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Panax ginseng improves glucose metabolism in streptozotocin-induced diabetic rats through 5′ adenosine monophosphate kinase up-regulation

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ABSTRACT

5′ AMP-activated protein kinase (AMPK), insulin receptors and transporters are distorted in diabetes mellitus. In this study, the effect of *Panax ginseng* was assessed on glucose manipulating enzymes activities and gene expression of AMPK, IRA and GLUT2 in streptozotocin-induced diabetic male rats. Forty male albino rats were randomly divided to four groups 10 rats of each, group I, normal control group (received saline orally); group II, normal rats received 200 mg/kg of *Panax ginseng* orally; group III, Streptozotocin (STZ) –induced diabetic rats and group IV, STZ-induced diabetic rats received 200 mg/kg of *Panax ginseng* orally. The duration of experiment was 30 days. Results showed the ability of *Panax ginseng to* induce a significant decrease in the blood glucose and increase in the serum insulin levels, hepatic glucokinase (GK), and glycogen synthase (GS) activities with a modulation of lipid profile besides high expression levels of AMPK, insulin receptor A (IRA), glucose transporting protein-2 (GLUT-2) in liver of diabetic rats. In conclusion, the obtained results point to the ability of *Panax ginseng* to improve the glucose metabolism in diabetic models.

1. Introduction

Many enzymes are concerned with energy homeostasis among them 5 AMP activated protein kinase (EC 2.7.11.31) that regulates the energy homeostasis inside the cell (Winder and Hardie, 1999). The enzyme has been confirmed to be expressed in liver (Huang et al., 2018), and muscles (Dial et al., 2018). Activation of the enzyme leads to activation of fatty acids oxidations in muscles and liver, ketogenesis, glucose uptake by the cells and inhibition of cholesterol synthesis, lipogenesis, triglycerides synthesis and modulation of insulin secretion by beta cells of pancreas (Winder and Hardie, 1999). Several studies which have been used agents for controlling of Diabetes Mellitus (DM) pointed AMPK as target (Jung et al., 2017; Liu et al., 2018a; Xiong et al., 2018). DM as a chronic metabolic disorder is attractive for many researchers. There is a daily continuous seeking for a modulator or preventer for DM, however the success of the modulator depends on its target inside the body and its relevance to the disorder (Sangeetha et al., 2017). Natural agents' especially medicinal plants now constitute the major targets used for controlling of DM (Neamsuvan et al., 2015). *Panax ginseng* is a medicinal plant contributed for the controlling of many disorders (Ru et al., 2015), in the same line, its pharmacological action have been demonstrated in disorders such as cancer including; breast, lung, liver, colon and skin cancer (Majeed et al., 2018), cardiovascular diseases (Zheng et al., 2017), acute menopausal symptoms (Kargozar et al., 2017), acute pancreatitis (Liu et al., 2018b), it has been used for stimulating immune activity (Kang and Min, 2012; Yu et al., 2018), as a neuroprotective agent (Luo et al., 2018), and for its antioxidant activities (Shergis et al., 2014), anti stress (Wang et al., 2018) and anti-aging (Bjorklund et al., 2018) activities. Ginseng now is one of the most

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famous natural agents used in controlling the metabolic syndromes such as DM and its complications (Deng et al., 2017; Kim et al., 2017; Wang et al., 2017; Xu et al., 2017). Recently, it has been demonstrated that ginseng can improves the glucose intake, attenuates insulin resistance and reduce fat mass in high fat diet-obesity mice (Dai et al., 2018). The matter which gives us the impetus to study the effectiveness of Panax ginseng on enzymes and proteins related to DM. We considered AMPK as a target in the present study. Its mRNA expression levels were measured in the all experimental animals, beside the expression levels of glucose transporter-2 (GLUT-2), Insulin receptor A (IRA) and activities of Glucokinase (GK), Glycogen synthase (GS) in livers of experimental groups. Blood glucose, insulin, total Cholesterol (TC), triacylglycerol (TAG), HDL-cholesterol (HDL-c), LDL-cholesterol levels were also determined.

2. Material and methods

2.1. Animals selection and grouping

Forty male albino rats 6 month of age and weighing 120 ± 20 g were obtained from the animal house of Zagazig University, Egypt. The animal were housed in individual suspended stainless steel cages at 22 ± 2 °C with a 12 h light/dark cycle and allowed to acclimatize for period of 7 days before beginning the experiment. Rats were divided randomly into 4 groups (n = 10) and were allowed free access to food and water. Rats were divided randomly into 4 groups. (n = 10) in each. Group I served as non-treated control group, group II received daily dose of ginseng 200 mg/kg Bwt for constitutive 30 days, group III STZ-induced diabetic rats did not receive any type of treatment, group IV STZ-induced diabetic rats received 200 mg/kg Bwt of ginseng for constitutive 30 days.

2.2. Chemicals

Streptozotocin (STZ) (Sigma-Aldrich Co. St. Louis, Missouri, USA). Root powder of Korean ginseng (*Panax ginseng* C.A. Meyer).

2.3. Ethical statement

All procedures of the current experiment have been approved by the Ethical Committee of the Faculty of Vet. Med. Zagazig University Egypt.

2.4. Induction of experimental diabetes mellitus

Diabetes mellitus was induced by a single intra-peritoneal injection of 100 mg/kg B.wt. of a freshly prepared Streptozotocin powder "STZ" (Sigma-Aldrich Co. St. Louis, Missouri, USA) dissolved in 0.01 M cold sodium citrate buffer (pH 4.5) immediately before use. The rats with STZ were given 5% W/V glucose solution next 24 h to prevent the hypoglycemia. After 72 h rats with fasting blood glucose more than 250 mg/dL had been selected as diabetic rats (Alkaladi et al., 2014).

2.5. Sampling protocol

After 12 h fasting, the blood samples were collected from median canthus of eye and the sera were separated by centrifugation and stored at −20 °C for biochemical determinations. Liver tissues were collected and divided in to two parts. The first part was used to prepare a tissue homogenate for biochemical measurements of enzymes activities and the other part collected on liquid nitrogen, preserved at −80 °C until the extraction of RNA and was used for gene expression investigation.

2.6. Biochemical determinations

The blood glucose concentrations were determined using glucose oxidase method and the kits provided by SPINREACT (Sant Esteva de Bas, Girona, Spain). The insulin levels in serum were estimated using IMMULITE Insulin kits (Catalog Number: LKIN1, Avenue, Silver Spring, MD 20993) following manufacture instructions (Chevenne et al., 1998). Serum total cholesterol (Richmond, 1973), triacyl-glycerols (Fossati and Prencipe, 1982), HDL - cholesterol (Lopes-Virella et al., 1977), and LDL-cholesterol (Glatter, 1984) were determined in the sera of all experimental animals. The activities of hepatic GK (Pakoskey et al., 1965) and GS (Brady, 2003) were determined in the liver tissue homogenates of all experimental rats.

2.7. Molecular biological determinations

2.7.1. RNA extraction and cDNA synthesis

Total RNA was extracted from liver tissue using RNeasy Mini Kit (Qiagen, Cat. No. 74104) and following the manufacturer instructions. The amount of extracted RNA was quantified and qualified by NanoDrop® ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, Delaware USA. The first strand cDNA was synthesized by using RevertAidTM H Minus (Fermentas, life science, Pittsburgh, PA, USA).

2.7.2. Primers for experimental genes

The primers pairs were designed according to the previously published data as following AMPKα1 (ID: 65248); forward, 5′-ATCCGCAGAGAGATCCAGAA-3′ and reverse 5′-CGTCGACTCTCCTTTTCGTC-3′ (McCrimmon et al., 2006), IRA (ID: 24954); forward, 5′-TTCATTCAGGAAGACCTTCGA-3′ and reverse, 5′-AGGCCAGAGATGACAAGTGAC-3′, GLUT-2 (ID: 25351); forward, 5′-TTAGCAACTGGGTCTGCAAT-3′, and reverse 5′-TCTCTGAAGACGCCAGGA AT-3′ (Alkaladi et al., 2014), and ß-actin (ID: 81822); forward, 5′- AGCCATGTACGTAGCCAT -3′ and reverse 5- CTCTCAGCTGTGGTGGTGAA -3′ (Batalha et al., 2016).

2.7.3. Real time PCR

One μL of cDNA was mixed with 12.5 μL of 2x SYBR® Green PCR mix from BioRad, 5.5 μL of autoclaved water, and 0.5 μL (10 pmol/μL) of each forward and reverse primer for the measured genes. The house keeping gene β-actin was used as a control for normalization. Fold change was calculated using the ($2^{-\Delta\Delta ct}$) method to quantitate mRNA levels, according to Litvak and Schmittgen (2001).

2.8. Statistical analysis

The obtained data was analyzed by using the statistical package for social science (SPSS, 18.0 software, 2011). Differences among groups was evaluated using one way ANOVA. Results were expressed as mean ± SE. P values less than 0.05 were considered to be significant.

3. Results

3.1. Fasting blood glucose (mg/dL) and serum insulin (μIU/mL) levels.

There were a significant decrease in the blood glucose levels in diabetic rats received ginseng as compared to diabetic non-treated rats. The insulin levels were significantly increased in diabetic rats received ginseng also if compared with diabetic non-treated groups at $P < 0.05$ (Table 1).

3.2. Glucokinase (U/gm tissue) and glycogen synthase (mU/mg protein) activities in liver tissues.

The activities of glucokinase and glycogen synthase were significantly increased in diabetic rats received ginseng if compared with diabetic non-treated rats at $p < 0.05$ (Table 2).

3.3. Serum lipid profiles (mg/dL).

There were a significant decrease in the concentrations of total cholesterol, tri-acylglycerols and LDL-cholesterol (mg/dL) in diabetic rats received ginseng if compared with the diabetic non-treated rats. The level of HDL-cholesterol was significantly decreased in diabetic rats received ginseng when compared with diabetic non-treated rats at $P < 0.05$ (Table 3).

3.4. mRNA expression levels of AMPK, IRA, and GLUT2.

The mRNA expression levels of AMPK, IRA and GLUT2 proteins was increased in the diabetic rats received ginseng if compared with diabetic non-treated rats at $P < 0.05$ (Table 4).

4. Discussion

The present work studied the molecular and biochemical effects of Panax ginseng on some enzymes and proteins related to glucose metabolism in diabetic rats. The effect on AMPK mRNA expression levels was estimated. The mRNA of AMPK showed the lowest expression level among diabetic rats (0.45 ± 0.26), while its expression level was modulated and increased in all rats received ginseng, normal (1.02 ± 0.28) and diabetic (0.96 ± 0.11) respectively. The results explained the great association between AMPK action and the incidence of diabetes mellitus the matter which making AMPK a popular target when study DM. recently it has been demonstrated that activation of AMPK could promote the glucose uptake by the cells (Na et al., 2018) and the ability of ginseng to induce an up-regulation of AMPK expression in diabetic rats lead to decline the blood glucose in experimental rats received ginseng 1.02 and 1.78 times in normal and diabetic rats respectively. The same observations have been obtained in human by Choi et al. 2018, ginseng has induced lowering in blood glucose of diabetic patients specially those of fasting blood glucose (FBG) of 110 mg/dL or more (Choi et al., 2018). We also reported the effect of ginseng on serum insulin levels. Insulin level was increased by 1.42 times in diabetic rats received ginseng when compared with non treated rats. In the same line, other studies reported the hypoglycemic effect of ginseng in diabetic/obese models (Kim et al., 2017; Lee et al., 2017b; Sun et al., 2018), it has been reported to induce an increase in serum insulin levels (Wang et al., 2017), and improved the insulin tolerance (Deng et al., 2017) in diabetic mice. There is a great prove now that the activation of AMPK can lead to increasing insulin secretion and improve its tolerance (Jung et al., 2018; Wu et al., 2018; Zhao et al., 2018). We can indicate the effect of ginseng to reduce serum blood glucose and increase serum insulin levels to its ability to induce up-regulation of AMPK expression in diabetic rats. Coming with this up-regulation there was a significant up-regulation of IRA and GLUT2 (Fig. 1). Ginseng significantly increased the expression of IRA and GLUT2 0.87 and 0.91 fold respectively in diabetic rats received ginseng when compared to diabetic non-treated rats 0.36 and 0.48 fold respectively. In a similar study the effect of ginseng on insulin receptor has been reported to induce high expression in insulin receptor B (IRB) and insulin receptor substrate-1 (IRS) (Dai et al., 2018). Another study reported the ability of ginseng to induce up-regulation of insulin receptors in muscles of metabolic syndrome rats (Kho et al., 2016). