Hypoglycemic and antioxidant effect of *Juniperus procera* extract on rats with streptozotocin-induced diabetes

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ABSTRACT

Juniperus procera, a coniferous tree in the cypress family, is one of the famous medicinal plants traditionally used in the southern area of the Arabian peninsula. This study examined the anti-hyperglycemic action of *Juniperus procera* extract (JPE) on diabetic rats. Sixty male rats were divided into 6 equal groups: control, control treated with JPE (200 mg/kg), diabetic, diabetic treated with insulin (1 U/kg), diabetic treated with JPE (200 mg/kg), and diabetic treated with both insulin and JPE. Blood and tissue samples were collected for serum chemistry, gene expression, and immunohistochemistry analyses, the results of which revealed hyperglycemia and inflammation following diabetes induction. Administration of JPE alone or with insulin reduced the hyperglycemia reported in diabetic rats by 25 %. The immuno-histochemical examination of pancreatic tissues demonstrated a moderate restoration of insulin and NF-κB expression in pancreatic and hepatic tissues. Significant recovery was observed for glutathione-S-transferase (GST), superoxide dismutase (SOD), and glutathione peroxidase (GPx) mRNA expression in the livers of rats treated with JPE. Administration of JPE led to similar amelioration of the mRNA expression of pyruvate kinase (PK) and phosphoenol pyruvate carboxy kinase (PEPCK) in the livers of diabetic rats. In addition, diabetic rats treated with insulin, JPE, or a combination of these agents demonstrated an improvement in the mRNA expression of IRS-1 and IRS-2 in hepatic and pancreatic tissues, reaching levels approaching normal. Our findings led us to conclude that JPE has a powerful anti-inflammatory effect accompanied by a moderate hypoglycemic effect that occurs via different mechanisms.

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1. Introduction

The increasing worldwide incidence of diabetes mellitus, one of the most predominant diseases in this century, represents an important health problem [1]. In fact, since the numbers of adults with diabetes mellitus could double over the next 25 years, this disease could be referred to as a pandemic [2]. Current statistics predict that 380 million people will have diabetes by 2025 [3]. Over the last two decades, the incidence of diabetes in Saudi Arabia has grown faster than expected, with Saudi Arabia becoming one of the top ten countries affected by this disease [4]. Several reasons, all related to a massive shift in lifestyle, underlie this acceleration: abandonment of the traditional lifestyle, becoming more sedentary/low physical activity, and consumption of a diet higher in sugar [5,6].

Diabetes mellitus, a metabolic disorder, is characterized by hyperglycemia due to inadequate secretion of insulin and/or the efficiency of the secreted insulin [6,7]. Recently, the chronic hyperglycemia of diabetes and the accompanying impaired metabolism of carbohydrates, lipids, and proteins has been found to be related to specific microvascular complications and to non-specific macrovascular disease [8]. Diabetes mellitus is divided into two

main classes. The first, which is dependent on insulin (diabetes type 1), represents ∼10 % of all cases. This type is caused by an auto-immune reaction that leads to β-cell destruction; while this usually happens during childhood, it can occur at any age [8]. The second type is not dependent on insulin (diabetes type 2) [7]. This type is characterized by either an insufficient amount of insulin secretion or an increased resistance to the action of insulin [9]. Both classes of diabetes are characterized by long acting hyperglycemia-induced dysfunction in the kidneys, nerves, eyes, and heart, as well as stroke, peripheral vascular disease, and other general symptoms such as polyuria, polydipsia, and weight loss [7,10,11]. Several factors contribute to the development of diabetes and its complications, including obesity, genetics, diet, sedentary lifestyle, and age [12].

In the search for effective treatments for many diseases, including diabetes, medicinal plants have been considered a potential source of anti-inflammatory compounds that might provide good replacements for the non-steroidal agents. Over the last four decades, many scientific investigations of plants have identified some plant extracts that are able to decrease inflammation to some degree, which may improve blood glucose and prevent the complications of diabetes mellitus [13,14]. One of the most valued medicinal plants commonly used in the Arabian peninsula for the treatment of some diseases, such as liver disease and digestive problems, is *Juniperus procera*, known locally by its Arab name 'Arar.' This tree is found in the mountainous areas around Ta'if and south into Yemen. Many published reports of research concerning the antioxidant properties of some species of *Juniperus* show that plants of this genus have antioxidant and anti-inflammatory properties, with the potential to be used for diseases such as digestive problems, and that even high doses of *Juniperus procera* extract are considered safe [15,16]. Therefore, our study aimed to examine the possible therapeutic effect of *Juniperus procera* extract (JPE) on diabetes and its associated disorders and complications.

2. Materials and methods

2.1. Animals and materials

Adult male Wistar rats (aged 3 months, 220−270 g) were purchased from the Institute of King Fahad for Scientific Research, Jeddah, Saudi Arabia. Streptozotocin (STZ) and the TriZol reagent for the RNA extraction were purchased from Sigma Aldrich (St. Louis, MO, USA). Oligo dT, chloroform, and ethanol were purchased from Wako Pure Chemicals (Osaka, Japan) and primers were purchased from Macrogen Company (Seoul, South Korea). The reverse transcriptase enzyme was purchased from Sibzyme, Ltd., Ak (Novosibirsk, Russia). The Master Mix for PCR was purchased from Promega Corporation (Madison, WI, USA). Biochemical measurements were conducted using a fully automated analyzer (Cobas 6000 Roche).

2.2. Juniperus procera ethanol extraction

Leaves of *Juniperus procera* were dried in the dark, ground to a powder, and 200 g of powder dissolved in an equal amount of ethanol and water (in a 50:50 ratio) to create 30 g of *Juniperus procera* extract as described previously [15,16].

