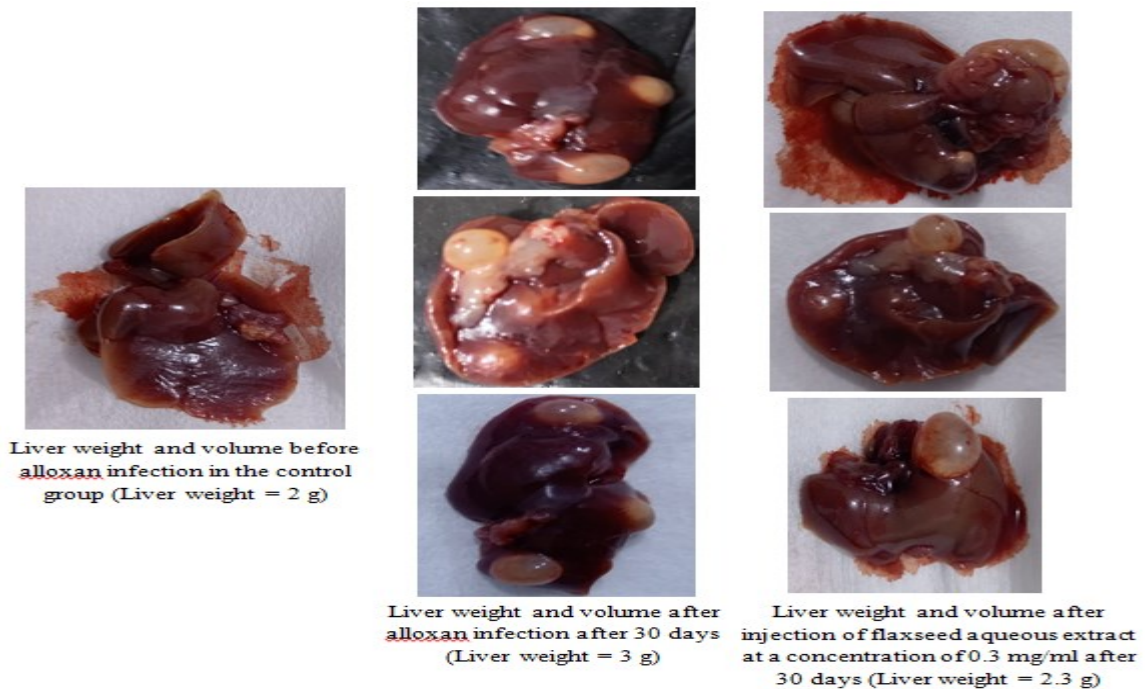


Figure 11. shows an increase in liver size and weight compared to uninfected livers (liver of uninfected mice) 30 days after alloxan infection.

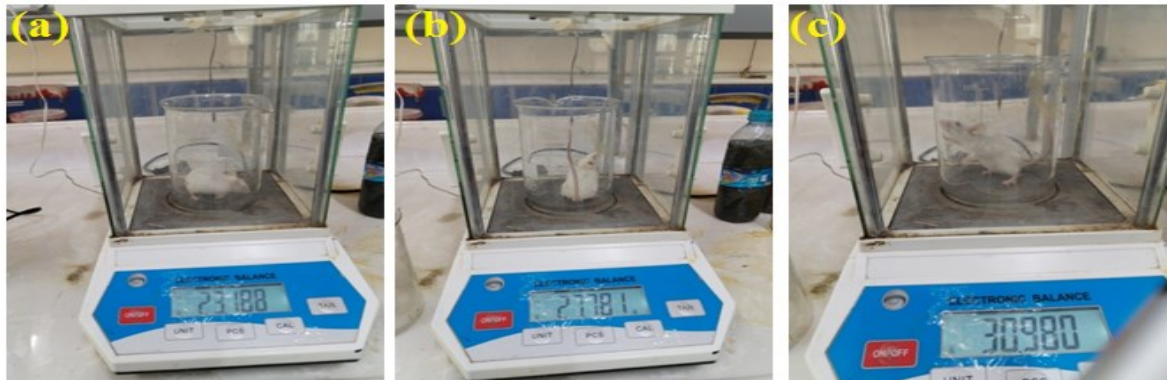


Figure 12. shows the difference in weights of the (a) control group of mice (23.188 g), (b) the group injected with aqueous solution of flaxseed extract (27.781 g), and (c) the group injected with alloxan solution (30.980 g).

This result is consistent with that of [26], who indicated that liver weight gain in rats and mice is due to hepatic cell hypertrophy resulting from the toxic effects of stimulating substances on liver cells. Liver enlargement also occurs as a result of liver enzyme induction, which is caused by these substances.

## 5. CONCLUSION

Flaxseed (*Linum usitatissimum L.*) is a multi-purpose crop, and its consumption is beneficial for human health. The results of the present study revealed that water is the most suitable solvent for extracting the total phenolic compounds and flavonoids from flaxseeds. Therefore, flaxseeds may constitute a good source of healthy compounds useful in the treatment of diabetes mellitus. Flaxseed extract yielded good results in the liver. Spectroscopic methods confirmed the presence of phenols, flavonoids, and lignin in the aqueous extract of flaxseeds, which play an important role in controlling blood sugar levels. Conversely, the aqueous extract of flaxseeds had a clear effect in repairing liver damage caused by alloxan injection in mice. These results

show flaxseed aqueous extract's promising therapeutic potential as a natural antidiabetic. The bioactive substances found seem to have both hepatoprotective and antioxidant properties, especially phenols, flavonoids, and lignin. Additionally, the improvement in liver tissue structure that was seen after therapy suggests that flaxseed extract not only helps control blood glucose levels but also lessens organ damage caused by oxidative stress. All things considered, flaxseeds are a readily available and efficient source of natural substances that may aid in the treatment of diabetes and its related issues.

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