

Full Length Research Paper

Effect of Persian shallot (*Allium hirtifolium* Boiss.) extract on glucokinase (GCK), glycogen phosphorylase and phosphoenolpyruvate carboxykinase (PEPCK) genes expression in diabetic rats

Mehdi Mahmoodi¹, Seyed-Mostafa Hosseini-Zijoud^{*2}, Mohammad Kazemi Arababadi¹, Hossein Khorramdelazad¹, Hemen Moradi-Sardareh², Younes Moradi³, Gholamhossein Hassanshahi¹, Behrooz Hashemi³, Hasan Ghasemi², Mohammad-Nabi Moradi² and Nejad Mohamadi²

¹Department of Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran.

²Department of Biochemistry, Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.

³Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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There has been a growing interest in hypoglycaemic agents from natural products, especially those derived from plants. In the current survey, hypoglycaemic properties of Persian shallot (*Allium hirtifolium* Boiss) was evaluated by studying mRNA expression levels of the key enzymes involved in carbohydrate metabolism in liver, Glucokinase (GCK), phosphoenolpyruvate carboxykinase (PEPCK), and glycogen phosphorylase. Thirty two male rats were divided into 4 groups of 8, diabetic groups received 100 and 200 mg/kg Persian shallot extract, diabetic control and normal control received 0.9% saline for 30 days. At the end of the experimental period blood and liver samples were collected. FBS and insulin levels were measured and followed by analysis of the gene expression by Real-Time PCR based methods. Findings indicated that the Persian shallot significantly reduces the FBS level in parallel with slight enhancement of insulin in diabetic rats' serum. Investigations of gene expression showed that Persian shallot gently increased GCK and glycogen phosphorylase but decreased PEPCK gene activity, in diabetic rats. In conclusion, the data suggest that Persian shallot is an effective hypoglycaemic agent, the observed effect may be via its ability to enhance insulin secretion and GCK gene expression and to decrease hepatic glucose output by reducing PEPCK.

Key words: Persian shallot, Glucokinase, glycogen phosphorylase, phosphoenolpyruvate carboxykinase, diabetes.

INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia due to defects in insulin production and/or function (Abel et al., 2001). Liver is an

insulin-sensitive tissue and plays a major role in maintaining glucose homeostasis via regulating process of the glucose utilization and gluconeogenesis. The liver produces glucose by two pathways, gluconeogenesis (de novo synthesis of glucose) and glycogenolysis (enzymatic break-down of glycogen by glycogen phosphorylase catalytic activity) (Ferre et al., 1996). In the liver, insulin suppresses transcription of genes encoding gluconeogenic

*Corresponding author. E-mail: seyedo2@yahoo.com. Tel: +98 919 5350501.

and glycogenolytic enzymes and stimulates transcription of genes encoding glycolytic enzymes, thus, leading to decreased glucose level (Barthel and Schmolli, 2003). The glucose which is taken up by mammalian cells has to be converted into glucose 6-phosphate by Glucokinase (GCK) as a prelude for further utilization in glycolysis, the pentose phosphate pathway or glycogen synthesis. In the liver, expression of GCK is very closely dependent on the presence of insulin. Hence, GCK mRNA and protein disappear from the livers of insulin deficient rats and is restored following insulin treatment. The central role of GCK in facilitating glucose disposal by the liver on the one side, and insulin secretion by the islets of Langerhans on the other side, provide a strong rationale for the search of small molecule activators of GCK in drug discovery programs aimed at developing a new class of antidiabetic drugs (Ilyedjian et al., 1998).

Another important enzyme in carbohydrate metabolism in mammals, including human being is phosphoenolpyruvate carboxykinase (PEPCK), which is involved in regulation of the circulating glucose level. Gluconeogenic tissues, such as kidney and liver, convert lactate and other non-carbohydrate molecules to glucose, which in turn is released into the circulation (Matte et al., 1997). In mammals, gluconeogenic genes are mainly under insulin control. Insulin itself inhibits expression of PEPCK enzymes at the transcriptional level. The importance of PEPCK in carbohydrate metabolism in humans is that it has the potential to be employed as a potential drug target in the treatment of diabetes mellitus (Barthel and Schmolli, 2003).

Also, increasing number of reports suggested that glycogenolysis inhibitors are probably useful in the treatment of diabetes. Glucose production from the catalysis of glycogen to glucose-1-phosphate is rate-limited by glycogen phosphorylase, a well-studied enzyme that is regulated by multiple covalent, substrate, and allosteric effectors (Newgard et al., 1989).

The underlying goal for all types of diabetes treatment and management is to maintain blood glucose on an adequate level. Considering the heterogeneity of this disease, current therapies are often limited. And to achieve that, the investigation of new compounds with improved antidiabetic action is of paramount importance (Celik et al., 2009). Investigations showed green tea components have glycaemic effects and mimic insulin, and reduces gene expression of the gluconeogenic enzyme PEPCK, and increase Glucokinase mRNA expression in the liver of rats in a dose dependent manner (Nakagawa et al., 2002).