2.3. Experimental design

Sixty adult male Wistar rats weighting 220−270 g were used for this experiment. Rats were allowed to adapt for 1 week to overcome any effects due to stress and maintained at room temperature ($26 \pm 4\,^\circ$C) in the Ta'if area. Rats were divided into six groups: normal control; normal rats treated with 200 mg/kg of JPE orally;

diabetic rats; diabetic rats treated with 1U/kg insulin; diabetic rats treated with 200 mg/kg JPE; and diabetic rats treated with insulin and JPE. The dose of JPE (200 mg/kg) used was based on that used in a previous study [17]. After approval of the project (#4825/1437/1) by the Animal Care Committee of Ta'if University, all institutional animal care procedures were followed. Rats were fasted for 12 h, after which experimental diabetes was induced in the rats in the diabetes groups by a single intraperitoneal injection of 60 mg/kg STZ in citrate buffer (0.05 M, pH 4.5) as described previously [18,19]. Following overnight fasting, rats with glucose levels between 200 and 250 mg/dL were used in the experiments for further treatments [20,21]. Glucose levels were measured randomly for 4 successive weeks to confirm the occurrence of diabetes. Four weeks after treatment was begun, rats were fasted overnight and anesthetized for the harvesting of blood and organs using diethyl ether.

2.4. Sampling

Serum was extracted from blood collected from the retro-orbital venous plexuses. Liver and pancreatic tissues were preserved in Trizol reagent at −70 °C pending RNA extraction. Other organs were immersed in 10 % buffered neutral formalin for immunohistochemistry and histopathology analysis.

2.5. Biochemical analysis

Glucose, triacylglycerols (TG), total cholesterol (TC), high density lipoproteins (HDL), creatinine, C-peptide, and urea serum levels were measured spectrophotometrically using a Cobas 6000 Roche analyzer.

2.6. RNA extraction, cDNA synthesis, and semi-quantitative RT-PCR analysis

RNA was extracted from the pancreas and liver tissues of experimental rats as described previously [18]. A Polytron 300 D homogenizer was used for sample homogenization. The pellets of RNA were dissolved in diethyl pyrocarbonate (DEPC) water after washing with 70 % ethanol. The concentration of RNA was determined spectrophotometrically at 260/280 OD. For synthesis of cDNA, a mixture of 5 μg RNA and oligo dT primer (0.5 ng) were reverse transcribed in a PCR thermal cycler (Bio-Rad T100™) at 65 °C for 10 min for denaturation. Using a mixture of 10X RT-buffer (2 μL), 10 mM dNTP (2 μL), and 100 U M-reverse transcriptase (1 μL) in a total volume of 20 μL, cDNA was synthesized after incubation at 37 °C for 1 h and at 90 °C for 10 min to inactivate the enzyme. For RT-PCR analysis, the primers shown in Table 1 were designed and ordered from Macrogen (Macrogen Company, Seoul, South Korea). PCR was carried out [1 μL cDNA, 1 μL 10 pM primer (forward and reverse), and 12.5 μL PCR Master Mix (Promega Corporation, Madison, WI, USA)]. PCR conditions were 94 °C for 5 min of one cycle, followed by denaturation at 94 °C for 1 min, annealing as shown in Table 1 and 1 min extension at 72 °C, with an end point extension for 10 min at 72 °C. Expression of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA was used as a reference. PCR products were visualized under UV light after being run on a 1.5 % agarose gel and photographed using a gel documentation system. The band intensities were densitometrically quantified using Image J software (Version 1.47).

2.7. Immunohistochemical examinations

Pancreatic and liver tissues were fixed in 10 % buffered neutral formalin, washed, dehydrated, cleared, immersed in paraffin, casted, and then sectioned. Sections were deparaffinized and soaked with 3 % H_2O_2 for 10 min. Sections were heated in 10 mM

Table 1
PCR conditions and reactions.

PCR cycles and annealing	Reverse primer (5'-3')	Forward primer (5'-3')	mRNA expression
30 cycles, 52 °C 1 min	TCGTAGACAAGGGGGCAC	TTTACTGGGAAGGCATCGAT	**PEPCK (236 bp)**
35 cycles, 52 °C 1 min	CCCGCATGATGTTGGTATAG	ATTGCTGTGACTGGATCTGC	**PK (229 bp)**
35 cycles, 53.5 °C 1 min	CATCGTGAAGAAGGCATAGG	GCCAATCTTCATCCAGTTGC	**IRS-1 (337 bp)**
35 cycles, 56.5 °C 1 min	CCAGGGATGAAGCAGGACTA	CTACCCACTGAGCCCAAGAG	**IRS-2 (151bp)**
35 cycles, 57 °C 1 min	CGT CTG GAC CTA CCA GGA ACT T	AAG GTG CTG CTC ATT GAG AAT G	**GPx (406 bp)**
35 cycles, 55 °C 1 min	TCTACAGTTAGCAGGCCAGCAG	AGGATTAACTGAAGGCGAGCAT	**SOD (410 bp)**
35 cycles, 55 °C 1 min	GTCCTGACCACGTCAACATAG	GCTGGAGTGGAGTTTGAAGAA	**GST (575 bp)**
25 cycles, 52 °C 1 min	TCCCTCAAGATTGTCAGCAA	AGATCCACAACGGATACATT	**GAPDH (309 bp)**

PCR cycle of respective genes are shown, while temperature and time of denaturation and elongation steps of each PCR cycle were 94 °C, 30 s and 72 °C, 60 s, respectively.

citrate buffer at 121 °C for 30 min for antigen retrieval and blocked in 5 % normal serum for 20 min. Sections were incubated with an anti-insulin primary antibody (dilution 1:100; sc9168; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Other sections were incubated with an antibody for NF-κB p50 (1:100 dilution; sc-7178; Santa Cruz Biotechnology, Inc.) in PBS at 4 °C overnight. Sections were washed 3 times with PBS and re-incubated with a goat anti-rabbit IgG as a biotin-conjugated secondary antibody (1:2000; sc 2040; Santa Cruz Biotechnology, Inc.) at 32 °C for 20 min. Antibody binding activity was visualized using diaminobenzidine after counter staining with hematoxylin and incubation with horseradish peroxidase-labeled streptavidin.

