

Plantago ovata (Psyllium Husk) Ameliorates Streptozotocin-Induced Diabetes in Rats via Modulating Oxidative Stress and the Expression of PPAR- γ , Nrf2, and TNF- α

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ABSTRACT

Background: Plantago ovata (psyllium husk) has untapped potential and is emerging as a promising candidate for diabetes management. Yet, its bioactive properties and underlying mechanisms remain inadequately investigated.

Aim: This study aims to investigate the phytochemical profile, antioxidant capacity, safety, and antidiabetic potential of methanolic Plantago ovata husk extract compared to its crude fibre in streptozotocin (STZ)-induced diabetic rats.

Methods: In vitro characterisation of the phytochemicals and their functional groups using GC–MS and FTIR revealed the presence of phenols, carboxylic acids, alkenes, and fatty acids. The methanolic extract is considered safe and exhibits potent in vitro antioxidant scavenging potential ($IC_{50} = 42.72$ mg/ml).

Results: In vivo evaluation of the acute oral toxicity, antihyperglycemic, antihyperlipidemic, antioxidant, liver and kidney function, and antioxidant effects of Plantago ovata husk extract (100 or 200 mg/kg BW) and fibre (0.5 g/kg BW) in STZ-induced diabetic rats manifested a significant decrease in blood glucose, AST, ALT, ALP, urea, and creatinine. Moreover, the antihyperlipidemic action of psyllium husk is evidenced by significant decreases in TG, TC, and LDL levels and a significant increase in HDL levels compared with untreated diabetic rats. Oxidative stress markers significantly improved. Histological alterations of the liver, kidney, and pancreas were examined microscopically. Psyllium husk fibre and extract diminished the detrimental effects of peroxidation on vital organs and sustained tissue integrity. Mechanistic insights were further explored through gene expression and in silico molecular docking studies, suggesting possible modulation of PPAR- γ , Nrf2, TNF, and inhibition of SGLT2.

Conclusion: Plantago ovata husk can serve as a promising antidiabetic and antioxidant pharmaceutical alternative and a talented nucleus for designing supplementary effective and selective therapeutic agents of nutritional origin, especially as an antioxidant and potentially for alleviating diabetes.

Keywords: Plantago ovate; Husk, Antidiabetic; Antioxidant; GC-MS, Docking.

INTRODUCTION

Diabetes is listed as the fastest increasing chronic disorder worldwide (Abutair et al., 2016). Data indicate that the rising case counts of diabetes have already reached epidemic proportions. Moreover, 40% to 50% of prediabetic or diabetic patients are undiagnosed (Abouzid et al., 2022). Diabetes is not a single disorder, but rather a cluster of conditions collectively diagnosed by elevated blood glucose levels, which is the ultimate pathway converging the disparate metabolic derangements. Clinical presentation and progression of diabetic patients vary, dreadful by the devastating complications, including nerve damage, blindness, cardiovascular complications such as angina, stroke & atherosclerosis, renal failure, overall poor life quality, and mortalities

(Cole and Florez, 2020). Metabolic disorders in both human and veterinary medicine are mostly represented by diabetes, dyslipidemia, and obesity. The most explicit sign spotted by the owners of diabetic pets is polyuria-polydipsia syndrome (Fernoagă et al., 2016). Diabetes, dyslipidemia, and visceral fat are controversially connected, yet disconnected (Molnar et al., 2023). Hyperlipidemia is a common risk factor for diabetes, and diabetes increases the risk of hyperlipidemia. Both are highly prevalent chronic inflammatory disorders. Several studies implied the mutual relationship between diabetes and hyperlipidemia (Zhou et al., 2015).

Psyllium is the common name for *Plantago ovata* and is taxonomized under the genus *Plantago*, the largest genus within the Plantaginaceae family. Its seeds resemble horses' ears and are generally distributed in tropical regions such as India, Egypt, China, and Japan (Guo et al., 2009). Psyllium was originally collected from selected wild species and was introduced as a medicinal plant. Particularly, psyllium fibre, which attributes many pharmaceutical properties; besides, it is listed as a low-calorie food and is used as an ingredient in many low-calorie products (Verma and Mogra, 2013). Psyllium husk has been used in traditional medicine in areas of India and China. It can return the body to normal physiological homeostasis and reverse physiological deviations such as hyperglycemia (Feinglos et al., 2013), high cholesterol and LDL levels (Brum et al., 2018), and obesity (Jane et al., 2019). Additionally, it has a potent antioxidant effect and free radical scavenging activity as it contains natural bioactive metabolites, especially polyphenols and flavonoids (Khedher et al., 2022).

This study aims to identify the bioactive compounds of psyllium husk methanolic extract by GC–MS and FTIR analyses, to determine its antioxidant potential through DPPH assay, and to determine the antidiabetic and antioxidant effects of psyllium husk in streptozotocin-induced adult male Wistar albino rats for 21 consecutive days through biochemical and histological alterations. Furthermore, to compare between the antidiabetic potency of psyllium husk methanolic extract and its crude psyllium husk fibre in rats, and to assess the regulation of hepatic, renal, and pancreatic gene expression, and finally to predict the potential hypoglycemic mechanism by *in silico* molecular docking techniques, to contribute to the discovery and production of innovative therapeutics derived from psyllium husk.

MATERIALS AND METHODS

Plant material and extraction

Psyllium husks were purchased from a trusted wholesaler of dried botanicals, herbs, and spices, the Indian herbs store, Cairo, Egypt. *Plantago ovata* L. husk has been identified, taxonomized, authenticated, and approved at Cairo University's herbarium, Egypt, under the voucher specimen CAI.99.468.1403.111.

500 grams of psyllium husk have been screened and minced into very fine powder with an electric grinder. Subsequently, the grounded husks were soaked in absolute methanol in glass containers at ambient temperature for 72 hours with frequent shaking. The extract was then filtered, and methanol was evaporated under vacuum by the rotary vacuum evaporator at 45°C to avoid thermal degradation. Extraction was conducted several times till the exhaustion of the plant material. The extract was incubated at 35° C till complete dryness. The extraction yield was 39.64 grams. The extraction yield percentage was evaluated to be 8%.

Identification of the bioactive compounds

Screening of the extract was conducted by gas chromatography-mass spectrometry (GC-MS) along with Fourier transform infrared spectroscopy (FTIR) analyses.

2.1.GC/MS-MS Agilent 7000E Triple Quadrupole Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS-MS having an Elite-5MS (5% diphenyl/ 95% dimethyl polysiloxane) with a capillary column (30 x 0.25 µm ID x 0.25µm df). The ionizing energy was 70 eV. Helium (99.999%) flowed at a constant flow rate of 1ml/min with an injection volume of 2µl (split ratio of 30:1); the injector temperature was 250°C; the ion-source temperature was 200°C. The oven temperature was set to 110°C (isothermal for 2 min), which increased 10°C/min, reaching 200°C, followed by a 5° C/min increase till it reached 280°C, terminating by isothermal 9

minutes at 280°C. Mass spectra were considered at 70 eV. Every scan interval continued for half a second. fragments were (45-450Da). The run continued for 36 minutes. Calculation of the relative area of each compound was done by comparing its average peak area to the total area. Turbomass was software utilized to operate mass spectra along with chromatograms. Analysis of the mass spectrum was assisted by the National Institute of Standards and Technology database (NIST), which has patterns exceeding 62,000. The name, molecular weight, as well as structures of all compounds were ascertained (Ezhilan and Neelamegam, 2012).

2.2. FTIR analysis Identification of the functional groups of psyllium husk methanolic extract in a frequency range of 400–4000 cm⁻¹ with the aid of Fourier transform infrared spectroscopy technique (Shimadzu IRAffinity-1S, Japan) according to the manufacturer's instructions.

Assessment of in vitro radical scavenging and antioxidant effect by DPPH (2,2-Diphenyl- 1-picrylhydrazyl) assay

The principle of the DPPH assay is to measure the extent of scavenging of the DPPH radicals by the antioxidants found in the plant samples. A hydrogen atom is donated by the antioxidants to the nitrogen in the DPPH radical, which has an unpaired electron. This reduced form of DPPH radical and the resultant hydrazine (DPPH-H) lets the violet colour disappear considerably to pale yellow. The intensity is measured calorimetrically by spectroscopy (Gulcin and Alwasel, 2023). Three extract strengths of 50, 100, and 200 mg/ml were prepared. 1 ml of DPPH (0.2 ml/molar) was added to the prepared extract concentrations. All were placed in a dim area for thirty minutes. The analysis conditions were 24.3 °C, and the RH% was 47%. The intensity of the discolouration was estimated calorimetrically using a spectrophotometer at 517 nm. Absolute methanol was blank, and the DPPH solution alone was the control. Measurements were done in triplicate, and the average values were considered (Ansari et al., 2013). The results were recorded. Radical scavenging activity was calculated by the DPPH activity (%) = $(A_0 - A_1) / A_0 \times 100$. A₀ is the DPPH solution absorbance solely, and A₁ represents the direct extract absorbance from the spectrophotometer. Considering any decrease in the extracts ' absorption in comparison to the control (Oktay et al., 2003).

Drugs and chemicals

Streptozotocin was purchased from MP Biomedicals, California, USA. CAS NO. 18883-66-4. Daonil® Tablets, as a standard antidiabetic drug, glibenclamide, produced by Sanofi, Egypt, under the license of Sanofi Aventis / France. Carboxymethyl cellulose (CMC), Advent, India.

Acute toxicity (LD50) study

Estimation of the acute toxicity (LD50) of the psyllium husk's methanolic extract was executed in mice according to the method of Lorke (Lorke, 1983). Six groups, each with 5 mice, were administered the tested methanolic extract at 1000, 2000, 3000, 4000, and 5000 mg/kg suspended in 0.2% CMC solution. The doses were administered orally using oral gavage feeding tubes. The mice were monitored for 72 hours for any symptoms of poisoning or mortality.

