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Effect of *prickly pear cactus* seeds oil on the blood glucose level of streptozotocin-induced diabetic rats and its molecular mechanisms

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Abstract
This study was undertaken to investigate the hypoglycemic effect of the prickly pear cactus seeds oil (PPO) in streptozotocin-induced diabetic rats (STZ) and its molecular mechanisms. Oil extracts exhibited strong antioxidant properties when compared to α-tocopherol. Unsaturated fatty acids were the most fatty acids content representing up to 80.9%. Administration of PPO at different doses to STZ induced diabetic rats, resulted in significant decrease (P<0.05) in plasma glucose level in a dose dependent manner. Treatment with PPO elicited an increase in the expression level of glucose transporter 2 (Slc2a2) gene but reduced the expression of phosphoenolpyruvate carboxykinase (PCK1) gene. More fore, PPO treated rats, showed significantly lower levels of serum alanine transaminase (ALT), aspartate aminotransferase (AST) and urea levels compared to STZ untreated rats. Prickly pear cactus oil is beneficial for improvement of hyperglycemia by increasing gene expression of liver Slc2a2 and reducing expression of PCK1 genes in streptozotocin-induced diabetic rats.

Keywords: *Prickly pear cactus oil*; fatty acid; antioxidant;(STZ)-induced diabetic rats; genes expression.

1. Introduction
The prickly pear cactus, is a Native American plant that has become widely spread throughout the world, especially in the drier countries of Africa, the Mediterranean and the Middle East. Many uses of cladodes and cactus pear fruit are reported [1]. Cladodes are consumed as fresh vegetables or added to casseroles [2]. The seeds are made of two different tissues, the endosperm and the pericarp in the relative proportion of 1:9, respectively. Analysis of the main constituents of prickly pear seeds showed a significant amount of polysaccharides, cellulose and hemicelluloses, and the structure of their glucuronoxylans has been identified by [3]. Prickly pear seeds oil has reported to exhibit hypocholesterolemic effects, probably due to the fatty acid composition of the prickly pear seeds oil [4] and anti-diabetic effect [5]. The cultivation of the prickly pear in a number areas of Yemen, is gaining the status of Agriculture and occupation due to its advantages of economic and multiple nutritional values, after the plant was not common between the farmers and the population in Yemen, some farmers in some areas starting to have a private association for prickly pear after they sensed that the feasibility of cultivation and production of prickly pear. The Gyeman area which located in Bani Bahloul, Sana'a is considered the first area that tended to farming prickly pear as a basic agricultural product alternative to Qat tree. Our experiments are shown that the PPO extracted from the prickly pear seeds to have potent antioxidant capacity similar to vitamins E. As a potent antioxidant capacity, PPO should be explored to establish new pharmacological possibilities for its future development and application. The present study was undertaken to evaluate the potential hypoglycemic activity of the PPO in streptozotocin (STZ)-induced diabetic rats. Effects of PPO at different doses were compared to glibenclamide which used as a reference hypoglycaemic drug. An understanding of the many diverse signals that regulate glucose metabolism will aid in the development of pharmacological agents from PPO to treat diabetes. Since several anti-diabetic agents, including plant derived drugs target the liver to decrease hepatic glucose production, changes in hepatic expression of the glucose metabolism regulating genes including glucose transporter 2 (Slc2a2) and phosphoenolpyruvate carboxykinase (PCK1) were determined by real time PCR to explore the hypoglycemic mechanism(s) of action of PPO.

2. Materials and methods

2.1 Chemicals

Standard fatty acid methyl esters were purchased from Sigma-Aldrich (Sigma-Aldrich Co., Missouri, USA), Petroleum ether, n-hexane, sodium hydroxide and Boric acid were purchased from Fisher (Fisher Scientific Co Ltd., Ottawa, Canada). Chloroform and methanol were purchased from BDH (BH15 ITD2, England). 1,1-diphenyl-2-picrylhydrazyl (DPPH), was purchased from Sigma-Aldrich (St. Louis, MO). Vitamin E was purchased from Fisher Scientific (Loughborough, UK). Methanol and DMSO were purchased from Fisher Scientific (Fisher Scientific Co Ltd., Ottawa, ON).

2.2 Plant material

The prickly pear cactus fruit were collected from the Gyeman area which located in Bani Bahloul Sana'a Yemen. Seeds were obtained from the fruit, dried, powdered and stored and protected from light at -20 ^{0}C prior to further use.