Studies have highlighted the benefits of medicinal plants with combined antidiabetic and antioxidant properties (Nakagawa et al., 2002). Persian shallot (*Allium hirtifolium* Boiss) is a nutritive plant with special taste that belongs to Liliaceae family. It is one of the important edible onions in Iran. It is a native Iranian plant

and grows wildly in the Zagross Mountains. Biochemical analysis of Persian shallot extracts has confirmed its hypoglycaemic and hepatoprotective effects (Hosseini et al., 2012. Hosseini-zijoud et al., 2012). There are several reports that emphasized shallot medicinal effects as antioxidant (Leelarungrayub et al., 2006), immune system regulating (Jafarian et al., 2003) and anticancer (Ghodrati et al., 2008). The Persian shallot extract is a stronger hypoglycaemic agent compared to garlic extract and it could be a useful supplemental remedy in diabetes (Leelarungrayub et al., 2006). Since Persian shallot grows as a wild plant only in some mountains of Iran, limited information is available regarding different aspects of this species. Therefore, the present study was designed to investigate the possible hypoglycaemic effects of two different doses (100 and 200 mg/kg) of Persian shallot in streptozotocin-induced diabetic rats; a suitable model for type 1 diabetes (Kopp et al., 2008). We have evaluated glucose homeostasis, the expression of genes regulating glycolysis, gluconeogenesis and glycolysis in liver, as well as insulin and FBS levels.

MATERIAL AND METHODS

Preparation of hydroalcoholic extract of Persian shallot

Fresh Persian shallot (*A. hirtifolium* Boiss) bulbs were obtained from Kangavar (Kermanshah-Iran). The genus and species of the bulbs were confirmed by the botanists (Department of Botany, Valiasr University Rafsanjan-Iran). Then, 100 g of fresh bulbs was well crushed and 400 ml distilled water/ethanol (25/75) was added. After 48 h incubation, the solution was filtered using a filter paper through a Buchner funnel. The filtered resultant solutions obtained from this stage, concentrated by means of a vacuum distillation and decanted to dry powder was used to prepare the needed concentrations (Momeni, 2000).

Induction of diabetes and Persian shallot treatments

In this study 32 male albino Wistar rats weighing 180 to 230 g were recruited. Twenty-four rats were injected (intraperitoneal injection) with 45 mg/Kg body weight of streptozotocin (STZ) (diabetic type-1 rats) and eight rats were included as normal group. After being matched according to body weight, the rats were allocated to four groups of 8:

Group 1: diabetic rats received daily 200 mg/kg Persian shallot extract (2 ml) for 30 days.

Group 2: diabetic rats received daily 100 mg/kg Persian shallot extract (2 ml) for 30 days.

Group 3: diabetic rats received daily 0.9% saline (2 ml) for 30 days (diabetic control).

Group 4: normal rats received daily 0.9% saline (2 ml) for 30 days (normal control).

The solutions (2 ml) given to animals by using a gavage syringe. The animals were then housed in cages and had free access to water and standard food. Animal handling was performed in accordance with the guidelines of Iranian animal ethics society, Rafsanjan University of Medical Science rules and under supervi-

sion of Professor Mehdi Mahmoodi. At the end of 30 days treatment, blood and liver samples were collected and the levels of FBS and insulin were measured in all study groups and gene expression analyzed by Real-Time PCR.

Biochemical analysis

Plasma insulin concentrations were assayed by ELISA method using a commercial kit (Mercodia, Sweden) and FBS was measured by BT-3000 autoanalyzer.

Extraction of RNA

For the isolation of tissue RNA, rats were humanly sacrificed and under aseptic situations the liver tissues were removed and immediately frozen in liquid nitrogen. Prior to RNA extraction, liver samples were homogenized in TRIZOL™ reagent (Invitrogen) using Mixer 301. Total RNA was extracted according to the manufacturer's guidance. RNA samples were electrophoresed in agarose gels and visualized with ethidium bromide for quality control.

cDNA synthesis and quantitative Real-Time PCR

Three micrograms of RNA were reverse transcribed with reverse transcriptase for 1 h at 37°C for synthesis of cDNA. Quantitative changes in mRNA expression were assessed with real-time quantitative Real-Time PCR (Bio-Rad CFX) using SYBR-Green detection consisting of SYBR Green PCR Master Mix (Aria-tous, Iran). The PCR master mix was made up by 0.5U of *Taq polymerase*, 2 µL of each primer and 3 µL of each cDNA samples in a final volume of 20 µL. All amplifications were repeated three times. Oligonucleotide primer sequences are illustrated in Table 1. β_2 -microglobulin was used as endogenous control, and each sample was normalized on the basis of its β_2 -microglobulin content. Relative quantification of the mRNA expression levels of target genes was calculated using the $2^{-\Delta\Delta C_t}$ method (Celik et al., 2009) (Table 2).

$$\Delta\Delta C_t = (C_t \text{ gene studied} - C_t \beta_2\text{-microglobulin})_{\text{treated}} - (C_t \text{ gene studied} - C_t \beta_2\text{-microglobulin})_{\text{control}}$$

Statistical analysis

Results are presented as mean±SD. Statistical difference between the means of the various groups were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple test. Data were considered statistically significant if $p < 0.05$.