Experimental animals

Sixty male albino Wistar rats were fetched from the Holding Company for Biological Products and Vaccines (VACSERA). Rats of seven weeks old weighing 200±50 g. Rats were housed in the Department of Pharmacology, Veterinary Medicine, Cairo University, Egypt, under hygienic laboratory standards. All rats had free access to water and were fed on balanced commercial pellets. Rats were assorted in clean, bedded cages fitted with upper wire mesh lids at ambient temperature with a source of ventilation. The light-dark cycle was 12:12 hours, and the relative humidity was 50–60%. Rats were acclimatized for 14 days before the experimental intervention. The protocol complied with the National Research Council's guidelines. The Institutional Animal Care and Use Committee (IACUC) approval reference number is Vet CU 09092023778. Concerning ethical affairs, every effort was implemented to treat the experimental animals with empathy and compassion. This study is reported in accordance with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

Induction of diabetes

Fifty overnight fasted rats were injected with a single dose of freshly formulated streptozotocin with buffered citric acid of 0.1 M (pH 4.5). To induce diabetes, a single dosage of 35 mg/kg was injected intraperitoneally. The blood glucose was monitored frequently using Biodiagnostic kits according to the method of Trinder 50. After 72 hours, blood sugar exceeding 250 mg/dl was regarded as diabetic; otherwise, rats were excluded (Chakraborty and Das, 2010).

Experimental design

Random setting of sixty rats into six sets, each of ten rats:

Group 1: Normal non-diabetic rats received the vehicle, CMC carboxymethyl cellulose 0.2% in water.

Group 2: Control positive diabetic group (treated with CMC vehicle only).

Group 3: Diabetic rats treated with 100 mg/kg psyllium husk methanolic extract (Wahid et al., 2020).

Group 4: Diabetic rats treated with 200 mg/kg psyllium husk methanolic extract.

Group 5: Diabetic rats treated with 0.5 g/kg crude psyllium husk ground fibre suspension (Ahmed et al., 2010).

Group 6: Diabetic rats treated with 5 mg/kg glibenclamide suspended in 0.2% CMC (Sabitha et al., 2011) as a standard treatment.

All treatments were administered daily throughout the experimental period, which continued for 3 weeks. The treatments were administered orally using orogastric feeding gavage (16 gauge) with weekly body weight tracking and blood glucose monitoring.

Sampling

Blood specimens were drawn from the retro-orbital plexus by heparinized micro-haematocrit capillary tubes into heparinized tubes at the end of the study period after the last administration. Samples were subsequently fractioned for ten minutes with a centrifugation force of 3000 rpm. The pipetted plasma was conducted for several analyses.

Biochemical analysis

Plasma was frozen at -20°C for subsequent analysis. Biodiagnostic commercial kits, for diagnostic and research reagents in Egypt, were utilized for blood glucose determination (Trinder, 1969), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) (Lippi and Guidi, 1970), as well as alkaline phosphatase (ALP) (KIND and KING, 1954) levels in respective aspects.

Lipid profile assay

Measurement of plasma cholesterol in compliance with Abel et al., (1952), and triglycerides assessment was done according to Bucolo and David, 1973. High-density lipoprotein cholesterol (HDL-C) level was measured using the method described by Assmann et al., 1983, and low-density lipoprotein cholesterol (LDL-C) according to Friedewald et al., (1972).

Oxidative stress and lipid peroxidation markers in blood

The colourimetric method employed by Biodiagnostic kits, for diagnostic and research reagents in Egypt, was utilized to estimate plasma superoxide dismutase (SOD) (Durak et al., 1993), malondialdehyde (MDA) (Ohkawa et al., 1979), as well as glutathione peroxidase (GPX) (Paglia and Valentine, 1967).



Histopathological examination

Rats were euthanized by cervical dislocation for histopathological sampling. The liver, kidney, and pancreas were dissected from each rat to be assessed histopathologically. The organs were immersed in a solution of 10% neutral buffered formalin for fixation. Ascending alcohol concentrations were used to dehydrate the sampled organs. They were then cleared using xylene. Routine processing and sectioning at 3-5 μm thick sections stained with Hematoxylin and Eosin (H&E) (Suvarna et al., 2018). A conventional microscope (Olympus, Tokyo, Japan) was utilized at different magnification powers. Analysis continued to screen for any histological alterations.

Gene expression study

Total RNA was extracted from the tissue samples according to the manufacturer's instructions using the RNeasy mini extraction kit (Qiagen). The Nanodrop was employed at a wavelength of 260 to determine the concentration and the 260:280 ratio to select the pure samples in the range of 1.8 and 2.0 (Abdel Aziz et al., 2023). The complementary DNA (cDNA) was produced using a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. The primer sets for measuring the mRNA levels of genes were generated using the *Rattus norvegicus* sequences in GenBank (Table 1). The primer3 tool was used to design the primers. SYBR Green PCR Master Mix was used in real-time PCR analysis to assess the relative expression of the selected genes (Thermo Scientific Cat number: 4309155). Applied Biosystems ABI Prism StepOnePlus Real-Time PCR System as directed by the manufacturer (Abo El-Ela et al., 2024). For each sample, the PCR reactions were conducted twice. The expression levels were normalized to the housekeeping gene beta-actin. The fold change in expression was calculated using the $2^{-\Delta\Delta\text{CT}}$ method (Livak and Schmittgen, 2001).

Molecular-docking study

In silico trials were implemented to gain insight into the possible interactions between the phytochemicals of the psyllium husks and the body targets. Among the many receptors implicated in glucose pharmacodynamics, sodium-glucose cotransporter 2 (SGLT2) was selected for its relevance. This receptor is a very crucial transport system for absorbing back 80 to 90% of the blood sugars consistently filtered by the renal glomeruli. Interestingly, SGLT2's competitive inhibition enhances glucose excretion, and it is a revolutionary therapy to alleviate not only diabetes but also obesity (Idris and Donnelly, 2009). Homo sapiens sodium-glucose cotransporter 2 (SGLT2) ID: 8HDH was procured from the Protein Data Bank (<https://www.rcsb.org>) (Hiraizumi et al., 2023). BIOVA Discovery Studio Visualizer was used to prepare the receptor by removing co-crystallized ligands and solvents (BIOVA Discovery Studio Visualizer, n.d.). Ligands were selected according to GC-MS with the aid of FTIR analyses of the husks, and dapagliflozin was selected as a Reference Listed Drug (RLD). Dapagliflozin is an SGLT2 inhibitor approved by the US Food and Drug Administration (FDA) in January 2014 (Saeed and Narendran, 2014). All the ligands, including dapagliflozin, were procured from the NCBI, National Center for Biotechnology Information web server, USA: PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). Docking was processed and Gibbs free energy (ΔG) was estimated by the virtual screening software for computational drug discovery, PyRx (Dallakyan and Olson, 2015). BIOVA Discovery Studio Visualizer, as well as UCSF ChimeraX were used for the results analysis (Pettersen et al., 2021; BIOVA, 2023).

Statistical analysis Raw data were collected and generated with the aid of one-way variance analysis (ANOVA) through SPSS version 14 (SPSS, Chicago, IL, USA). Duncan's Multiple Range Test was used to determine the mean difference. Remarking that any p-value below 0.05 is considered statistically significant. The Shapiro-Wilk W test examined normality for the entire data.

RESULTS

1. GC-MS and FTIR analyses

GC-MS analysis was conducted, and 21 compounds were identified, including fatty acids and the dicarboxylic

acids in the husk methanolic extract. The fatty acids were found in varying amounts, including 9-octadecenoic acid, decanoic acid, hexadecenoic acid, hexanoic acid, and oleic acid. The most abundant fatty acid was found between 6–15-octadecadienoic acid, with a concentration percentage of 58% (Table 2). The FTIR spectra generally showed characteristic absorption peaks representing a mixture of several fatty acids that exist in psyllium husk; however, insignificant amounts of proteins and amide compounds were detected (Fig. 1). The peak 1734 cm^{-1} represents the C=O bonds found in 9-octadecenoic acid, 6-octadecenoic acid, decanoic acid, and methyl 8,9-octadecadienoate. The bands between 1641 and 1426 cm^{-1} are from twisting and C–C stretch vibrations, representing the presence of aromatic moieties. The range between 1252–899 cm^{-1} wavenumbers showed peak strengthening at 1082 cm^{-1} , which is generally allocated to C–O bond vibrations in the ring moieties besides the C–O–C bond attached to C–C and C–O–H groups. The peak 3006 cm^{-1} indicates the stretching of olefinic=CH unsaturated fatty acids.

2. In vitro antioxidant activity

A significant in vitro DPPH scavenging action consistent with high antioxidant potential was found. The methanolic extract of psyllium husk showed an IC₅₀ of 42.72 mg/ml. The free radical scavenging activity (RSA)% of psyllium husk methanolic extract exhibited an increase with increasing concentration, as the concentrations of 50, 100, and 200 mg/ml produced 58.52, 80.98, and 83.16% scavenging activity, respectively.

3. Acute toxicity study

Psyllium husk methanolic extract showed no sign of acute toxicity nor mortality for 3 days when administered orally using an oral gavage feeding tube in doses from 1000 to 5000 mg/kg. According to Lork's acute toxicity studies, the median lethal dose (LD₅₀) is considered safe if it is more than 5000 mg/kg BW (Lorke, 1983). This will help to avert unforeseen danger inherent in overdosing of herbal products by virtue of being confirmed to have an LD₅₀ greater than 5,000 mg/kg body weight. Thus, the extract is non-toxic and entirely safe in mice up to 5000 mg/kg BW.

4. Effects on body weight

Eventually, at the termination of the experimental period, the STZ-diabetic control (Group II) body weights were significantly reduced compared to the normal rats (Group I). Nevertheless, oral doses of psyllium husk extract, fibre, and glibenclamide to diabetic rats kept the body weights close to normal (Table 3). Psyllium husk extract at 100 and 200 mg/kg produced a 15.68% and 23.71% increase in the body weights, respectively, when compared to the diabetic rats that induced a loss of body weight by 21.25%.