2.8. Statistical analysis

Data are represented as means ± standard error of the means (SEM). Our findings were analyzed using ANOVA (analysis of variance) and post hoc descriptive tests using SPSS software for Windows. Values with $p < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of JPE on biochemical parameters in diabetic rats

As shown in Table 2, diabetic rats demonstrated a significant increase in serum glucose levels ($p < 0.05$) compared to those of control treated animals. This dramatic increase in glucose levels was reduced by about 25 % in diabetic rats treated with JPE. However, insulin injection was significantly more effective for diabetic rats ($p < 0.05$). In addition, C-peptide data indicate slight differences in pancreas function. Lipid profile data indicate that, while JPE decreased levels of both total cholesterol and triglycerides in diabetic rats, insulin injection caused a more significant reduction ($p < 0.05$), as shown in Table 2. It is noteworthy that administration of JPE with insulin demonstrated a partially synergistic effect (Table 2).

3.2. Immunohistochemistry of insulin and NF-κB in pancreas and liver tissues after JPE administration

Pancreas tissue from control rats and those treated with JPE showed normal immunoreactivity to insulin in the Islets of Langerhans (Fig. 1A & 1B, respectively). Pancreas tissue from the diabetic group showed mild reactivity to insulin in the Islets of Langerhans (Fig. 1C). Pancreas tissue from rats with diabetes treated with insulin showed moderate reactivity to insulin in the Islets of Langerhans (Fig. 1D). Pancreas tissue from the diabetic group treated with JPE showed moderate restoration of insulin immune reactivity in the Islets of Langerhans (Fig. 1E). Pancreas tissue from the diabetic group treated with JPE and insulin showed a higher recovery of insulin expression in the Islets of Langerhans (Fig. 1F). Livers from the control and JPE groups did not show any reactivity for NF-κB in hepatic tissue (Fig. 2A & B, respectively). Livers from diabetic rats showed high reactivity for NF-κB in hepatic tissue (Fig. 2C). Livers from rats in the diabetic group treated with insulin showed a moderate immunoreactivity for NF-κB in hepatic tissue (Fig. 2D). Diabetic rats treated with JPE showed a moderate recovery with mild NF-κB expression in hepatic tissue (Fig. 2E). Livers of rats in the diabetic group treated with JPE and insulin showed an absence of NF-κB expression in hepatic tissue (Fig. 2F). Rats in the control and JPE treated groups showed an absence of expression of NF-κB in the pancreatic tissue (Fig. 3A & B, respectively). Rats in the diabetic group showed a high expression of NF-κB in pancreatic tissue (Fig. 3C). Rats in the diabetic group treated with insulin showed a moderate reactivity for NF-κB in pancreatic tissue (Fig. 3D). Pancreatic tissue from rats in the diabetic group treated with JPE showed moderate restoration of normal expression, with a mild reactivity to NF-κB (Fig. 3E). Rats in the diabetic group treated with JPE and insulin showed mild NF-κB expression in pancreatic tissue (Fig. 3F).

3.3. Effect of JPE on mRNA expression of antioxidants in the livers of diabetic rats

As shown in Fig. 4, liver tissue from rats with diabetes demonstrated downregulated mRNA expression of glutathione-S-transferase (GST), superoxide dismutase (SOD), and glutathione peroxidase (GPx) relative to expression in controls. Rats with diabetes given insulin and JPE showed significant amelioration ($p < 0.05$) of the mRNA expression of GST, SOD, and GPx relative to expression in rats with diabetes. When insulin was co-administered with JPE, both were shown to increase the m RNA levels of the antioxidants examined (Fig. 4).

3.4. Effect of JPE on mRNA expression of PK and PEPCK in diabetic rats

Expression of the rate-limiting enzyme of glycolysis, pyruvate kinase (PK), was downregulated in diabetic rats relative to that in controls. Administration of insulin alone or together with JPE to rats with diabetes upregulated PK mRNA expression as compared to that in diabetic rats, as shown in Fig. 5. Partial recovery in PK mRNA expression after treatment with JPE alone relative to normal control was also observed (Fig. 5). Fig. 5 shows that the expression of a key enzyme of gluconeogenesis in the liver, phosphoenolpyruvate carboxykinase (PEPCK), was upregulated in rats with diabetes compared to that of the normal control group. Rats with diabetes treated with insulin, JPE, or co-administered both showed significant ($p < 0.05$) downregulation in PEPCK mRNA expression relative to that in diabetic rats (Fig. 5).

3.5. Effect of JPE administration on mRNA expression of IRS-1 and IRS-2 in liver of diabetic rats

STZ diabetic rats showed a decrease in serum blood glucose levels. Consequently, the mRNA expression of insulin receptors

Table 2
Effect of JPE and insulin administration on biochemical parameters in diabetic rats.

| Diabetic + Juniperus + Insulin	Diabetic + Juniperus	Diabetic + Insulin	Diabetic	Juniperus	Control	Parameter/Group
213 ± 16.2#	293 ± 28.2#	163 ± 5.6#	414 ± 10.1*	111 ± 5.1	106 ± 10.3	Glucose
0.11 ± 0.001	0.15 ± 0.001	0.13 ± 0.001	0.17 ± 0.001	0.18 ± 0.001	0.19 ± 0.002	C-peptide
38.2 ± 6.2#	48.1 ± 2.2#	44.5 ± 3.4#	62 ± 3.2*	43 ± 3.1	39 ± 4.2	Total Cholesterol
66.3 ± 6.7#	71.7 ± 4.7#	81 ± 5.3#	138 ± 12.8*	57 ± 1.6	66 ± 3.3	TG
18.1 ± 1.0#	21.1 ± 0.9#	19.9 ± 1.2#	18.8 ± 0.8	23.1 ± 1.4	22.4 ± 1.1	HDL