RESULTS

FBS and insulin

The FBS concentrations of four groups of rats during experimental period are shown in Figure 1. There was a significant difference in FBS level among all groups and Persian shallot consumption reduced significantly FBS level in diabetic treated groups in dose dependent manner ($P < 0.05$).

The Fasting plasma Insulin levels of four groups of rats during experimental period are displayed in Figure 2. Diabetic groups showed statistically lower insulin levels in compare to normal control. Although Persian shallot consumption slightly increases insulin level in diabetic rats but this elevation was not significant.

The mRNA levels of glycolytic and gluconeogenic enzymes in liver

The expression level of the genes in the normal control group was considered as 100% and the expression in the other groups were accordingly calculated (Table 2). When compared with control rats, diabetes was found to suppress GCK gene expression in liver (Figure 3). The Persian shallot elevated hepatic glucokinase gene expression when compared with the control group ($P < 0.05$) (Figure 3 and Table 2). In contrast, PEPCK and glycogen phosphorylase genes expression were higher in the diabetic rats (Figures 4 and 5) while, treating with Persian shallot increased glycogen phosphorylase and decreased PEPCK gene expression however the observed effect was not significant (Table 2).

The level of mRNA encoding target genes were detected by Real-Time PCR and were normalized with β_2 -microglobulin mRNA (as housekeeping gene) (using the $2^{-\Delta\Delta C_t}$ method). Group 1: diabetic rats received 200 mg/kg Persian shallot. Group 2: diabetic rats received 100 mg/kg Persian shallot. Group3: diabetic rats received 0.9% saline.

DISCUSSION

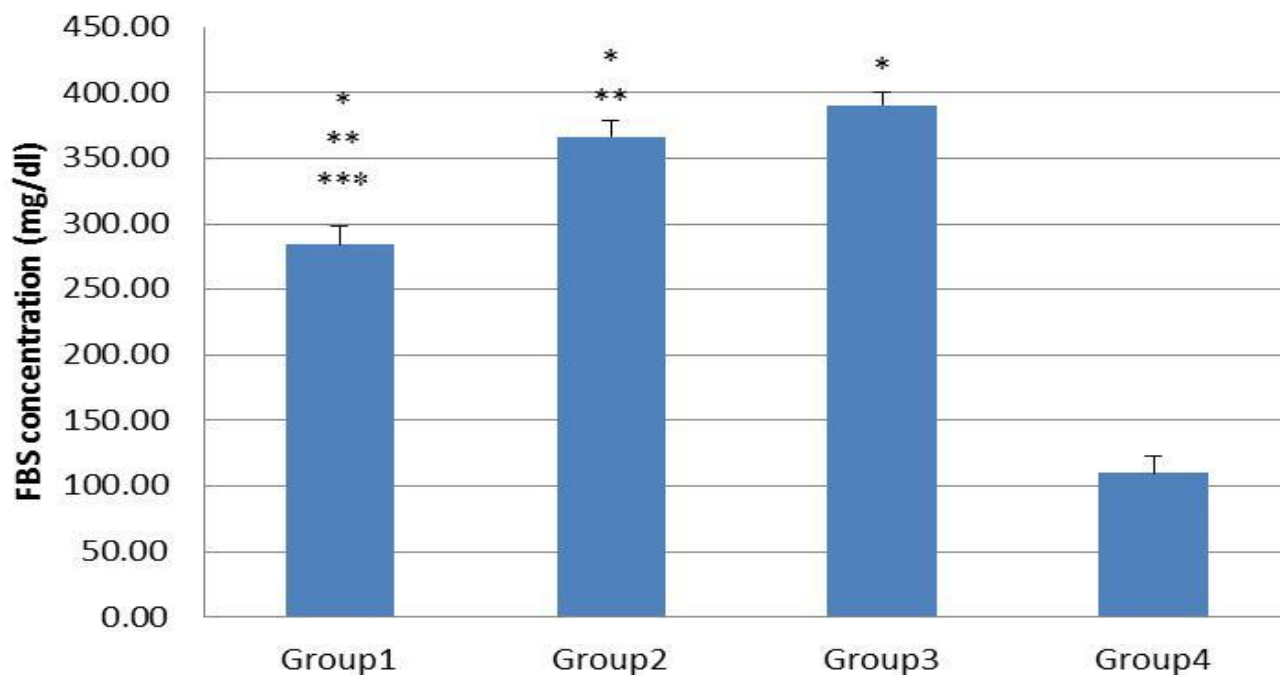
Chronic insulin deficiency and insulin insensitivity are the major causes of the decreased hepatic glucose utilization and increased glucose production in diabetes, because insulin decreases the hepatic glucose output by activating glycogen synthesis and glycolysis, and by inhibiting gluconeogenesis (You-Gui et al., 2011). Recently there has been a growing interest in hypoglycaemic agents from natural products, especially those derived from plants, because plant sources are usually considered to be less toxic, with fewer side effects than synthetic sources. Several bioflavonoids, ubiquitously present in Persian shallot (*A. hirtifolium* Boiss.) have been reported to improve hyperglycemia in diabetes mellitus (Leelarungrayub et al., 2006). In our study Persian shallot significantly reduced FBS while gently increasing serum insulin level. Recently, several studies have shown that the activities of many enzymes such as GCK, and glycogen phosphorylase in liver of diabetic mice were significantly affected (Palsamy and Subramanian 2009, Zhang et al., 2009). Zhang et al. (2009) reported that glucokinase enzyme activity was decreased by more than