2.3 Oil extraction, GC analyses of fatty acids and total antioxidant activity of the oil

2.3.1 Oil extraction

The prickly pear cactus seeds were finely ground using an electrical grinder (Waring Blender, Tokyo, Japan) at speed 6 for 2 min and was passed through a 35 mm (42 mesh) sieve. Homogenized and ground samples were soaked overnight in three different solvents 1:5 (w/v):*n*–hexane, petroleum ether and chloroform-methanol (2:1 v/v) at room temperature. The mixture of samples and solvent was covered with aluminum foil, kept overnight at room temperature. The mixture was filtered through a filter paper (Whattman No. 2). The extraction procedure was repeated twice and the solvent was removed using a rotary evaporator (Rikakika Co. Tokyo, Japan) at 50°C and 90 rpm. The extracted oil was transferred into glass sealed amber dark bottles and then stored in a freezer (−20 °C) for subsequent analyses.

2.3.2 GC Analyses of fatty acids

Fatty acids composition of the oil was determined using gas chromatography according to the International Union of Pure and Applied Chemistry (IUPAC, 1979) method. Fatty acid methyl ester was prepared as the following: the oil was mixed with 0.95 ml hexane in 2 ml screw capped vial and was vortexed. 0.05 ml of 1M-sodium methoxide (prepared by dissolving 1.15 g sodium in 50 ml MeOH) was added into the mixture and was shacked again for 5 seconds. The clear upper layer of the methyl ester was injected into a Hewlett- Packard GC apparatus (Model 439 USA), equipped with a hydrogen flame ionization detector. The carrier gas used was helium, at a flow rate of 3 ml/min and a glass column, [DB 23 cis/trans (50% cyanopropyl) - methylpolysiloxane] was used as the stationary phase. The column was set at 90°C, injection and detection temperatures were 300 °C and column temperature reached 400°C.

2.3.3 Determination of DPPH radical scavenging activity

The antioxidant activity of the prickly pear cactus oil was used for the antioxidant analysis, on the basis of the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, were determined by the method described by Benzie and Strain (1996). Vitamin E was used as standard antioxidants and methanol was used as the control. An aliquot of 0.5 ml of a methanolic solution of DPPH (50 mg DPPH/100 mL MeOH) was added into the different concentration (1, 0.5, 0.25 mg/ml) of each extract and Vitamin Eas long as control samples (both extract and ascorbic acid were dissolved in methanol). All samples were incubated in the dark at room temperature for 30 min before absorbance values were read at 517 nm (Amersham 2100Pro, UV-vis spectrophotometer, UK). The decrease in absorbance was calculated as an IC_{50} and expressed as µg/ml, which is the concentration of sample required for 50% scavenging of DPPH radicals in the specified time period.

The radical scavenging effect was calculated as follows:

Radical scavenging effect (%) = Ac – As/Ac × 100

Ac = absorbance of control and as = absorbance of test sample. Where control is the absorbance of the DPPH radical+ methanol.

2.4 Animals and treatment

Male Sprague-Dawley rats aged 6-8 weeks (180-250g) were sued in this study. They were randomly selected and housed under standard environmental conditions (25±1°C, 12 h/12 h light/dark cycle). Animals had free access to water and a standard laboratory diet (carbohydrates; 30%, proteins; 22%, lipids; 12%, vitamins; 3%). Experiments were carried out according to the guidelines for the use of animals and approved by The Animal Care and Use Committee of the Faculty of Agriculture, University of Sana`a, Yemen

2.4.1 Induction of experimental diabetes

After an overnight fast, diabetes was induced by intraperitoenal (i.p.) injection of a freshly prepared solution of streptozotocin (STZ) (Sigma, St. Louis, Mo) dissolved in 0.1 M cold sodium citrate buffer (pH 4.5) at a dose of 55 mg/kg body weight. Animals were then allowed to drink 5% glucose solution overnight to overcome the drug-induced hyperglycaemia. Control rats were injected with citrate buffer alone. After 1 week, rats with fasting blood glucose levels of greater than 13.8mmol/l were considered as diabetic and used in the present study according to previous study [6].