Values are means ± SEM for 10 different rats per each treatment. Values are statistically significant at *$p < 0.05$ vs. control; #$p < 0.05$ vs. diabetic rats.
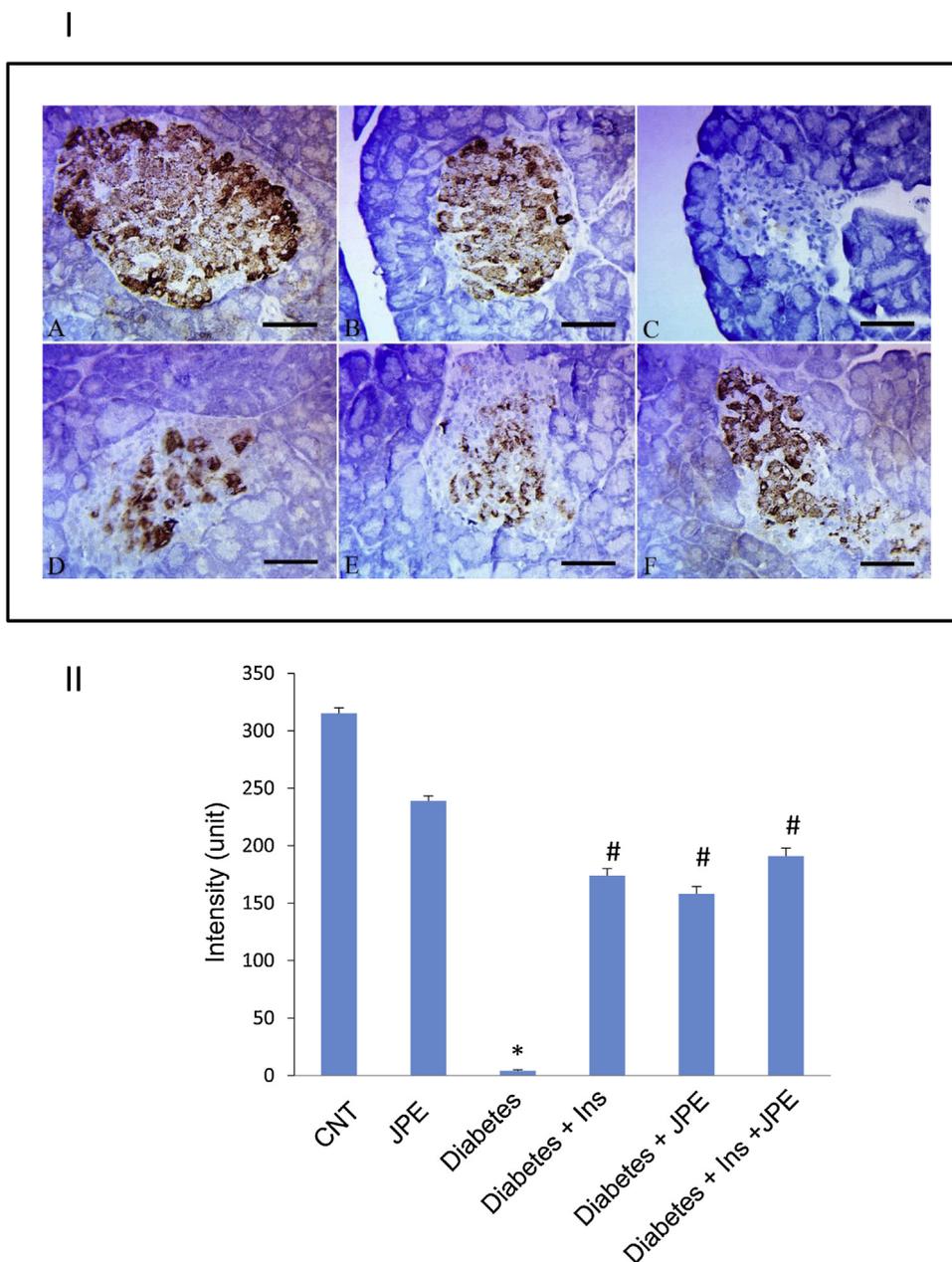
Fig. 1. I:(A, and B) Pancreas tissue from control and JPE groups, respectively, showed normal expression of insulin in the Islets of Langerhans. (C) Pancreas tissue from the diabetic group showed mild expression of insulin in the Islets of Langerhans. (D) Pancreas tissue from the diabetic group treated with insulin showed moderate expression of insulin in the Islets of Langerhans. (E) Pancreas tissue from the diabetic group treated with JPE showed moderate restoration of insulin expression in the Islets of Langerhans. (F) Pancreas tissue from the diabetic group treated with JPE and insulin showed a greater restoration of insulin expression in the Islets of Langerhans (scale bar = 50 μm). **II:** Image *J* analysis of data from the upper figure showed a significant reduction in insulin expression in the Islets of Langerhans in tissue from the diabetic group when compared to that of the control group. Significant restoration of insulin expression was detected in diabetic groups treated with JPE or insulin, with even more restoration seen in the group treated with both of them.
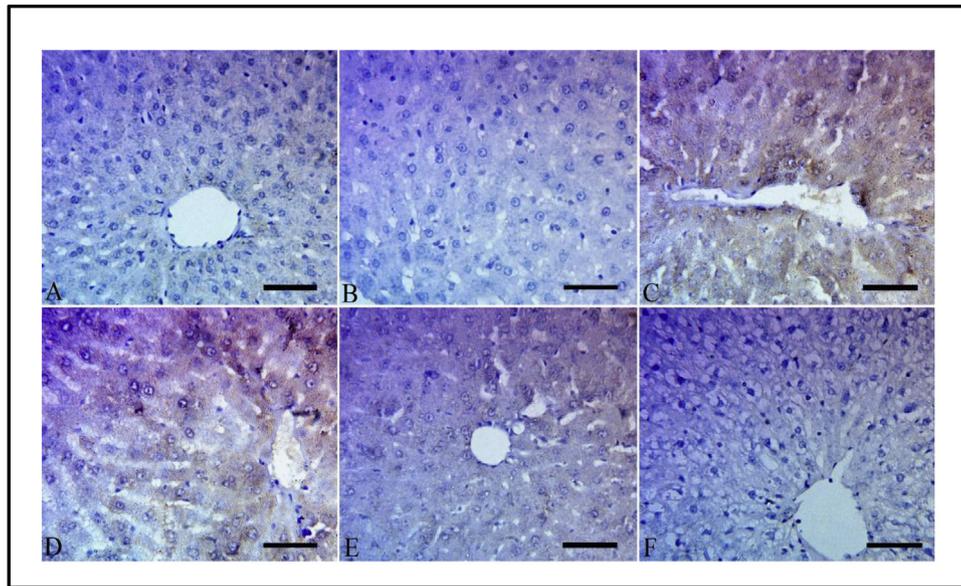
Fig. 2. (A, and B) Livers from the control and JPE groups, respectively, showed an absence of expression of NF-κB in hepatic tissue. (C) Livers from the diabetic group showed high expression of NF-κB in hepatic cells. (D) Livers from the diabetic group treated with insulin showed moderate expression of NF-κB in hepatic tissue. (E) Livers from the diabetic group treated with JPE showed moderate restoration, with mild NF-κB expression in hepatic tissue. (F) Livers from the diabetic group treated with JPE and insulin showed an absence of NF-κB expression in hepatic tissue (scale bar = 50 μm).
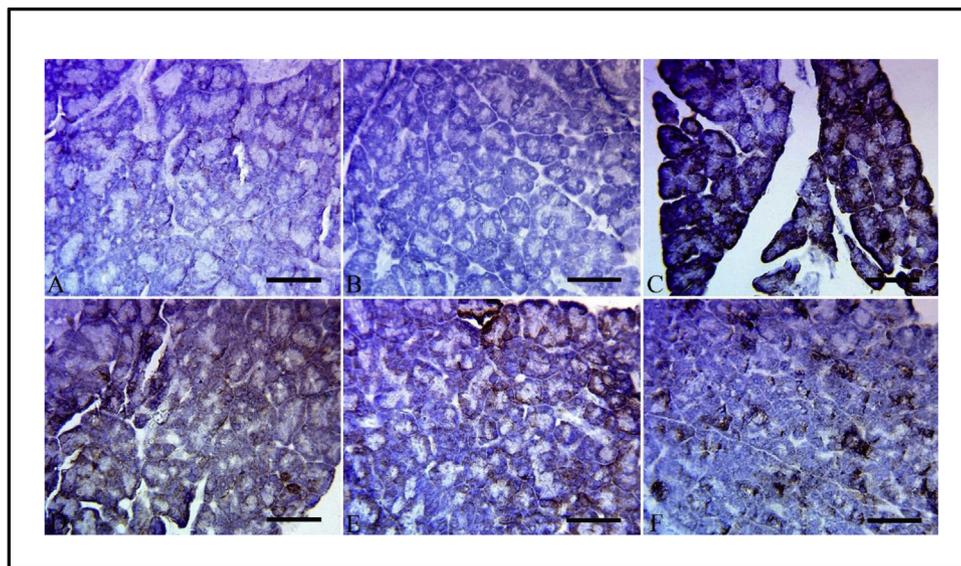
Fig. 3. (A, and B) Pancreas tissue from control and JPE groups, respectively, showed an absence of expression of NF-κB in pancreatic tissue. (C) Pancreas tissue from the diabetic group showed high expression of NF-κB in pancreatic tissue. (D) Pancreas tissue from the diabetic group treated with insulin showed moderate expression of NF-κB in pancreatic tissue. (E) Pancreas tissue from the diabetic group treated with JPE showed moderate restoration with mild NF-κB expression in pancreatic tissue. (F) Pancreas tissue from the diabetic group treated with JPE and insulin showed mild NF-κB expression in pancreatic tissue (scale bar = 50 μm).