Table 1. Primers sequences.

Transcripts	Primer sequences
Glucokinase (GCK)	F-5'ACTGACTATCCGGCTACATG3' R-5'GATTCCTGCTTGAATAGTGC3'
phosphoenolpyruvate carboxykinase (PEPCK)	F-5'GTCACCATCACTTCCTGGAAGA3' R-5'GGTGCAGAATCGCGAGTTG3'
Glycogen phosphorylase	F-5'CCCGAGCACCCAATGACTTTAACC3' R-5'GCGAGTGCGGGATGTGTGTCA3'
β 2-microglobulin	F-5'TTCTGGTGCTTGTCTCACTGA3' R-5'CAGTATGTTGCGCTTCCCATTG3'

Table 2. Real-Time PCR results for selected genes.

Groups	Group 1 (Real-Time PCR fold changes)	Group 2 (Real-Time PCR fold changes)	Group 3 (Real-Time PCR fold changes)
Glucokinase (GCK)	1.46	1.28	1.01
Phosphoenolpyruvate carboxykinase (PEPCK)	1.22	1.47	1.89
Glycogen phosphorylase	0.67	0.56	0.51

**Figure 1.** The effect of different concentration of Persian shallot on FBS level (mg/dl). (Mean \pm SD) (P<0.05).

Group1: diabetic rats received 200 mg/kg Persian shallot. Group2: diabetic rats received 100 mg/kg Persian shallot. Group3: diabetic rats received 0.9% saline. Group4: normal rats received 0.9% saline. * Significant differences with Group 4 (P<0.05). ** Significant differences with Group 3 (P<0.05). *** Significant differences with Group 2 (P<0.05).