2.4.2 Experimental design

Rats were divided into six different groups; normal control rats were not induced with STZ and served as reference group. STZ diabetic control rats were given normal saline. Three STZ diabetic groups were treated with PPO at 0.2, 0.4 and 0.6 g/kg, twice daily continuously for 21 days. The fifth group received the reference drug, glibenclamide (6mg/kg) in aqueous solution orally for 21 days. The doses of the extract were chosen based on dose-response studies that were conducted earlier in our laboratory. Body weight, food and water consumption were measured weekly. Blood was collected from the eye at baseline and during treatment period after 1, and 2 weeks for glucose determination. After 21 dyes, blood was collected by cardiac puncture for o haematology and biochemistry tests. At the end of this period the rats were anaesthetized with diethyl ether and killed by cervical dislocation. Liver was excised, rinsed in ice-cold saline and snapped frozen with liquid nitrogen within the period of 2-5 min after death, and stored at −80°C for gene expression studies.

2.4.3 Parameters

Serum glucose, alanine transaminase (ALT), aspartate aminotransferase (AST) urea and creatinine levels were measured using reagent kits (Instrumentation Laboratory, USA) by ILab Chemistry Analyzer 300 PLUS (Instrumentation Laboratory, USA).

2.4.4 Gene expression studies

2.4.4.1 RNA isolation

Total RNA was isolated from fresh frozen liver tissues kept in liquid nitrogen, by TRI Reagent solution according to manufacturer's instructions (Ambion, Austin, Texas, USA). The total RNA concentration was determined by measuring the absorbance at 260 nm. Purity of the extracted RNA was determined by measuring the ratio of the optical density at 260 nm and 280 nm using a spectrophotometer (BioRad, USA) and ranged between 1.8 and 2.0. The integrity and size distribution of the total RNA was determined using a 1.5 agarose gel. The 18S and 28S RNA bands were visualized under UV light using gel image instrumentation.

2.4.4.1 Quantitative Real-Time PCR

Primers specific for Slc2a2, PCK1and beta-actin genes were designed from the gene sequence of rat (*rattus norvegicus*) adopted from the NCBI (National Center for Biotechnology Information) GenBank Database (www.ncbi.nlm.nih.gov) and supplied by Next Gene. GenBank accession number code forSlc2a2 is NM_012879.2, for PCK1 is NM_198780.3 and for beta actin gene is NM_031144.2. The sequences of the primers and the size are shown in Table 1.Real-time PCR using SYBR Green chemistry was performed on a Rotor Gene 6000 cycler with a 36 well rotor (Corbett Research UK). The run was performed using Sensi Mix one-step RT-PCR kit with SYBR Green (Quantace, London, UK) according to manufacturer's instructions. Briefly, a reaction volume of 25 µl contained 12.5 µl master mix, 1 µl of 5 µM each forward and reverse primers, and 1 µl of the template RNA at concentration of 100 ng. The reactions were performed under the following conditions: 42°C for 30 min (this step was included to synthesize cDNA), 95°C for 10 min, 40 cycles at 95°C for 15 s, 53°C for 30 min and 72°C for 4 s. Data were acquired on the SYBR channel at the end of each extension step. Melt curves were analysed to check for the absence of mispriming. The possibility of a genomic DNA influence on the results was eliminated by use of primers. Each experiment was performed three times and all samples were run in triplicates. Expression levels for each gene relative to beta-actin were calculated for all samples using the Rotor Gene software (Version 1.7, Corbett Research) and Microsoft Excel. Analysis of gene expression data was carried out by $\Delta\Delta C_T$ method of relative quantification (Kenneth and Thomas, 2001).

2.5 Statistical analysis

Data were expressed as mean±SD. Statistical analysis was performed using analysis of variance (ANOVA) followed by Bonferroni post test. Differences were considered to be significant when $P< 0.05$.

3. Results

3.1. Crude oil content in *prickly pear cactus seeds*

Oil yield extracted using chloroform: methanol produced a yield of 7.76 ± 0.43, (w/w) whereas, the yield extracted using hexane and petroleum ether were 5.0 ± 0.36 and 6.1± 0.41) respectively.

3.2. Fatty acid composition in *prickly pear cactus seeds* oil

As shown in Table 2, unsaturated fatty acids were the most fatty acids content representing up to 80.9 in the oil. However, the major unsaturated fatty acids were oleic acid (22.30, %) and linoleic acid (57.5 %). On the other hand the major saturated fatty acids of PPO were palmitic acid and stearic acid. In addition, arachidic (C20:0), behenic (C22:0) and lignoceric (C24:0) were detected as a saturated fatty acids as well as palmitoleic (C16: 1) and erucic (C22: 1n9) acids were detected as monounsaturated fatty acids in the three different samples. The ratio of saturated to unsaturated fatty acids (U/S %) was 4.4%.