5. Effects on blood glucose

The hypoglycemic effect of psyllium husk extract, fibre, and glibenclamide as a standard hypoglycemic drug compared with diabetic rats is displayed in Table 4. STZ injection led to a substantial rise in blood sugar levels in the diabetic control group in contrast to the control rats. Contrary to the diabetic group, psyllium husk extract at two different doses (100 and 200 mg/kg BW) led to a significant ($p < 0.05$) decline in plasma blood sugar level in the STZ-induced diabetic group in a dose-dependent mechanism. Psyllium husk fibres at 0.5 g/kg BW and glibenclamide (5 mg/kg) exhibited – 66.61% and – 75.35% reductions in plasma sugar levels, contrary to the diabetic group.

6. Effects on hepatic and renal function indicators

Blood levels of AST, ALT, ALP, urea, and creatinine substantially ($P < 0.05$) rose in diabetic rats in comparison to the non-diabetic control (Table 4). Notably, the psyllium-treated groups and glibenclamide showed significant decreases in all previous parameters with values brought toward close-normal levels.



7. Effects on lipid profile

The blood levels of TC, TG, and LDL were considerably ($P < 0.05$) higher, but HDL significantly declined in the diabetic group compared to the normal group. Conversely, psyllium extract and fibre administration significantly improved the lipid profile values (Table 4). The ratio of cholesterol to HDL for diabetic rats was high, with a value of 6.15, while the ratio in psyllium-treated rats and glibenclamide was very close to 3.00, the normal value of the control. The results indicate that psyllium is a potent anti-hyperlipidaemic agent.

8. Effects on oxidative stress and lipid peroxidation indicators

There were significant rises in SOD and GPx in the psyllium-treated groups ($p < 0.05$); nonetheless, the MDA level substantially declined ($p < 0.05$) as compared with the non-treated group (Fig. 2, 3, 4). Diabetic rats exhibited a significant reduction in SOD and GPx concentrations, but the MDA concentration increased ($p < 0.05$) substantially compared to the non-diabetic group.

9. Histopathological assessment

The non diabetic group's liver exhibited normal hepatic parenchyma, having polygonal hepatocytes, normal blood sinusoids, and a central vein, while the diabetic group showed massive and diffuse hepatic vacuolar degeneration; hepatocytes were swollen and vacuolated with an irregular arrangement and narrow blood sinusoids (Fig. 5a, b). The diabetic groups treated with psyllium husk extract, fibre, and glibenclamide showed healthy hepatic parenchyma; polygonal hepatocytes with normal blood sinusoids, and central vein (Fig. 5c, d, e, f).

Normal renal glomeruli and renal tubules were shown in control non-diabetic rats; on the contrary, the diabetic group showed massive and diffuse renal tubular epithelium vacuolation and necrosis, and glomerular epithelium vacuolation (Fig. 6a, b). The diabetic groups treated with psyllium husk extract, fibre, and glibenclamide showed normal renal parenchyma, glomeruli, and renal tubules (Fig. 6c, d, e, f).

The control group showed normal pancreatic acini and islets, while the diabetic group showed hyperplasia of pancreatic islets (Fig. 7a, b). The diabetic groups treated with psyllium husk extract, fibre, and glibenclamide showed normal pancreatic acini (Fig. 7c, d, e, f).

10. Molecular docking

The best seven molecular interactions were chosen according to their inhibitory potential and the Gibbs free energy expressed in kcal/Mol. All of them were chosen to be of the lowest mode and Root Mean Square Deviation (RMSD). The seven identified phytochemicals targeted the human active site, the SGLT2 receptor, with a G score ranging from -7.2 to -6.4 kcal/Mol as illustrated in Table 5. The docking methodology is supported by the Reference Listed Drug, dapagliflozin, which hit a binding affinity of -7.5 kcal/Mol. The 3D and 2D structures are demonstrated, and the interactions are elucidated by several conventional hydrogen bond interactions with the SGLT2 receptor (Fig. 8). The 2D-molecular docking of the seven bioactive compounds from the psyllium husk extract and dapagliflozin docked with SGLT2 active sites revealed diverse binding interactions according to the nature of the ligands (Fig. 9).

11. Gene expression study

Diabetic rats showed upregulation of the TNF gene and down regulation of the Nrf2 and PPAR- γ genes in all the studied organs. However, the transcription levels of the studied genes revealed that all the treatments significantly alleviated the dysregulation of the studied genes induced by diabetes in the pancreas, liver, and kidney tissues. These ameliorative effects were established by the downregulation of the pro-inflammatory TNF and upregulation of both the Nrf2 and PPAR- γ genes.

DISCUSSION

Antidiabetics include insulin, sulfonylureas, dipeptidyl peptidase-4 inhibitors (DPP-4), glucagon-like peptide 1 receptor agonists (GLP1), and SGLT2 inhibitors. Each class has its advantages and disadvantages based on the clinical evidence and the mechanism of action of each medication (Thrasher, 2017). Current medications are not adequately effective in maintaining long-term glycemic control in most patients, leaving diabetic patients susceptible to debilitating and life-threatening complications such as cardiovascular diseases, blindness, and kidney complications. In addition to other side effects such as CNS depression, hypoglycemia, gastrointestinal disturbances, hypertriglyceridemia, and metabolic disorders (Brown, 2008). Therefore, it is imperative to focus on developing advanced medications for the treatment of type 2 diabetes.

PPAR- γ (Peroxisome Proliferator-Activated Receptor Gamma) is a crucial glucose and lipid metabolism regulator (Villapol, 2018). In diabetic rats, PPAR- γ expression is downregulated, tributary to insulin resistance and metabolic disorders associated with diabetes (Rangwala and Lazar, 2004). Psyllium husk has been shown to upregulate PPAR- γ expression, which may improve insulin sensitivity and glucose homeostasis, leading to improved lipid metabolism, lipid profiles, and reduced blood glucose levels in diabetic rats (Ansari et al., 2023).

In diabetes, oxidative stress is a significant contributor to complications in the liver, kidney, and pancreas (Giacco and Brownlee, 2010). Nrf2 (Nuclear Factor Erythroid 2-Related Factor 2) is a key transcription factor that regulates antioxidant responses and protects against oxidative stress (Caturano et al., 2023). Psyllium husk upregulated the Nrf2 expression, enhancing the antioxidant defence mechanisms in these organs, which might help mitigate oxidative damage, thereby protecting cellular integrity and function in diabetic rats (Ansari et al., 2023).

TNF (Tumour Necrosis Factor) is a pro-inflammatory cytokine that plays a pivotal role in the inflammatory response associated with diabetes (Alzamil, 2020). Elevated TNF levels are linked to insulin resistance and various diabetic complications (Swaroop et al., 2012). Psyllium husk has been shown to downregulate TNF expression, thus decreasing the inflammatory changes in the liver, kidney, and pancreas. This anti-inflammatory effect of the psyllium husk is crucial for improving insulin sensitivity and lessening the risk of diabetes-related complications (Gholami et al., 2024).

In conclusion, Psyllium husk appears to play a beneficial role in controlling diabetes by modulating the expression of PPAR- γ , Nrf2, and TNF in the liver, kidney, and pancreas, suggesting that it could be a valuable dietary supplement for improving diabetes and reducing the risk of complications in diabetic patients.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to investigate the antioxidant efficacy. The unpaired electron in DPPH $\cdot$ exhibits an intense deep purple colour at 517 nm; however, while pairing up with another electron, it changes into colourless endpoints (Sadeer et al., 2020). The half-maximal inhibitory concentration of psyllium husk methanolic extract was concentration-dependent, with an IC₅₀ of 42.72 mg/ml. Psyllium is rich in vitamin C, fatty acids, polyphenols, and flavonoids, especially the latter two compounds, which possess substantial antioxidant effects along with their explicit effects in alleviating inflammation (Rafiee et al., 2021)(Sadeer et al., 2020). The phenolic compounds act as chief antioxidants as they contain the hydroxyl group that is responsible for the radical scavenging activity because of their reducing activities (Foti, 2008). The antioxidant activity of flavonoids and their metabolites depends on their structure and the arrangement of the functional groups. A double bond between the second and the third carbon atoms, along with a hydroxyl group attached to the third carbon, in addition to the fourth carbon atom possessing a carbonyl group, are all structural necessities essential for the antioxidant activity of flavonoids (Heim et al., 2002).

GC-MS analysis of the psyllium husk extract revealed the identification of fatty acids and dicarboxylic acids, including 9-octadecenoic acid, decanoic acid, hexadecenoic acid, hexanoic acid, and oleic acid. The most abundant fatty acids were 6–15-octadecadienoic acids with a concentration percentage of 58%. The phytochemical composition of the methanolic extract of psyllium extract with FTIR and GC-MS revealed that

9-octadecenoic acid is the major phytochemical, as it constitutes 31.91%. Octadecanoic acid is possibly the reason why STZ-diabetic rats manifested reduced blood glucose (Gazali et al., 2023).

The FTIR spectra generally showed characteristic absorption peaks representing a mixture of several fatty acids that exist in psyllium husk; however, insignificant amounts of proteins and amides were detected. The peak 1734 cm^{-1} represents the carbonyl moieties $\text{C}=\text{O}$ absorption peak (van der Weerd et al., 2005) that is found in 9-octadecenoic acid, 6-octadecenoic acid, decanoic acid, and methyl 8,9-octadecadienoate. The bands at 1641 and 1426 cm^{-1} are probably from twisting $\text{C}-\text{C}$ stretch vibrations, as the existence of aromatic moieties (Song et al., 2008). The range of 1252–899 cm^{-1} wavenumbers having a peak strengthened at 1082 cm^{-1} are in general allocated to $\text{C}-\text{O}$ bond vibration in the ring moieties, besides the $\text{C}-\text{O}-\text{C}$ bond attached to $\text{C}-\text{C}$ and $\text{C}-\text{O}-\text{H}$ groups (Lasalvia et al., 2022). The peak 3006 cm^{-1} may indicate the stretching of olefinic $=\text{CH}$ unsaturated fatty acids (Shapaval et al., 2014).