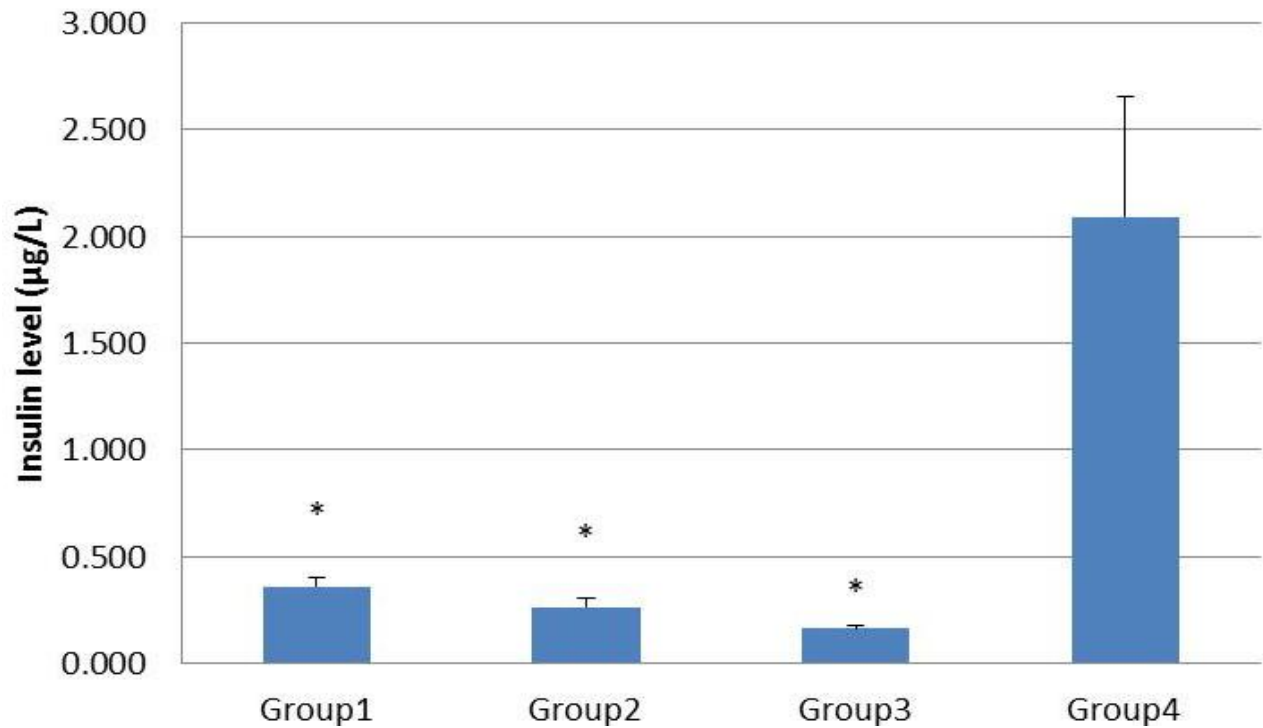


Figure 2. The effect of different concentration of Persian shallot on Insulin level (µg/L). (Mean±SD) ($P < 0.05$). Group1: diabetic rats received 200 mg/kg Persian shallot. Group2: diabetic rats received 100 mg/kg Persian shallot. Group3: diabetic rats received 0.9% saline. Group4: normal rats received 0.9% saline. * Significant differences with Group 4 ($P < 0.05$).

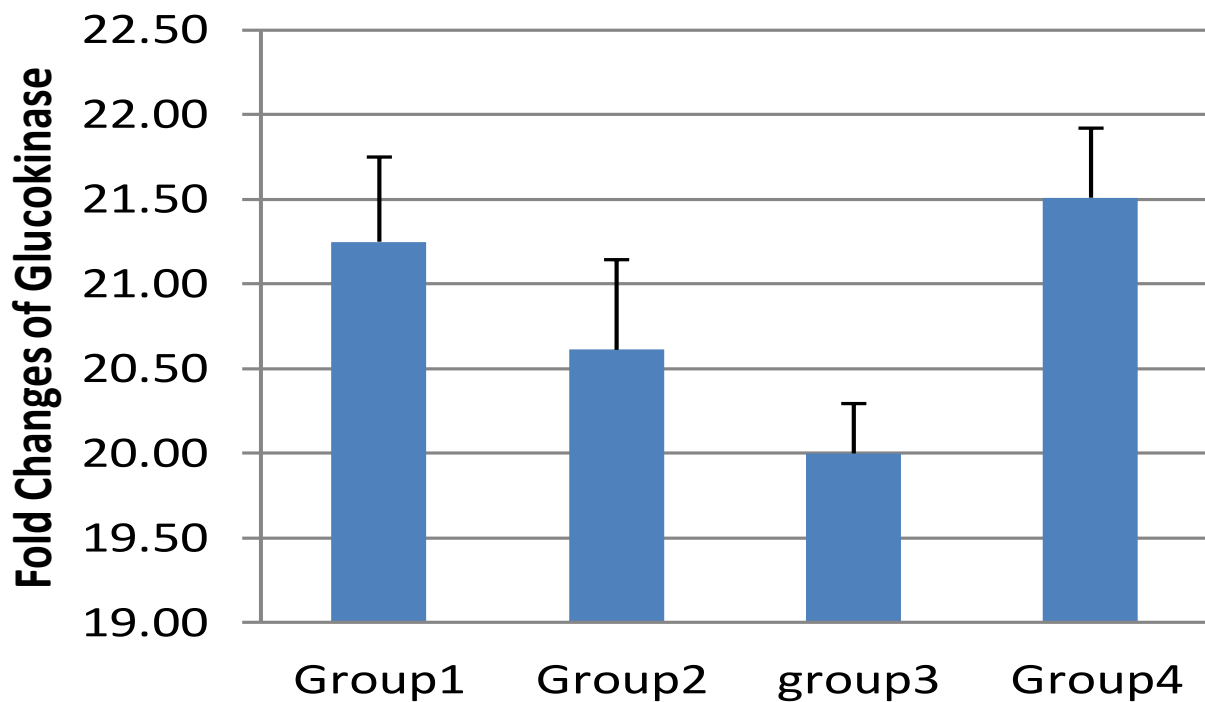


Figure 3. The expressed levels of Glucokinase mRNA (fold) in all groups (using ANOVA test, Mean±SD) ($P < 0.05$). Group1: diabetic rats received 200 mg/kg Persian shallot. Group2: diabetic rats received 100 mg/kg Persian shallot. Group3: diabetic rats received 0.9% saline. Group4: normal rats received 0.9% saline.