Table 2: Fatty acid composition of three different samples of *prickly pear cactus* oil

Fatty acid profile	% of FA profile in prickly pear cactus oil
Myristic C14 : 0	0 , 1 9 ± 0 , 0 2
Palmitic C16 : 0	1 4 , 3 0 ± 0 , 2 4
Palmitoleic C16 : 1	0 , 9 7 ± 0 , 0 3
Stearic C18 : 0	3 , 1 2 ± 0 , 0 4
Oleic C18 : 1	2 2 , 3 1 ± 0 , 2 4
Linoleic C18 : 2n6	5 7 , 6 0 ± 0 , 2 5
Linolenic C18 : 3n3	0 , 2 3 ± 0 , 0 1
Arachidic C20 : 0	0 , 3 7 ± 0 , 0 5
Eicosenoic C20 : 1	0 , 3 2 ± 0 , 0 3
Eicosadienoic C20 : 2	0 , 2 0 ± 0 , 0 3
Behinic C22 : 0	0 , 3 9 ± 0 , 0 4
U/S	4,44

Each value represents the mean of two replications. U/S= the ratio of total unsaturated fatty acids and total saturated fatty acids.

3.3. Antioxidant activityof prickly pear cactus seeds oil

The results of the free radical scavenging activity of PPOand the authentic antioxidant of vitamin E was summarized in Table 3A. Result from this assay clearly showed that the oil exhibited high antiradical activity towards DPPH radical. The scavenging activity of the pure antioxidant standard, vitamin E (α tocopherol) was 47% at lower concentration of 100µg/ml. The radical scavenging activity of the oil was concentration dependent and it increased by concentration to 80 and 87% at 500 and 1000µg/ml respectively. After 30 minutes of the reaction, the oil at 0.25 mg/ml scavenged more than 74% of the total radicals in the reaction system. Subsequently, the scavenging activity of the oil was gradually increased up to 87% of the total radicals, at higher concentration (1mg/ml). As shown in Table 3B, IC_{50} values of were135±2.6, 150±1.5 and 180±3.0 µg/ml respectively in oil extracted using chloroform: methanol, hexane and Petroleum ether whereas for vitamin E was 120±2.0.

Table 3A: The DPPH free radical scavenging activity of the PPO

Samples	Radical scavenging effect (%)			
	Concentration (mg/ml)			
	0.1	0.25	0.5	1.00
Vitamin E (α tocopherol)	47±5	79±6	85±5	90±4
PPO chloroform: methanol	45±5	74±3	80±9	87±8
PPO Hexane	42±9	76±6	78±7	86±6
PPO Petroleum ether	36±8	59±2	66±6	76±5

Table 3B: Antioxidant activities of the *selected leaves* extract and positive control using the (DPPH) free radical-scavenging assay

Samples	Antioxidant activity IC^{50}/ DPPH (µg/ml)
Vitamin E (α tocopherol)	120±8.0
PPO chloroform: methanol	135±2.6
PPO Hexane	150±5.5
PPO Petroleum ether	180±3.0

3.4. Plasma glucose levels

Plasma glucose levels of the experimental period is shown in Figure 1. At the end of the experiment the diabetic control rats and normal control rats had comparable levels of blood

glucose. The STZ control group had significantly ($p < 0.01$) higher of serum glucose level compared to normal group. After three weeks of treatment, the diabetic rats that received PPO at doses of 0.4 and 0.6g/kg and glibenclamide had significantly ($p < 0.05$, reduced blood glucose concentrations compared with STZ control group. There was no significant difference in serum glucose levels of STZ treated rats with PPO at different doses and STZ treated rats with glibenclamide at the end of the experiment period.