Fatty acids have inhibitory activity against the key enzymes related to type 2 diabetes. Fatty acids are potent anti- α -glucosidase. Fatty acids competitively inhibit the α -glucosidase enzyme by binding to the enzyme, forming a complex. They were found to be more potent than acarbose in inhibiting starch digestion in vitro. Fatty acids possess potent inhibitory effects against α -glucosidase activity in addition to the retardation of starch hydrolysis (Su et al., 2013). Talukder has reported the richness of psyllium husk with bioactive compounds, especially flavonoids, polyphenols, amino acids, and fatty acids. They correlated the high phenolic and flavonoid content of these compounds to their potent antioxidant activity. In addition, these compounds inhibited lipid peroxidation and thereby exerted their antioxidant capacity. Psyllium is a rich source of polyphenolic compounds, especially gallic acid and rutin. To a lesser degree, other polyphenols were also found. These polyphenolics have the highest potential for increasing antioxidant activity (Talukder et al., 2016).

The chronic high blood sugar levels in diabetic patients foster increased reactive oxygen species generation through several mechanisms, such as glucose auto-oxidation, activation of the polyol pathway, and the formation of advanced glycation end products. Additionally, the prevalent mitochondrial dysfunction in diabetes results in an additional release of reactive oxygen species (Caturano et al., 2023). The generation of reactive oxygen species, including superoxide anions (O_2^-) and peroxides (H_2O_2^-), depletes the antioxidant biomarkers such as SOD and GPx and consequently damage DNA, proteins, and lipids (Su et al., 2019). The oral administration of psyllium husk methanolic extract for 21 days significantly increased SOD and GPx blood levels and decreased the lipid peroxidation in a dose-dependent manner in diabetic rats.

The transfer of glucose across plasma membranes occurs via integral transport proteins. These transporters comprise two structurally and functionally distinct groups, whose members have been identified over the past two decades, namely: SGLT glucose co-transporters, and GLUT glucose co-transporters. The glucose transporters (GLUTs) equalize the glucose levels across the plasma membrane of the cells, whereas SGLT2 can create a glucose gradient force (Wood and Trayhurn, 2003). A novel antidiabetic mechanism has been developed that depends on SGLT2 inhibition. Inhibiting renal glucose uptake and enhancing its elimination through the urine. Subsequently, SGLT2 inhibitors can reduce blood glucose levels and prevent postprandial hyperglycemic complications (Wanner and Marx, 2018).

Since the mechanism of action of the antidiabetic effect of psyllium husks is not yet known, we suggest a potential mechanism of action using in silico studies and advanced computational techniques. The husks' phytochemicals were found to interrelate with SGLT2. The sodium-glucose cotransporter 2 is crucial for the reabsorption of up to 90% of blood glucose. Inhibitors of SGLT2 minimize glucose reabsorption and enhance urinary glucose excretion. SGLT2 inhibitors ameliorate diabetes, hyperlipidemia, and obesity (List and Whaley, 2011).

The binding affinity between SGLT2 and the husks' phytochemicals was found to be -7.2 kcal/mol for 12,15-Octadecadienoic acid, -7 kcal/mol for 6-Octadecenoic acid and 9-octadecenoic acid, -6.8 kcal/mol for methyl 8,9-octadecadienoate, -6.6 kcal/mol for 7-hexadecenoic acid, -6.5 kcal/mol for 9-Methyl-Z-10-pentadecen-1-ol

and -6.4 kcal/mol for 9,12-Octadecadienoic acid. Compared to -7.5 kcal/mol for dapagliflozin as a Reference Listed Drug (RLD). Dapagliflozin is a selective SGLT2 inhibitor that interacts with SGLT2 with one hydrogen bond and five hydrophobic bonds. The hydrogen bond was found to be connected to the oxygen atom of glutamine residue number 32 in the β chain of the SGLT2 transport system. The hydrophobic bonds include 3 pi-alkyl bonds with the amino acid residues of the α chain, including phenylalanine, valine, and leucine, one pi-pi stacked bond with phenylalanine, and one Pi-Sigma bond with the alanine residue of the SGLT2 receptor. 12,15-Octadecadienoic acid scored the closest binding affinity to dapagliflozin as stated in earlier antidiabetic literature (Joshi et al., 2020; Salau et al., 2020, 2022). 12,15-Octadecadienoic acid binds to SGLT2 with 4 hydrogen bonds, and 10 hydrophobic bonds, in addition to van der Waals interactions. The hydrophobic bonds range between alkyl, pi-alkyl, and pi-sigma bonds. Listijani Suhargo reported promising results of 6-Octadecenoic acid and 9-octadecenoic acid in his antidiabetic study (Suhargo et al., 2023). Both 6-octadecenoic acid and 9-octadecenoic acid have the same binding affinity. 6-octadecenoic acid binds with 3 hydrogen bonds, 4 alkyl bonds, and 6 pi-alkyl bonds, while 9-octadecenoic acid binds with 4 hydrogen bonds, 3 alkyl bonds, and 5 pi-alkyl bonds. Methyl 8,9-octadecadienoate can bind to SGLT2 with a conventional hydrogen bond to the serine residue number 287 (1.7 Å), a carbon-hydrogen bond to the asparagine residue number 75 (3.5 Å), 5 alkyl, 4 pi-alkyl and a pi-sigma bond. 7-hexadecenoic acid was also characterized in the metabolomics of avocado "*Persea americana*" seeds for evaluation of its antidiabetic activity (Younis et al., 2022). 7-hexadecenoic acid interacts with SGLT2 with three hydrogen bonds, 5 alkyl, and five pi-alkyl bonds. Modelling of 9-Methyl-Z-10-pentadecen-1-ol with SGLT2 with a hydrogen bond linked to tyrosine (2.3 Å), six alkyl bonds, and 4 pi-alkyl bonds. 9,12-Octadecadienoic acid forms a hydrogen bond with the glutamine residue number 451 in SGLT2 (2.1 Å), and other 7 alkyl bonds, along with 8 pi-alkyl bonds.

Interestingly, 9,12-Octadecadienoic acid (linoleic acid) has been reported to reduce postprandial blood glucose and inhibit glucose uptake. In an attempt to reveal the exact mechanism of action, scientists used cell culture to detect 9,12-Octadecadienoic acid's action on the sodium-glucose cotransporter-1 (SGLT1), which is responsible for the absorption of glucose from the small intestine, but the results were inconclusive, and this mechanism was excluded (Yamamoto et al., 2023). Inhibition of glucose uptake by 9,12-Octadecadienoic acid is not by direct action on SGLT1 in the absorptive cells of the small intestine, but rather it may act directly or indirectly on the sodium-glucose cotransporter 2 (SGLT2), inhibiting the reabsorption of the filtered renal glucose.

CONCLUSION

The potent radical scavenging activity could be attributed to the rich polyphenolic moieties in the psyllium husk extract. *Plantago ovata* husk (psyllium husk) can serve as a promising antioxidant reference for prospective pharmaceutical preparations. The isolated bioactive compounds showed significant antidiabetic activity, potentially by inhibiting the SGLT2 receptor. The *in silico* techniques and the molecular binding interactions demonstrated that psyllium husk extract phytochemicals exhibit target-specific interactions with SGLT2 receptors through hydrogen bonding and other hydrophobic interactions. Psyllium husk exhibited transactivation and modulation of PPAR- γ , Nrf2, and TNF gene expression in the liver, kidney, and pancreas. Based on our findings, psyllium husk is recommended as a potential antidiabetic agent and as a talented nucleus for designing effective supplementary and selective therapeutic preparations.

Ethical approval

The experimental protocol was approved by Cairo University's research committee on the ethics of animal use. The approval reference number is Vet CU 09092023778, which followed the general criteria of the National Institutes of Health Guide for the Care and Use of Laboratory Animals in Scientific Investigations.

Consent to participate

Not applicable. Consent to publish Not applicable.



CRediT authorship contribution statement

Khaled Abo-EL-Sooud designed the experiments, prepared the tables and figures, and critically revised the manuscript and drafted the manuscript. Hagar Hesham performed animal dosing and care, collected the samples, analysed the data, and performed molecular docking. Hesham Y. El-Zorba designed the experiments, performed the data analysis, and critically read the manuscript. Marwa A. Ibrahim performed gene expression study. All the authors read, revised, and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The materials and data used and analyzed in this research are available on request from the corresponding author.

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Table (1). The primer sets of assessed genes in the current study.

	Sense	Antisense	Amplicon	Accession number
<i>Nrf-2</i>	TGTAGATGACCATGAGTCGC	TCCTGCCAAACTTGCTCCAT	159	NM_031789.2
<i>TNF-α</i>	ACACACGAGACGCTGAAGTA	GGAACAGTCTGGGAAGCTCT	235	NM_012675.3
<i>PPAR-γ</i>	AGGGCGATCTTGACAGGAAA	CGAAACTGGCACCCTTGAAA	164	NM_013124.3
<i>Actb</i>	CCGCGAGTACAACCTTCTTG	CAGTTGGTGACAATGCCGTG	297	NM_031144.3

Table (2). GC-MS analysis of the psyllium husk methanolic extract

Peak	Name of the compound	Area Sum%	Retention Time
	9-Octadecenoic acid	36.91	26.865
	Methyl 8,9-octadecadienoate	11.17	45.224
	6-Octadecenoic acid	11.09	45.395
	Hexadecane, 1,1-dimethoxy	8.13	14.12
	Hexadecanoic acid	6.07	41.261
	Tridecanoic acid	1.94	46.015
	Decanoic acid	1.62	23.758
	Octanoic acid	1.53	20.112
	9,12-Octadecadienoic acid	1.37	6.489
	Octanoic acid	1.36	16.135
	12,15-Octadecadienoic acid	0.94	45.54
	2,2-Dimethylpropanoic acid	0.8	37.283
	Hexanoic acid	0.74	11.781
	Undecanoic acid	0.74	27.116
	9-Bromononanoic acid	0.74	33.897
	9-Methyl-Z-10-pentadecen-1-ol	0.69	32.495
	l-(+)-Ascorbic acid 2,6-dihexadecanoate	0.65	42.204
	7-Hexadecenoic acid	0.59	27.241
	9,12-Octadecadienoic acid	0.56	28.33
	Cyclohexanepropanoic acid	0.45	30.947
	Oleic acid	0.43	42.349

Table (3). Effect of psyllium husk extract and fibre on body weight change for 21 days