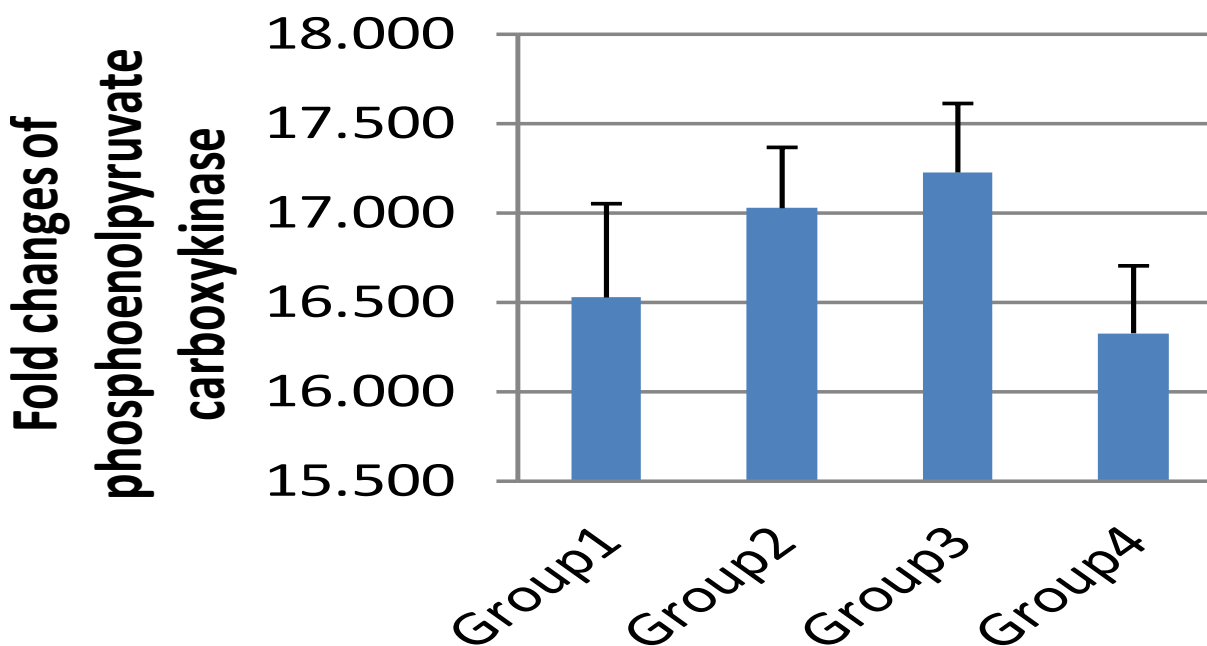


Figure 4. The expressed levels of phosphoenolpyruvate carboxykinase mRNA (fold) in all groups. (using ANOVA test, Mean \pm SD) ($P < 0.05$).

Group1: diabetic rats received 200 mg/kg Persian shallot. Group2: diabetic rats received 100 mg/kg Persian shallot. Group3: diabetic rats received 0.9% saline. Group4: normal rats received 0.9% saline.

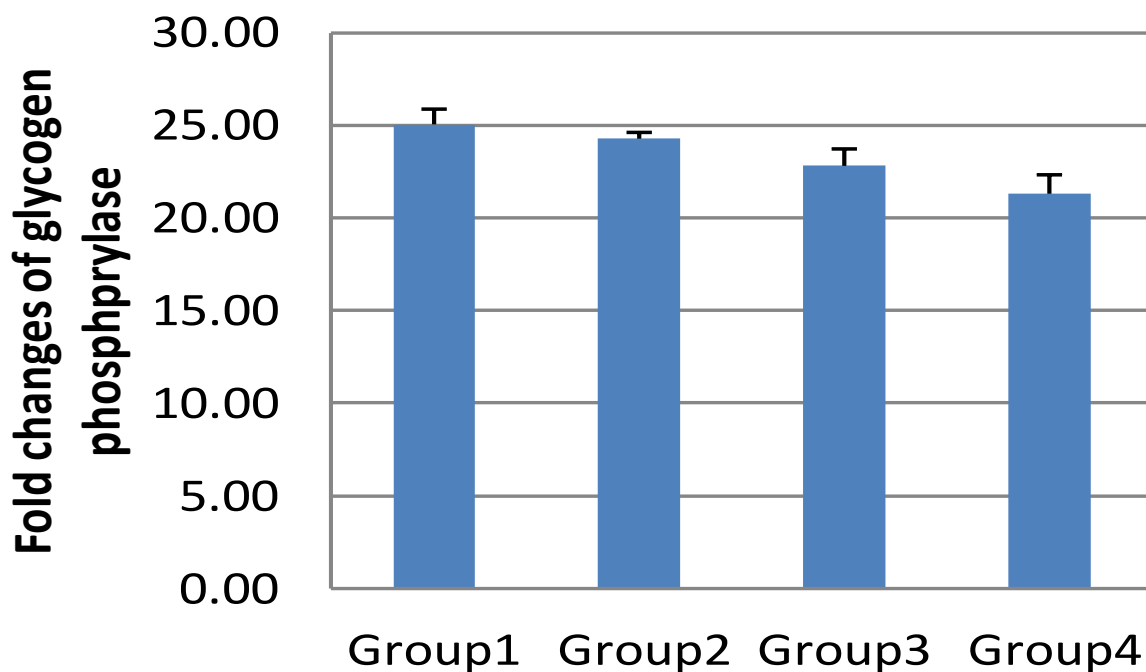


Figure 5. The expressed levels of glycogen phosphprylase mRNA (fold) in all groups (using ANOVA test, Mean \pm SD) ($P < 0.05$).

Group1: diabetic rats received 200 mg/kg Persian shallot. Group2: diabetic rats received 100 mg/kg Persian shallot. Group3: diabetic rats received 0.9% saline. Group4: normal rats received 0.9% saline.