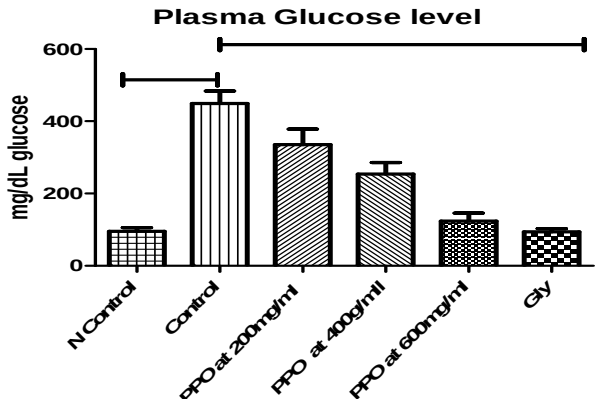

Fig. 1. Rats were randomly assigned into normal control group (N Control), adiabatic control group (Control), diabatic groups tretaed with PPO at doses of 200,400, and -600 mg/kg body wieght or with glibenclamide. STZ was administrted as a single dose (55 mg/kg i.p.) one week before treatment with PPO or glibenclamide. Data are presented as Mean ± SD. Lines indicate significant difference ($p < 0.05$; $n = 6$).

3.5. Body weight

STZ administration caused a significant decrease in the body weight when compared STZ treated rats with the PPO at 400 and 600 mg/kg body wieght (Figure 2) whereas no significant different was observed at 200 mg/kg body wieght when compared with STZ control rats.

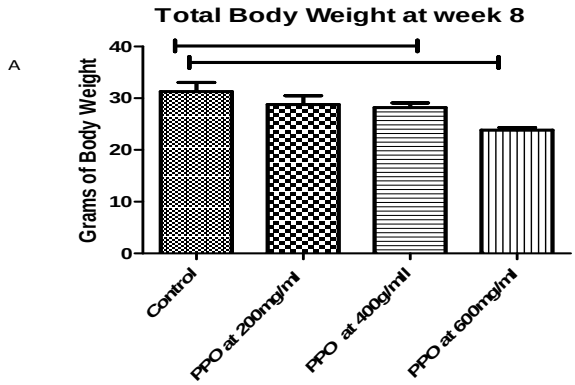

Fig 2: Data are presented as Mean ± SD. Lines indicate significant difference ($p < 0.05$; $n = 6$).

3.6. ALT, AST, Creatinine and Urea levels

The STZ control group showed significant elevations in ALT and AST levels compared to normal control group (Table 4). Administration of PPO at different doses (0.2, 0.4& 0.6 g/kg) to STZ diabetic rats caused reversal of the elevations in ALT and AST were elicited by STZ although the values were not lowered to the levels shown by normal non diabetic control group. Creatinine levels were not affected by STZ nor by PPO or glibenclamide treatment. Urea levels were significantly higher in STZ diabetic group compared to normal control group. Neither PPO nor glibenclamide had much effect on urea levels of diabetic rats.

Table 4: AST, ALT, creatinine and urea levels

Groups	AST (U/L)	ALT (U/L)	Creatine (mg/dl)	Urea (mg/dl)
NControl	90.0±24.4 [a]	54.0±20.10 [a]	0.42±0.04 [a]	26.0±7.60 [a]
stzControl	313.3±86.2[b]	230.2±63.60 [b]	0.30±0.08 [a]	70.8±13.50 [b]
stz0.2g/kg PPO	201.5±68.8[c]	199.0±98.00 [a]	0.31±0.10 [a]	67.3±14.10 [a]
stz0.4g/kg PPO	184.8±41.7[c]	64.80±27.00[a]	0.31±0.10 [a]	72.3±36.70 [a]
stz0.6g/kg PPO	154.8±63.0[c]	46.50±14.20[a]	0.55±0.09 [a]	42.3±27.40 [a]
Stz Glibenclamide	160.7±53.5 [c]	103.2±42.70[a]	0.29±0.10 [a]	54.3±26.60 [a]

Data are presented as Mean ± SD. Different letters within same column indicate significant difference ($p < 0.05$; $n = 6$).

3.7. Gene expression of glucose metabolism regulating enzymes in the liver.

Beta actin gene showed constant expression in different experimental groups and was therefore chosen as the reference gene transcript. Amplification and melting curves analysis were performed which resulted in single product specific melting temperatures as follows: beta actin at 84.3°C, Slc2a2 at 82.5 °C, and PCK1 at 82.3 °C. No primer-dimers were generated during the applied 40 real-time PCR amplification cycles. mRNA level of Slc2a2 in STZ diabetic control group were not significantly different from that of normal group (Figure3).Treatment with PPOcaused a significant increase in the expression of Slc2a2 mRNA level at different doses, compared to control diabetic rats. However no significant changes was observed in Slc2a2 mRNA level in treated rats with glibenclamidecompared to control diabetic rats.