Estimated parameter	Control Non-diabetic	Diabetic	Extract 100 mg/kg	Extract 200 mg/kg	Glibenclamide 5 mg/kg	crude fibre 0.5 g/kg
Initial body weight (g)	207.00±0.71	197.80±3.79	194.50±1.65	201.25±0.62	204.00±0.82	208.68±1.03
Final body weight (g)	250.00 ^a ±2.55	155.76 ^d ±5.20	225.00 ^a ±7.63	248.97 ^c ±1.65	247.44 ^a ±2.36	247.76 ^b ±0.71
Body weight change (g)	43.00 ^a ±3.08	-42.04 ^d ±7.36	30.50 ^a ±8.57	47.72 ^c ±4.08	43.44 ^a ±3.09	39.08 ^b ±3.18
%	20.77	-21.25	15.68	23.71	21.29	18.72

Means within the same row carrying different superscripts are significantly different at $p < 0.05$. The values shown are means $\pm$ SE. $n = 10$

Table (4). Effect of administration of psyllium husk extract and fibre for 21 days on

Estimated parameters	Control Non-diabetic	Diabetic	Extract 100 mg/kg	Extract 200 mg/kg	Glibenclamide (5 mg)	Crude fibre 0.5 g/kg
Blood Glucose (mg/dl)	104.38±4.22 ^a	455.55±7.63 ^d	225.65±3.11 ^c	146.27±2.75 ^b	110.08±2.85 ^a	152.10±2.24
AST (U/l)	93.26±2.85 ^a	154.19±3.75 ^c	126.96±2.87 ^c	118.96±3.07 ^b	110.47±2.11 ^a	117.23±3.22
ALT (U/l)	25.77±1.88 ^a	68.36±2.11 ^b	40.93±2.66 ^b	28.32±2.92 ^a	44.45±1.33 ^b	37.37±1.11
ALP (U/dl)	135.33±6.62 ^a	321.45±7.21 ^d	205.20±4.41 ^c	165.33±3.59 ^b	148.71±2.94 ^a	141.80±2.74
Urea (g/dl)	23.52±1.26 ^a	46.70±2.83 ^b	23.63±1.913 ^b	24.59±1.47 ^a	22.86±1.22 ^a	23.89±0.91
Creatinine (mg/dl)	1.18±0.09 ^{bc}	1.95±0.10 ^{cd}	1.44±0.13 ^{cd}	1.37±0.07 ^d	1.10±0.08 ^{cd}	1.12±0.07 ^b
Total cholesterol (mg/dl)	125.25±2.12 ^a	195.71±4.08 ^c	135.21±2.55 ^b	126.87±3.05 ^a	125.28±4.20 ^a	118.68±1.84
Triglyceride (mg/dl)	91.56±3.02 ^a	211.34±4.22 ^c	145.45±1.47 ^b	123.33±2.72 ^b	116.00±1.71 ^a	119.25±2.44
LDL (mg/dl)	81.31±2.09 ^a	151.21±2.21 ^c	116.47±2.20 ^b	110.33±2.08 ^b	122.36±1.97 ^c	97.13±1.45
HDL (mg/dl)	40.96±1.11 ^a	31.81±1.57 ^c	36.08±1.43 ^b	40.54±0.72 ^a	43.22±1.82 ^a	41.77±1.04
Ratio Cholesterol:HDL	3.06	6.15	3.75	3.13	2.9	2.86

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: alkaline phosphatase; LDL: low-density lipoprotein; HD: High-density lipoprotein. Means within the same row carrying different superscripts are significantly different at $p < 0.05$. The values shown are means $\pm$ SE. $n = 10$.

Table (5). Docking score and affinity of the sodium-glucose cotransporter 2 (SGLT2) with the isolated compounds from psyllium husk methanolic extract compared to dapagliflozin, as a selective SGLT2 inhibitor.

Compound name	Compound CID	Binding affinity (kcal/mol)	Dimensions XYZ (Å)
Dapagliflozin	9887712	-7.5	63.243987, 61.686318, 63.587917
12,15-Octadecadienoic	5.4E+07	-7.2	62.069524, 61.195381, 67.919190

acid			
6-Octadecenoic acid	5281125	-7	62.079905, 60.125524, 69.237905
9-octadecenoic acid	637517	-7	61.877714, 61.674857, 67.435095
Methyl 8,9-octadecadienoate	9.2E+07	-6.8	61.720048, 60.549381, 68.615286
7-hexadecenoic acid	543268	-6.6	61.572737, 60.604316, 68.366211
9-Methyl-Z-10-pentadecen-1-ol	5363327	-6.5	63.571444, 60.625278, 70.012111
9,12-Octadecadienoic acid	3931	-6.4	65.990286, 71.902429, 77.731571

PubChem unique identifier (compound identifier (CID) Root Mean Square Deviation (RMSD)

Figures:

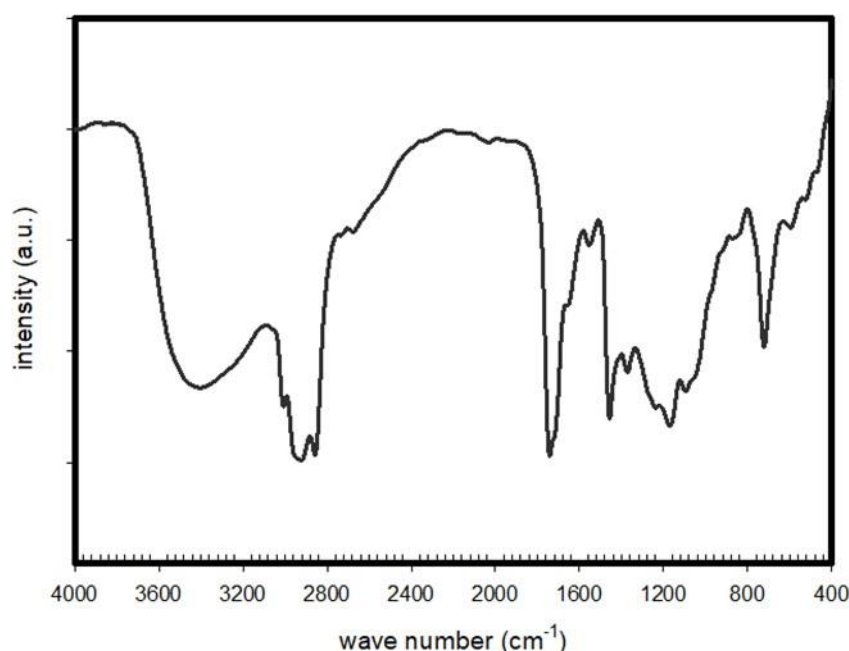


Fig. (1). FTIR spectra of psyllium husk methanolic extract.

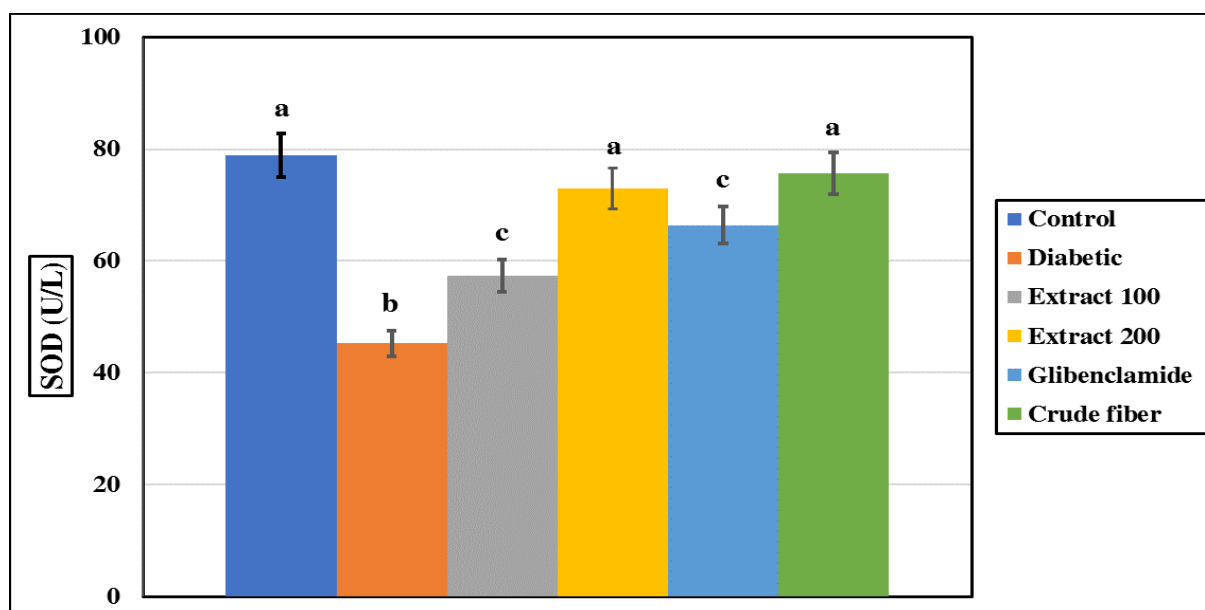


Fig. (2). Effects of psyllium husk extract and fibre on blood SOD (superoxide dismutase) indicator in male

Wistar rats for 21 days. Columns carrying different superscripts are significantly different (one-way ANOVA) ($P < 0.05$)

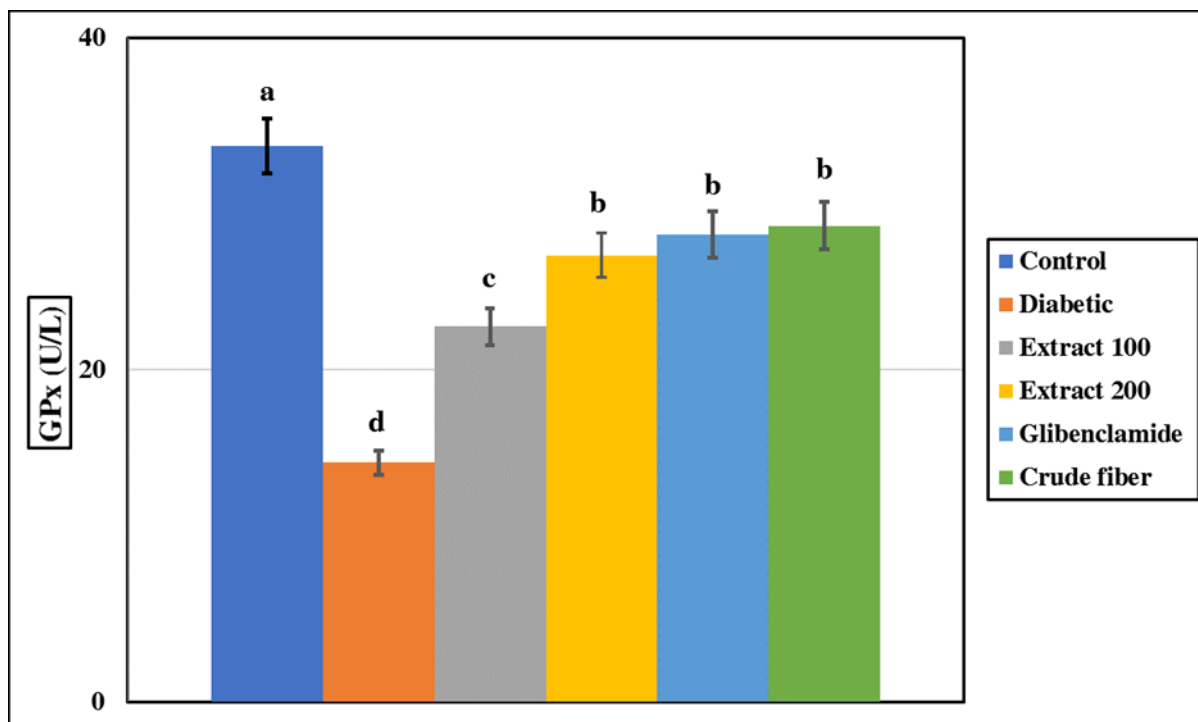


Fig. (3). Effects of psyllium husk extract and fibre on blood GPx (glutathione peroxidase) indicator in male Wistar rats for 21 days. Columns carrying different superscripts are significantly different (one-way ANOVA) ($P < 0.05$)

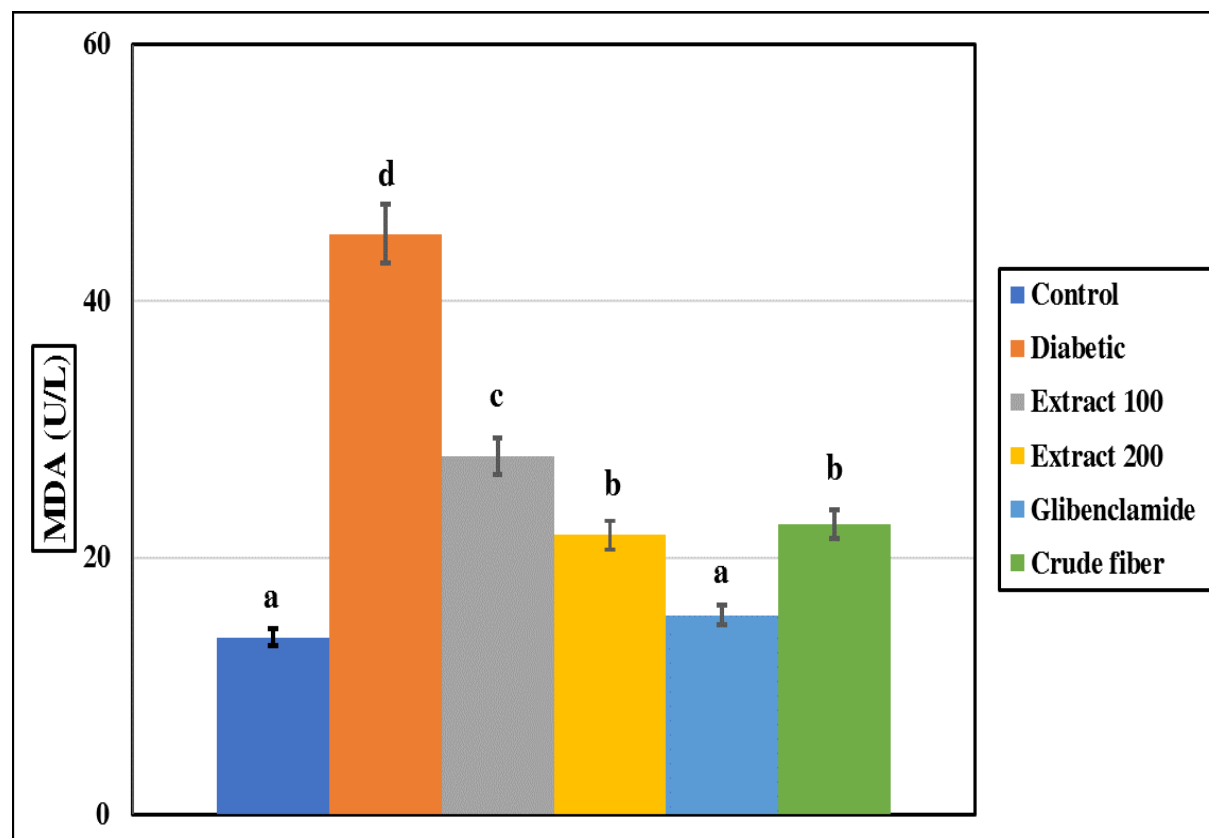


Fig. (4). Effects of psyllium husk extract and fibre on blood MDA (malondialdehyde) in male Wistar rats for 21 days. Columns carrying different superscripts are significantly different (one-way ANOVA) ($P < 0.05$)

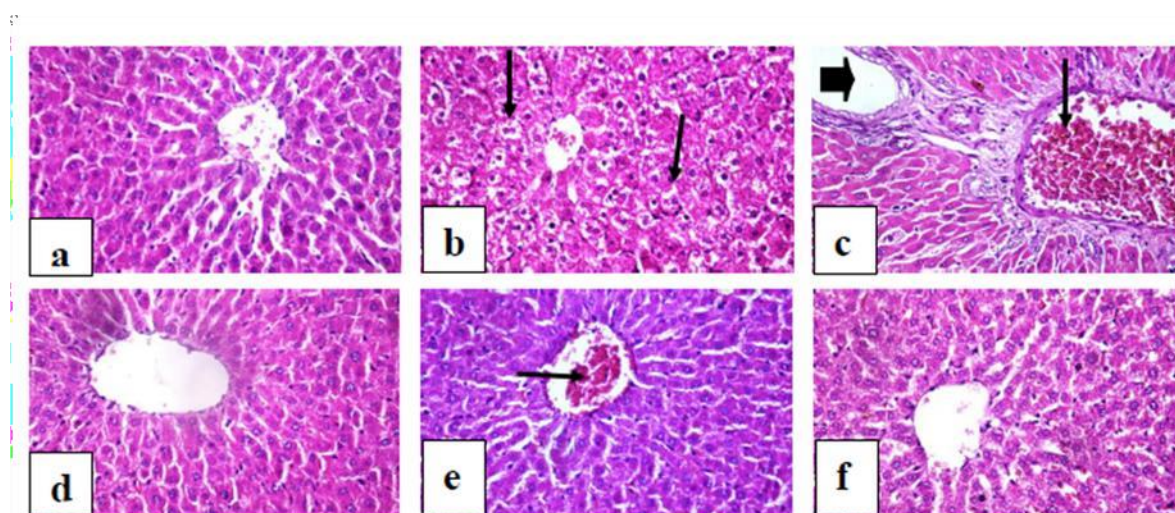


Fig. (5). Histopathological examination of liver stained with H&E×400. a: Control normal liver showed normal hepatic parenchyma with polygonal hepatocytes, normal blood sinusoids, and central vein. b: Diabetic liver showed massive and diffuse hepatic vacuolar degeneration; hepatocytes were swollen and vacuolated (arrows) with irregular arrangement and narrow blood sinusoids. c: Diabetic rats treated with 100 mg/kg psyllium husk extract showed normal hepatocytes and congestion of hepatoportal blood vessels (arrow), together with hyperplasia of the bile duct (arrowhead). d: Diabetic rats treated with 200 mg/kg psyllium husk extract showed healthy hepatic parenchyma; polygonal hepatocytes with normal blood sinusoids, and a central vein. e: Diabetic rats treated with 0.5 g/kg crude psyllium husk ground fibre suspension showed normal hepatic parenchyma, together with slight congestion of the central vein (arrow). f: Diabetic rats treated with 5 mg/kg glibenclamide showed normal hepatic parenchyma; polygonal hepatocytes with normal blood sinusoids, and a central vein.

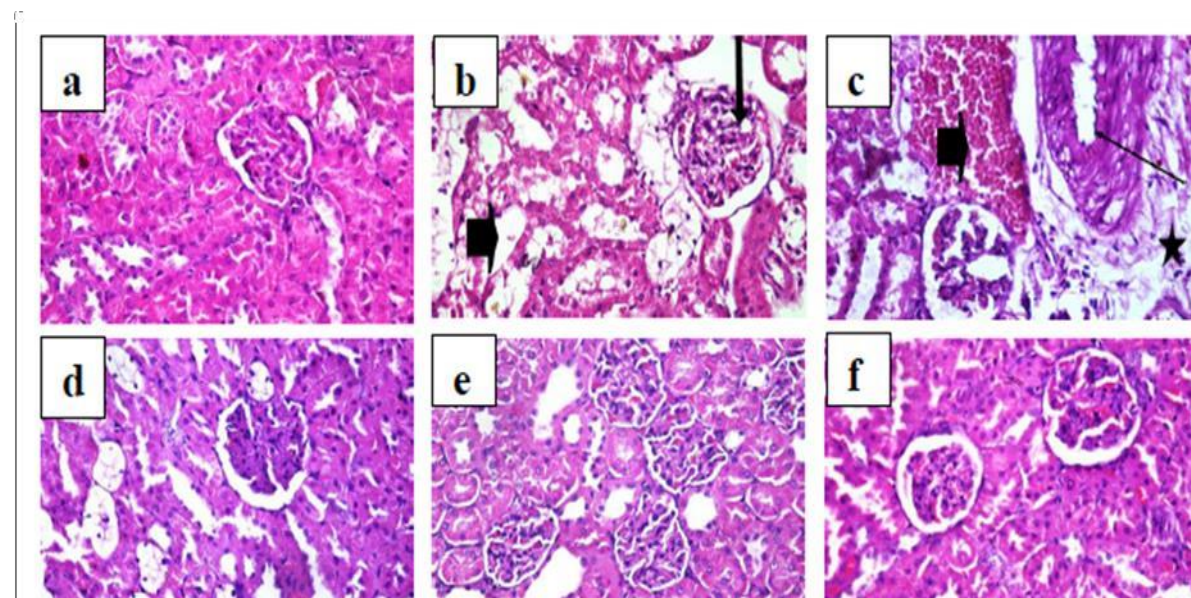


Fig. (6). Histopathological examination of the kidney stained with H&E×400. a: Control normal kidney showed normal renal parenchyma; note the normal renal glomeruli and normal renal tubules. b: Diabetic rats showed massive and diffuse renal tubular epithelium vacuolation, necrosis (arrowhead), and glomerular epithelium vacuolation (arrows). c: Diabetic rats treated with 100 mg/kg psyllium husk extract showed dilatation of interstitial blood vessels with thickened muscular walls (arrow) and perivascular edema (*), together with areas of hemorrhage (arrowhead). d, e & f: Diabetic rats treated with 200 mg/kg psyllium husk extract, 0.5 g/kg crude psyllium husk ground fibre and 5 mg/kg glibenclamide showed normal renal parenchyma; note the normal renal glomeruli and normal renal tubules.

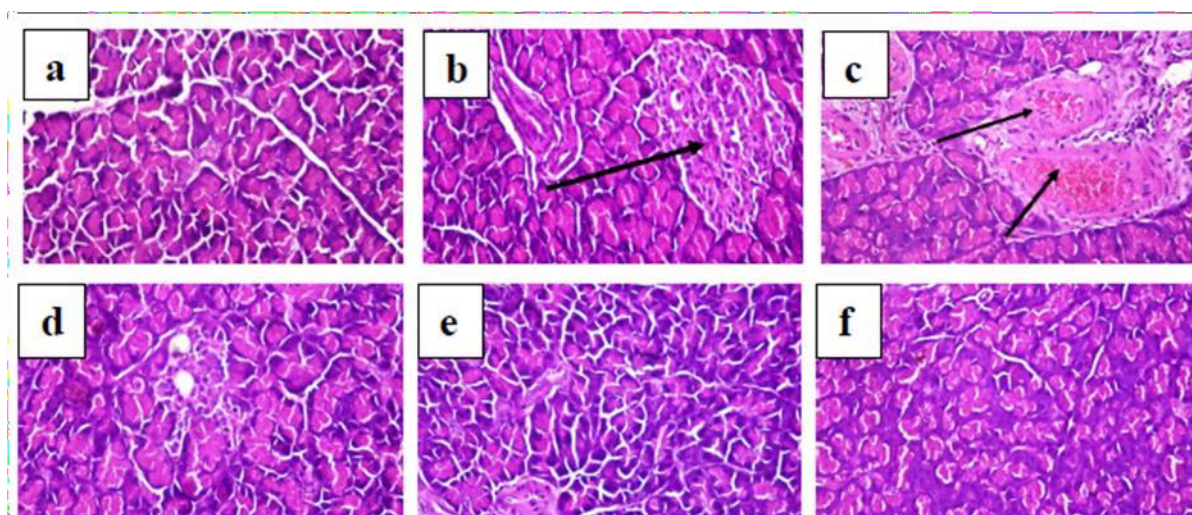


Fig. (7). Histopathological examination of the pancreas stained with H&E×400. a: Control normal pancreas showed normal pancreatic acini and islets. b: Diabetic rats showed hyperplasia of pancreatic islets (arrow). c: Diabetic rats treated with 100 mg/kg psyllium husk extract showed dilatation and congestion of the interstitial blood vessels (arrows). d, e & f: Diabetic rats treated with 200 mg/kg psyllium husk extract, 0.5 g/kg crude psyllium husk ground fibre, and 5 mg/kg glibenclamide showed normal pancreatic acini.

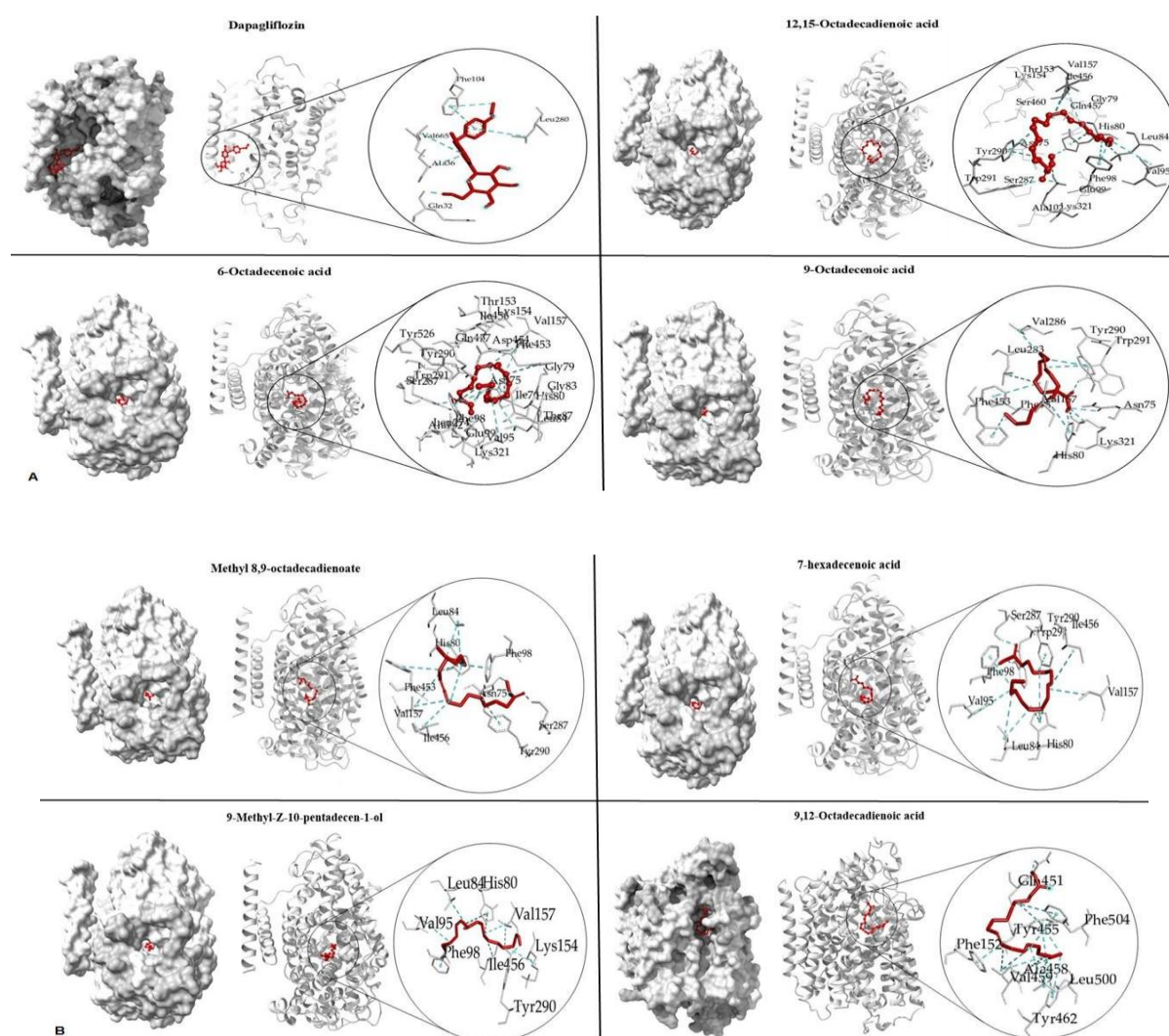


Fig. (8 A&B). 3D- molecular docking interaction between SGLT2 receptor active sites and the bioactive compounds of psyllium husk methanolic extract identified by GC–MS analysis compared with dapagliflozin.

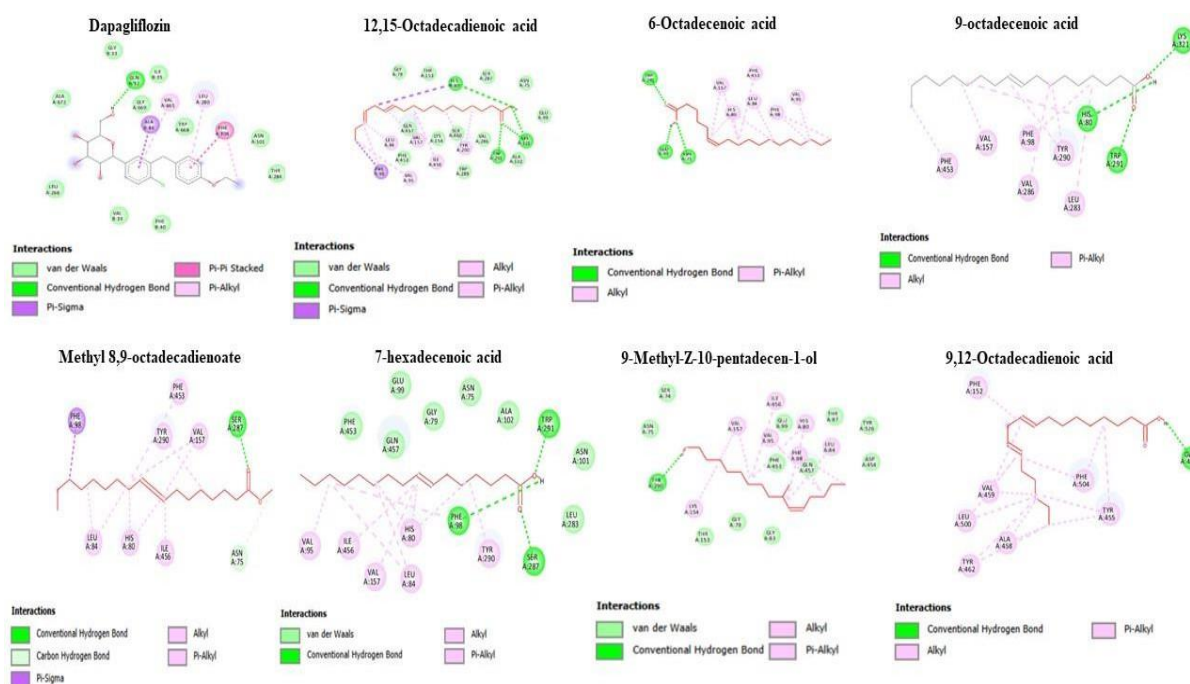


Fig. (9). 2D-molecular docking interaction illustrating the binding between the SGLT2 receptor and the ligands. The ligands are the seven bioactive compounds identified by GC–MS analysis of psyllium husk methanolic extract and dapagliflozin, all docked with the SGLT2 protein.