(IRS-1 and IRS-2) was downregulated in their hepatic and pancreatic tissues compared to those of the normal control group (Figs. 6 and 7, respectively). Insulin, JPE, or co-administration of both to rats in the diabetic group restored and normalized IRS-1 and IRS-2 mRNA expression in hepatic and pancreatic tissues to normal levels (Figs. 6 and 7).

4. Discussion

The rapid increase in the incidence of diabetes has prompted many research centers and researchers to seek diligently for both traditional and non-traditional treatments for this disease. Increasingly, natural plant extracts have caught the attention of many researchers in this field. *Juniperus procera* is a tree whose leaves have been used for medicinal purposes in the past. The findings from our study confirmed the hypoglycemic and antioxidant activities of JPE in diabetic rats. Moreover, JPE ameliorated the dyslipidemia associated with diabetes and normalized the alterations induced by diabetes in the genes associated with glucose metabolism.

Very few studies have examined the molecular mechanism and involvement of genes in the regulation of JPE. As shown in our results, diabetes induces oxidative stress (decreased mRNA expression of GST, SOD, and GPx). Our findings are in agreement with previously reported results from investigations that evaluated the enzymatic antioxidant defense systems in diabetes with differ-
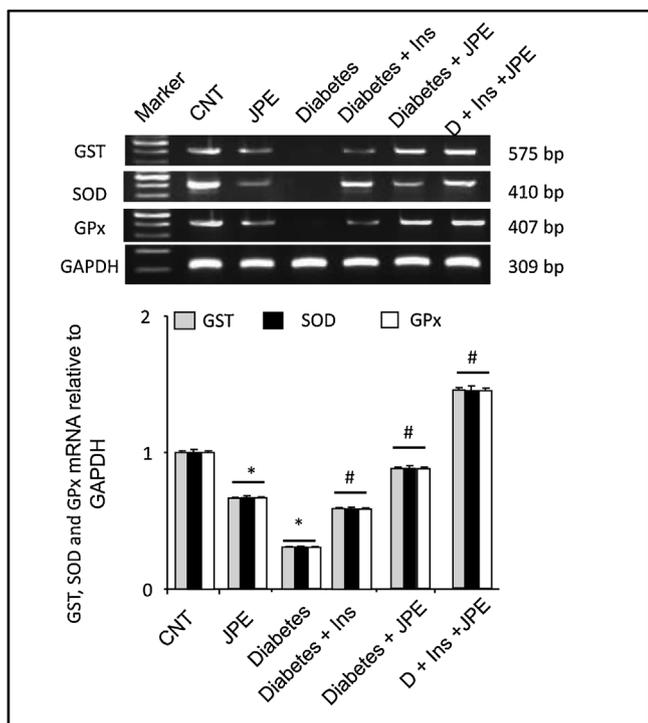
Fig. 4. Protective effect of JPE on altered antioxidant expression in liver. Semi-quantitative RT-PCR analysis of the mRNA expression of GST, GPx, and SOD and their corresponding G3PDH in liver. Experimental groups were administered JPE, insulin, or a combination as described in Materials and Methods. Values are means ± SEM obtained from 10 different rats. $^*p < 0.05$ vs. control group, $\#p < 0.05$ vs. diabetes.
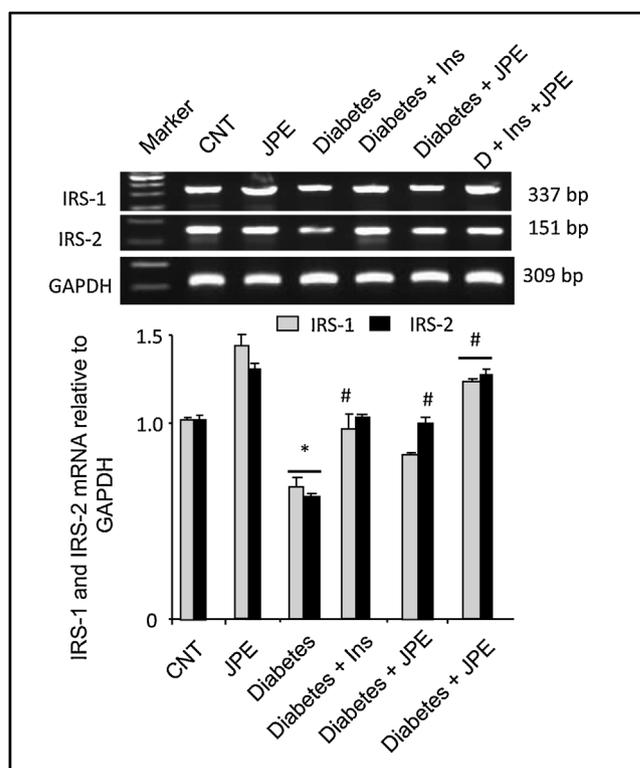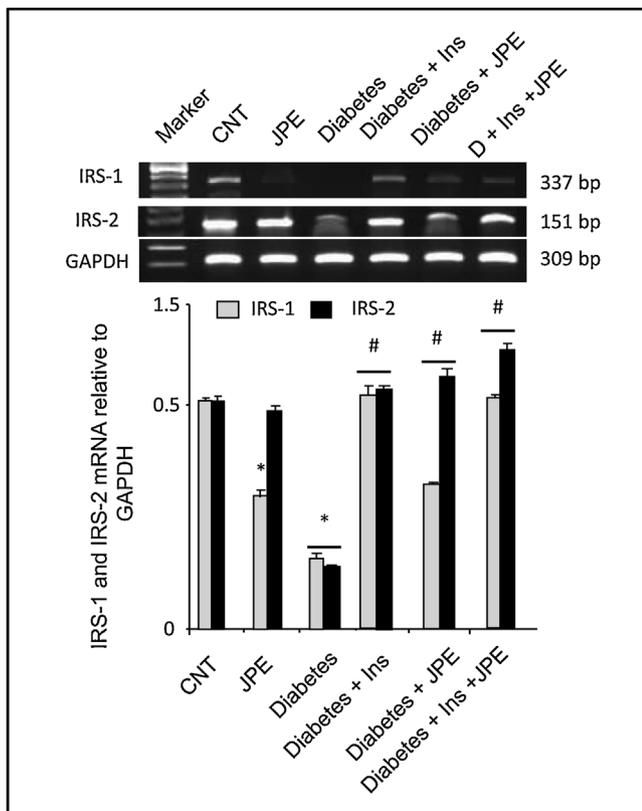

Fig. 6. Protective effect of JPE on changes in IRS-1 and IRS-2 mRNA expression. Semi-quantitative RT-PCR analysis of the mRNA expression of IRS-1 and IRS-2 and their corresponding G3PDH in liver. Experimental groups were administered JPE, insulin, or a combination as described in Materials and Methods. Values are means ± SEM obtained from 10 different rats. $^*p < 0.05$ vs. control group, $\#p < 0.05$ vs. diabetes.
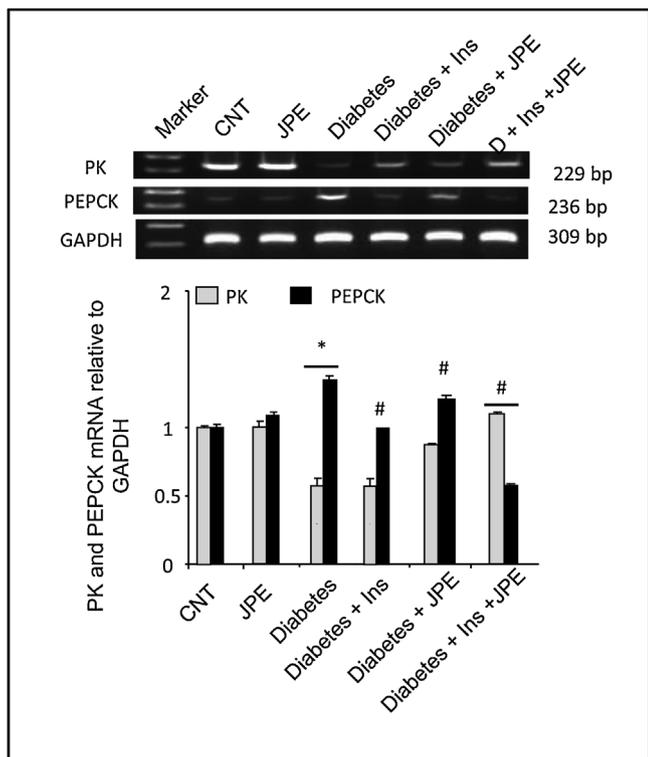
Fig. 5. Protective effect of JPE on changes in PK and PEPCK mRNA expression in liver. Semi-quantitative RT-PCR analysis the mRNA expression of PK and PEPCK and their corresponding G3PDH in liver. Experimental groups were administered JPE, insulin, or a combination as described in Materials and Methods. Values are means ± SEM obtained from 10 different rats. $^*p < 0.05$ vs. control group, $\#p < 0.05$ vs. diabetes.

ent results [22,23]. It has been shown that the therapeutic effects of plant-based medications used as alternative medicines are due mostly to their polyphenolic compounds [20,21]. We reported that JPE ameliorated the downregulation in mRNA expression of GST, SOD, and GPx reported in rats with diabetes. Administration of insulin plus JPE produced a more pronounced effect on the expression of abovementioned genes. The results reported in this study are in agreement with those of the Burits study [16] that confirmed the antioxidant properties of *Juniperus procera* oils used in the treatment of some inflammatory diseases [24].

PK is a known indicator of glycolysis in diabetes [25]. Insulin negatively regulates PEPCK, a rate-limiting enzyme in gluconeogenesis [26]. The increase in gluconeogenesis and decrease in glycolysis observed during hyperglycemia have been attributed to the downregulation of PK expression and upregulation of PEPCK expression described in our and other studies [21,27]. The results presented here demonstrate that treatment with insulin alone, or in combination with JPE, restores the downregulated mRNA of PK and normalized mRNA of PEPCK in rats with diabetes. Our results also show that the signals of the insulin receptors (IRS-1 and IRS-2) were downregulated in rats with diabetes, indicating a deterioration in insulin sensitivity. We also demonstrated that treatment with JPE alone or co-administered with insulin restored the expression of insulin signalling pathways in the liver. Activation of NF-kB, a marker of cellular response, in turn triggers the activation of most of the inflammatory components associated with cellular response and the decreased number of β-cells observed in diabetes. Since JPE has a positive effect on NF-κB immunoreactivity, it is possible that it could be used to decrease the expression of IRS-1 in insulin resistant diabetic patients [28], and provide a protective mechanism for β-cells against the apoptosis modulated by some cytokines [29]. In addition, upregulation in the expression of NF-

Fig. 7. Protective effect of JPE on changes in IRS-1 and IRS-2 mRNA expression in the pancreas. Semi-quantitative RT-PCR analysis of the mRNA expression of IRS-1 and IRS-2 and their corresponding G3PDH in pancreas. Experimental groups were administered JPE, insulin, or a combination as described in Materials and Methods. Values are means ± SEM obtained from 10 different rats. *$p < 0.05$ vs. control group, #$p < 0.05$ vs. diabetes.

κB has been reported in patients with diabetes [30,31]. NF-κB is a nuclear transcription factor that translocates into the nucleus and can bind to genomic DNA. Consequently, it regulates the expression of inflammatory cytokines, initiating or aggregating the inflammatory response and ultimately leading to the occurrence of insulin resistance (IR). Studies have suggested that IR can be prevented by the downregulation of NF-κB signaling [32]. Our study confirmed that the decrease in NF-κB immunoreactivity observed in JPE-treated rats significantly inhibits inflammatory reactions and ameliorates the diabetes induced by STZ in diabetic rats, which is nicely consistent with the biochemistry and gene expression data.

5. Conclusion

In conclusion, JPE has hypoglycemic and anti-oxidative properties due to its inhibition of the NF-κB signaling pathway and upregulation of GST, SOD, and GPx mRNA expression. Moreover, JPE improves and increases insulin sensitivity.

Declaration of competing interests

The authors declare that no conflicts of interest exist.

Author contributions

Conceived and designed the experiments: AA, MAN, and MMS. Performed experiments: MMS TAI, and MAN. Analyzed data: MMS. Biochemical measurements: MMS, AA, and EHM. Gene expression:

MMS, AA, and HHA. Data interpretations: MAN, AA, TAI, and MMS. Revision of manuscript: MMS, HHA, and EHM.

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References

[1] T.A. Elhadd, A.A. Al-Amoudi, A.S. Alzahrani, Epidemiology, clinical and complications profile of diabetes in Saudi Arabia: a review, Ann. Saudi Med. 27 (2007) 241–250.

[2] N. Chaturvedi, The burden of diabetes and its complications: trends and implications for intervention, Diabetes Res. Clin. Pract. 76 (Suppl 1) (2007) S3–12.

[3] P. Lefebvre, M. Silink, Diabetes fights for recognition, Lancet 368 (2006) 1625–1626.

[4] A.K. Alhowaish, Economic costs of diabetes in Saudi Arabia, J. Family Community Med. 20 (2013) 1–7.

[5] M.B. Schulze, K. Hoffmann, J.E. Manson, et al., Dietary pattern, inflammation, and incidence of type 2 diabetes in women, Am. J. Clin. Nutr. 82 (2005) 675–684, quiz 714-5.

[6] E. Ginter, V. Simko, Global prevalence and future of diabetes mellitus, Adv. Exp. Med. Biol. 771 (2012) 35–41.

[7] Diagnosis and classification of diabetes mellitus, Diabetes Care 36 (Suppl 1) (2013) S67–74.

[8] A.D. Deshpande, M. Harris-Hayes, M. Schootman, Epidemiology of diabetes and diabetes-related complications, Phys. Ther. 88 (2008) 1254–1264.