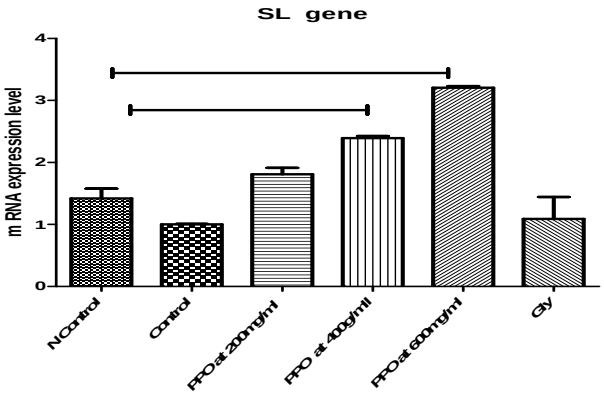

Fig 3. Effect of PPO at different doses on mRNA level of facilitated glucose transporter 2 gene (Slc2a2) in liver of diabetic rats. Rats were randomly assigned into normal control group (N Control), adiabatic control group (Control), diabatic groups tretaed with PPO at doses of 0.2, 0.4, 0.6 g/kg body

wieght or with glibenclamide. STZ was administrted as a single dose (55 mg/kg i.p.) one week before treatment with PPO or glibenclamide. Data represent the means±SD. Lines indicate significant difference (P<0.05) (n=6). Run was performed using real time PCR.

As shown in Figure 4, there was a significant reduction ($p < 0.05$) in the expression of PCK1in the groups treated with PPO at different doses when compared with the diabetic control group, whereas with glibenclamidetreatment statistically insignificant reduction was observed when compared with the diabetic control group.

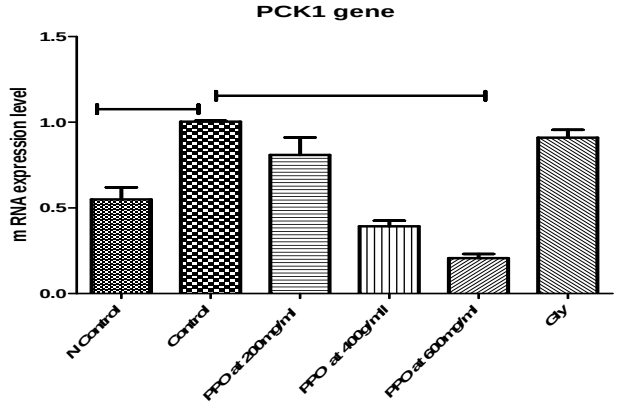

Fig 4. Effect of PPO at different doses on mRNA level of Pck1 in diabetic rat's liver. Data represent the means±SD. Lines indicate significant difference (P<0.05) (n=6). Run was performed using real time PCR.

4. Discussion

Diabetes mellitus (DM) is growing concern and represents one of the main threats to human health. Numerous studies have reported the potential hypoglycemic effect of plant oil extract in reducing the glucose level in blood in STZ induced diabetic rats [7, 8, 9]. The present study was undertaken to investigate the hypoglycemic effect of PPO in STZ induced diabetic rats.Glibenclamide was used as reference drug to compare the antidiabetic properties of PPO. For fatty acid composition, unsaturated fatty acids are the major component of cactus oil. Values are listed in Table-2. Together oleic and linoleic acids constitute 84% of the fatty acids. Linolenic acid was detected at a low level in cactus seed oil (<0.3%). The major unsaturated fatty acid detected was linoleic acid followed by oleic acid (Table 2). Cactus seed oil constitutes an important source of polyunsaturated fatty acid (PUFA) (61%) which has been reported to have many medical health properties.

The PPO showed low absorbance values compared to control samples, which indicated a high level of antioxidant activity. A concentration dependent reducing activity was observed for the PPO and for vitamin E as shown in Table.3. The PPO showed increased reducing power with the increased in the PPO concentration. The PPO extracted using chloroform: methanol however, contain overall higher reducing activity compared to the PPO extracted using hexane and petroleum ether.

Streptozotocin has been widely used for inducing type I diabetes in a variety of animals by affecting degeneration and necrosis of pancreatic b-cells [10]. Further, the STZ diabetic animals may exhibit most of the diabetic complications, namely, myocardial cardiovascular, gastrointestinal, nervous, vas deferens, liver, kidney, and urinary bladder dysfunctions through oxidative stress [11]. Several workers have reported that STZ-induced diabetes mellitus and insulin deficiency lead to increase blood glucose [12], by inducing necrosis of beta-cells of the islets of langerhans, thus causing hypo-insulin and hyperglycemia [13]. In our study, 3 weeks treatment with PPO daily at different doses significantly reduced fasting plasma glucose in STZ induced diabetic rat compared to STZ untreated control in a dose dependent manner (P<0.05). Administration of glibenclamide to STZ induced diabetic rats for 21 days, resulted in significant reduction of plasma glucose level in comparison to the STZ control group.

The rate-limiting step in the uptake and metabolism of glucose by insulin target cells is glucose transport, which is mediated by specific glucose transporters of the plasma membrane. Among various glucose transport systems, the liver plays a dual role, as glucose uptake occurs from circulation when gluconeogenesis and glycogenolysis are low; however, glucose is released when gluconeogenesis and glycogenolysis are activated [14]. In the present study, hepatic Slc2a2expression was higher in diabetic STZ animals compared to normal rats. Friedman *et al*., [15], have shown that the expression of hepatic Glut2 was increased in diabetic animals compared to normal rats. In contrast to the hepatic Glut2, the liver Slc2a2 expression was significantly higher in the treated groups with PPO at .4 and 0.6 g/kg when compared with the control group.

The treatment significantly reduced the expression of the gene encoding the regulatory enzyme of gluconeogenesis and glycogenolysis PCK1 in the liver of STZ rats. These results indicate that PPOaffected the last step in gluconeogenesis and glycogenolysis. Glucose is formed from gluconeogenic precursors in liver, and also from glycogen in liver. The present study reveals that PPO, represses hepatic PCK1 gene expression. Because of insulin level was not measured in the present study, so we were not able to address weather if any stimulation of insulin secretion is likely to have taken place after administration of the PPO. Subsequently, some hypothetical suggestions can be made, that the plasma glucose lowering effect in the absence of plasma insulin concentration suggests that PPO treatment may involve in an insulin independent mechanism. It can be postulated to involve an antioxidant mechanism as our results from this study has shown that PPO was shown to be a potent antioxidant. Glucose transporter 2 (Slc2a2), is present in the liver under both gluconeogenic and glycolytic conditions. Under the former condition, Slc2a2 activity would be essential for both glucose secretion and keeping the intracellular Glu-6-P concentration low and thus avoid permanent activation of glycolytic and lipogenic genes [16]. In the present study, thus, increase the expression of hepatic Slc2a2 might add another efficient mechanism through response to insulin action. Two groups [17, 18] reported in murine hepatocytes that Slc2a2 aand the insulin receptor (IR) seemed to colocalize and cointernalizein the endosomal fraction in response to insulin action. Such IR-Slc2a2 a complexes were increased in a mouse model of improved hepatic insulin sensitivity [18]. Serum ALT and AST levels were determined to evaluate the hepatic functions in hyperglycemia rats. Measurement of enzymatic activities of aminotransferase (AST and ALT) is of clinical and toxicological importance, as changes in their activities are indicative of tissue damage by toxicants or in disease conditions [19]. In normal rats, the normal level of serum AST, ALT in treated and untreated rats suggests that the extract may not be toxic at least to the liver at the concentration employed. However, the results of our study showed that STZ diabetes in rats caused significant increases in the levels of AST and ALT in diabetic rats, these findings were also reported by many other workers, [20] proposed that STZ in hyperglycemic

animals caused a time dependent rise in AST and ALT levels. Elevated levels of these transaminases could be responsible for the increased gluconeogenesis and ketogenesis that are observed in diabetes [21, 22]. Therefore, treatment with POO at different doses caused significant reduction of AST and ALT compared to untreated STZ diabetes rats in a dose dependent manner, that`s suggested that the PPO prevented liver damage further studies should carried out to investigate this activity and the mechanism of action. The diabetic hyperglycemia induces elevation of the serum levels of urea which were considered as significant markers of renal dysfunction [23]. The result show there was significant increase in the level of serum urea in the diabetic control groups compare with the non-diabetic control groups.

5. Conclusion. The PPOwas found to be effective for improving the hyperglycemia in STZ induced diabetic rats by reducing the expression of PCK1 gene while increasing the expression of Slc2a2gene in the liver tissue.

6. Acknowledgments
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