90% in the liver of diabetic rats. So in this study, to evaluate the antidiabetic mechanism(s) of Persian shallot, the key enzymes of carbohydrate metabolism such as GCK, PEPCK, and glycogen phosphorylase were investigated in liver at mRNA level using Real-Time PCR. We showed that hepatic GCK was down regulated, but hepatic PEPCK and glycogen phosphorylase were up-regulated evidently in diabetic rats. It also increased insulin level in serum, these results suggest that the antioxidants could restore the damaged pancreas and stimulate the secretion of pancreatic insulin at the same time. The Persian shallot has probable ability to accelerate the hepatic glucose metabolism may be via regulating the expression of the functional genes of PEPCK, and GCK. The results of Real-Time PCR studies provided supportive evidence for FBS analyses (Jung et al., 2004). In fact, this hypoglycaemic action of Persian shallot is likely to be associated with a marked enhancement of the GCK mRNA expression in the liver. Current results is consistent with previous studies that showed GCK mRNA expression increase in Naringin (Jung et al., 2004) and epigallocatechin gallate, a main polyphenolic constituent of green tea (Waltner et al., 2002) treated rats. Hepatic GCK has a major effect on glucose homeostasis and is a potential target for pharmacological treatment of diabetes, and rats overexpressing GCK in the liver had reduced blood glucose. The elevation of hepatic GCK can cause an increased utilization of the blood glucose for energy production or glycogen storage in the liver (Jung et al., 2006). A low hepatic GCK activity is also reported to favor the release of glucose synthesized by gluconeogenesis into the circulation. The PEPCK is a key enzyme that controls gluconeogenesis and glucose output from the liver. It is involved in the synthesis of glucose-6-phosphate from non-carbohydrate precursors. Enhanced expressions of the PEPCK gene in liver was present in most models of diabetes, and is thought to contribute to the increased hepatic glucose output seen in this disease. Insulin is the most important hormone that inhibits gluconeogenesis. At the gene transcription level, insulin down-regulated the mRNAs encoding PEPCK (Davies et al., 2001). In the present study PEPCK gene expressions was increased in diabetic rats whereas reduced in Persian shallot treatment group. The present finding is in agreement with Jung et al. (2006) observation that Caffeic acid phenethyl ester markedly reduces PEPCK mRNA levels in diabetic rats. In addition, previous investigations showed that Naringin (Jung et al., 2004) and epigallocatechin gallate, a main polyphenolic constituent of green tea (Waltner et al., 2002) suppress PEPCK mRNA expression. This is consistent with present findings. In addition to PEPCK, GCK activity is also reported to be controlled primarily at the level of transcription, being regulated by insulin. High insulin levels have been shown to inhibit hepatic glucose pro-

duction by means of stimulation of GCK gene transcription (Friedman et al., 1997). In fact in the present study, the changes in hepatic glucose-regulating enzymes could be partly attributed to insulin levels because plasma insulin level was elevated in Persian shallot-supplemented diabetic rats in comparison to the control (Jung et al., 2006). Further, previous investigations indicated that hepatic glycogenolysis plays a major role in the regulation of plasma glucose levels in diabetic rats which suggest that glycogen phosphorylase inhibitors may be useful in the treatment of diabetes (Martin et al., 1998). Surprisingly Persian shallot increases glycogen phosphorylase in diabetic rats. It has been demonstrated that insulin inhibits glycogenolysis via stimulation of glycogen phosphorylase activity (Celik et al., 2009), but in the current study Persian shallot increased insulin and glycogen phosphorylase activity simultaneously.

Conclusions

Overall, based on the finding of the present study it could be suggested that Persian shallot has complimentary potency to develop as a hypoglycaemic agent for treatment of diabetes mellitus. Moreover, Persian shallot perhaps also modulates the hepatic glucose metabolism by balancing (up/down-regulating) the expression of rate-limiting enzymes (GCK, PEPCK and glycogen phosphorylase) in STZ-induced diabetic mice. The antioxidant activity of Persian shallot is possibly capable of protecting pancreatic islets from STZ-induced damage by scavenging the free radicals and repairing the destroyed pancreatic β -cells, and in fact guarantees the normal secretion of insulin in serum.

REFERENCES

- Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB (2001). Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature* 409:729-733.
- Barthel A, Schmol D (2003). Novel concepts in insulin regulation of hepatic gluconeogenesis. *Am. J. Physiol. Endocrinol. Metab.* 285:685-692.
- Celik S, Erdogan S, Tuzcu M (2009). Caffeic acid phenethyl ester (CAPE) exhibits significant potential as an antidiabetic and liver-protective agent in streptozotocin-induced diabetic rats. *Pharmacol. Res.* 60:270-276.
- Davies GF, Khandelwal RL, Wu L, Juurlink BH, Roesler WJ (2001). Inhibition of phosphoenolpyruvate carboxykinase (PEPCK) gene expression by troglitazone: a peroxisome proliferator-activated receptor-gamma (PPARgamma)-independent, antioxidant-related mechanism. *Biochem. Pharmacol.* 62:1071-1079.
- Ferre T, Pujol A, Riu E, Bosch F, Valera A (1996). Correction of diabetic alterations by glucokinase. *Proc. Nat. Acad. Sci. USA* 93:7225-7230.
- Friedman JE, Sun Y, Ishizuka T, Farrell CJ, McCormack SE, Herron LM, Hakimi P, et al (1997). Phosphoenolpyruvate carboxykinase (GTP) gene transcription and hyperglycemia are regulated by glucocorticoids in genetically obese db/db transgenic mice. *J. Biol.*