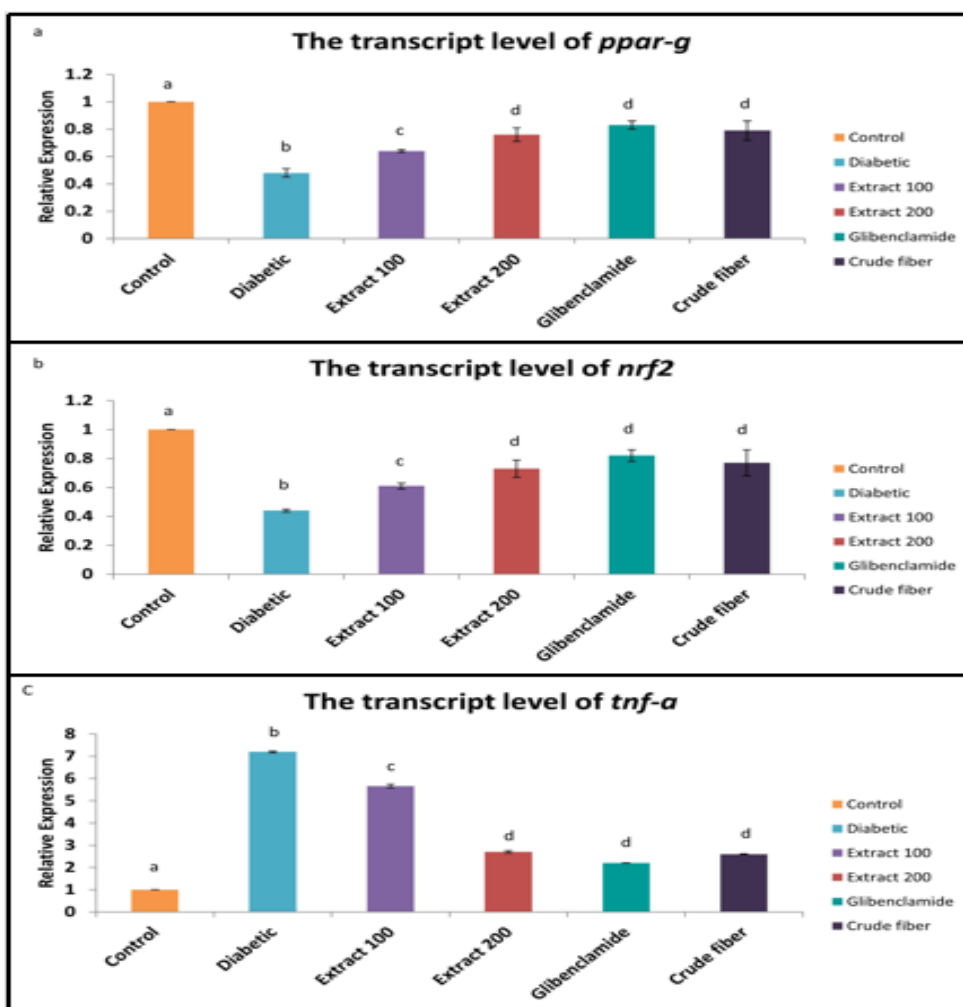


Figure (10). Bar charts of the target genes' expression levels in the hepatic tissue (a) PPAR- γ , (b) Nrf2, (c) TNF- α , (d) data are expressed as (Mean $\pm$ SEM). Values are statistically significant when $p \leq 0.05$.

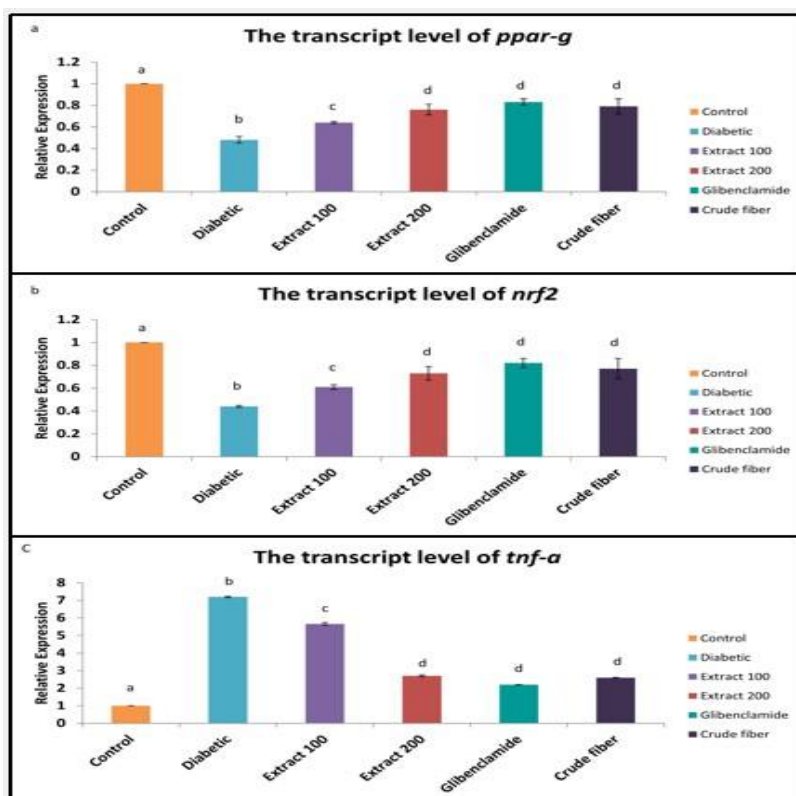


Figure (11). Bar charts of the target genes' expression levels in the renal tissue (a) PPAR- γ , (b) Nrf2, (c) TNF- α , (d) data are expressed as (Mean $\pm$ SEM). Values are statistically significant when $p \leq 0.05$.

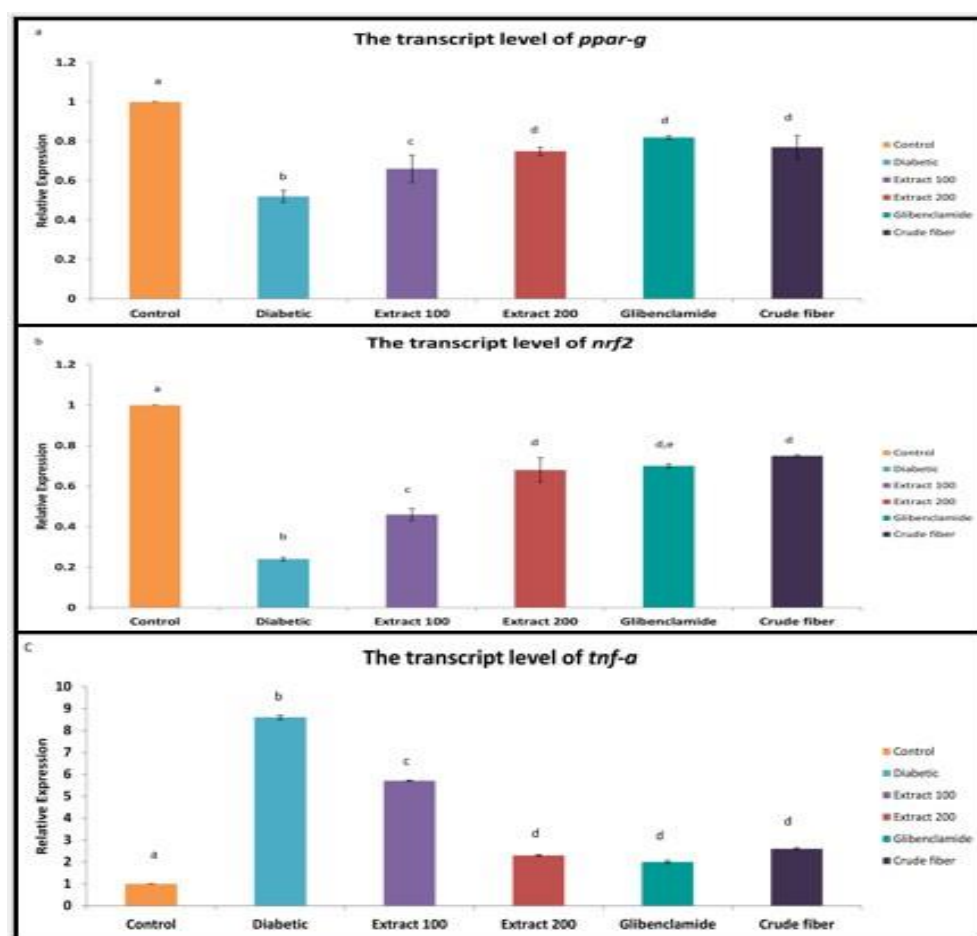


Figure (12). Bar charts of the target genes' expression levels in the pancreatic tissue (a) PPAR- γ , (b) Nrf2, (c) TNF- α , (d) data are expressed as (Mean $\pm$ SEM). Values are statistically significant when $p \leq 0.05$.