[9] A.E. Butler, J. Janson, S. Bonner-Weir, R. Ritzel, R.A. Rizza, P.C. Butler, B-cell deficit and increased β-Cell apoptosis in humans with type 2 diabetes, Diabetes 52 (2003) 102–110.

[10] M. Laakso, Hyperglycemia as a risk factor for cardiovascular disease in type 2 diabetes, Prim. Care 26 (1999) 829–839.

[11] A.I. Adler, H.A. Neil, S.E. Manley, R.R. Holman, R.C. Turner, Hyperglycemia and hyperinsulinemia at diagnosis of diabetes and their association with subsequent cardiovascular disease in the United Kingdom prospective diabetes study (UKPDS 47), Am. Heart J. 138 (1999) S353–9.

[12] J. Papak, D. Kansagara, Management of hyperglycemia in a hospitalized patient with diabetes mellitus and cardiovascular disease, Am. J. Cardiol. 110 (2012) 24B–31B.

[13] L. Rubio, M.J. Motilva, M.P. Romero, Recent advances in biologically active compounds in herbs and spices: a review of the most effective antioxidant and anti-inflammatory active principles, Crit. Rev. Food Sci. Nutr. 53 (2013) 943–953.

[14] C.M. Chan, Y.W. Chan, C.H. Lau, et al., Influence of an anti-diabetic foot ulcer formula and its component herbs on tissue and systemic glucose homeostasis, J. Ethnopharmacol. 109 (2007) 10–20.

[15] S.I. Alqasoumi, M.S. Abdel-Kader, Terpenoids from Juniperus procera with hepatoprotective activity, Pak. J. Pharm. Sci. 25 (2012) 315–322.

[16] M. Burits, K. Asres, F. Bucar, The antioxidant activity of the essential oils of Artemisia afra, Artemisia abyssinica and Juniperus procera, Phytother. Res. 15 (2001) 103–108.

[17] A.M. Al-Attar, A.A. Alrobai, D.A. Almalki, Protective effect of olive and juniper leaves extracts on nephrotoxicity induced by thioacetamide in male mice, Saudi J. Biol. Sci. 24 (2017) 15–22.

[18] M.M. Soliman, M. Abdo Nassan, T.A. Ismail, Immunohistochemical and molecular study on the protective effect of curcumin against hepatic toxicity induced by paracetamol in Wistar rats, BMC Complement. Altern. Med. 14 (2014) 457.

[19] M.M. Soliman, M. Abdo Nassan, T.A. Ismail, Origanum majoranum extract modulates gene expression, hepatic and renal changes in a rat model of type 2 diabetes, Iran. J. Pharm. Res. 15 (2016) 45–54.

[20] D. Cheng, B. Liang, Y. Li, Antihyperglycemic effect of Ginkgo biloba extract in streptozotocin-induced diabetes in rats, Biomed. Res. Int. 2013 (2013), 162724.

[21] T.A. Ismail, M.A. Nassan, A.Q. Alkhedaide, M.M. Soliman, Modulation of aquaporins expressions and its impact on alleviating diabetic complications in rats: role of Citrullus colocynthis, J. Physiol. Pharm. Pharmacol. 6 (2016) 310–322.

[22] A. Manzari-Tavakoli, I. Pouraboli, M.-M. Yaghoobi, M. Mehrabani, S.-M. Mirtadzadini, Antihyperglycemic, antilipid peroxidation, and insulin secretory activities of Otostegia persica shoot extract in streptozotocin-induced diabetic rats and in vitro C187 pancreatic β-cells, Pharm. Biol. 51 (2013) 253–259.

[23] R. Gupta, R. Gupta, Antidiabetic efficacy of Mangifera indica seed kernels in rats: a comparative study with glibenclamide, Diabetol. Croat. 40 (2011) 107–112.

[24] M. Burits, K. Asres, F. Bucar, The antioxidant activity of the essential oils of Artemisia afra, Artemisia abyssinica and Juniperus procera, Phytother. Res. 15 (2001) 103–108.

[25] G.S. Prasath, S.P. Subramanian, Modulatory effects of fisetin, a bioflavonoid, on hyperglycemia by attenuating the key enzymes of carbohydrate

metabolism in hepatic and renal tissues in streptozotocin-induced diabetic rats, Eur. J. Pharmacol. 668 (2011) 492–496.

[26] P.G. Quinn, D. Yeagley, Insulin regulation of PEPCK gene expression: a model for rapid and reversible modulation, Curr. Drug Targets Immune Endocr. Metabol. Disord. 5 (2005) 423–437.

[27] M. Xie, D. Chen, F. Zhang, G.R. Willsky, D.C. Crans, W. Ding, Effects of vanadium (III, IV, V)-chlorodipicolinate on glycolysis and antioxidant status in the liver of STZ-induced diabetic rats, J. Inorg. Biochem. 136 (2014) 47–56.

[28] G. Boden, P. She, M. Mozzoli, et al., Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver, Diabetes 54 (2005) 3458–3465.

[29] F. Habibi, F. Ghadiri Soufi, R. Ghiasi, A.M. Khamaneh, M.R. Alipour, Alteration in inflammation-related miR-146a expression in NF-KB signaling pathway in diabetic rat Hippocampus, Adv. Pharm. Bull. 6 (2016) 99–103.

[30] E. Roggli, A. Britan, S. Gattesco, et al., Involvement of microRNAs in the cytotoxic effects exerted by proinflammatory cytokines on pancreatic beta-cells, Diabetes 59 (2010) 978–986.

[31] S. Patel, D. Santani, Role of NF-kappa B in the pathogenesis of diabetes and its associated complications, Pharmacol. Rep. 61 (2009) 595–603.

[32] L. Yang, Z. Wang, L. Jiang, W. Sun, Q. Fan, T. Liu, Total flavonoids extracted from Oxytropis falcata bunge improve insulin resistance through regulation on the IKKβ/NF-κB inflammatory pathway, Evid. Based Complement. Altern. Med. 2017 (2017).