- Chem. 272:31475-31481.
- Ghodrati AH, Ghaffari SM, Riazi GH, Ahmadian S, Vahedi F (2008). Antiproliferative activity of chloroformic extract of Persian Shallot, *Allium hirtifolium*, on tumor cell lines. *Cytotechnol.* 56(3):179-185.
- Hosseini J, Hosseini-zijoud S-M, Oubari F, Abbasi Oshaghi E, Rajabi Gilan N, Ghasemi SR, Mahmoodi M, Hashemi B (2012). Hepato-protective effects of hydroalcoholic extract of *Allium hirtifolium* (Persian shallot) in diabetic rats. *J. Basic Clin. Physiol. Pharmacol.* 23(2):83-87.
- Hosseini-zijoud SM, Hosseini J, Mahmoodi M, Behrooz H, Hosseini-zijoud SM (2012). The effects of Persian shallot extract on the levels of some blood biochemical parameters in streptozotocin-induced diabetic rats. *Afr. J. Agric. Res.* 7(22):3308-3313.
- lynedjian P, Gjinovci A, Renold A (1998). Stimulation by insulin of glucokinase gene transcription in liver of diabetic rats. *J. Biol. Chem.* 263:740-744.
- Jafarian A, Ghannadi A, Elyasi A (2003). The effects of *Allium hirtifolium* boiss. on cell-mediated immune response in mice. *Iran. J. Pharmaceut. Res.* 2: 51-55.
- Jung UJ, Lee MK, Jeong KS, Choi MS (2004). The hypoglycaemic effects of hesperidin and naringin are partly mediated by hepatic glucose-regulating enzymes in C57BL/KsJ-db/db mice. *J. Nutr.* 134:2499-2503.
- Jung U, Lee M, Park Y, Jeon S, Choi M (2006). Antihyperglycemic and antioxidant properties of caffeic acid in db/db mice. *J. Pharmacol. Exp. Ther.* 318:476-483.
- Kopp U, Cicha M, Yorek M (2008). Impaired responsiveness of renal sensory nerves in streptozotocin-treated rats and obese Zucker diabetic fatty rats: role of angiotensin. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294:858-866.
- Leelarungrayub N, Rattanapanone V, Chanarat N, Gebicki JM (2006). Quantitative evaluation of the antioxidant properties of garlic and shallot preparations. *Nutr.* 22:266-274.
- Martin WH, Hoover DJ, Armento SJ, Stock IA, McPherson RK, Danley DE, Stevenson RW, Barrett EJ, Treadway JL (1998). Discovery of a human liver glycogen phosphorylase inhibitor that lowers blood glucose in vivo. *Proc Natl Acad Sci USA* 95: 1776-1781.
- Matte A, Tari L W, Goldie H, Delbaere L (1997). Structure and mechanism of phosphoenolpyruvate carboxykinase. *J. Biol. Chem.* 272:8105-8108.
- Momeni T (2000). *Herbal Extracts*. Reza press-Tehran, 43-47(Persian).
- Nakagawa T, Yokozawa T, Terasawa K, Shu S, Juneja LR (2002). Protective activity of green tea against free radical- and glucose-mediated protein damage. *J. Agric. Food Chem.* 50:2418-2422.
- Newgard C, Hwang P, Fletterick R (1989). The family of glycogen phosphorylases: structure and function. *Crit. Rev. Biochem. Mol. Biol.* 24:69-99.
- Palsamy P, Subramanian S (2009). Modulatory effects of resveratrol on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. *Chem. Biol. Interact.* 179:356-362.
- Waltner-Law M, Wang XL, Law BK, Hall RK, Nawano M, Granner DK (2002). Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. *J. Biol. Chem.* 277:34933-34940.
- You-Gui L, Dong-Feng J, Shi Z, Zhi-Qiang L, Tian-Bao L, Shi C (2011). Hybrid of 1-deoxynojirimycin and polysaccharide from mulberry leaves treat diabetes mellitus by activating PDX-1/insulin-1 signaling pathway and regulating the expression of glucokinase, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase in alloxan-induced diabetic mice. *J. Ethnopharmacol.* 134:961-970.
- Zhang X, Liang W, Mao Y, Li H, Yang Y, Tan H (2009). Hepatic glucokinase activity is the primary defect in alloxan-induced diabetes of mice. *Biomed. Pharmacother.* 63:180-186.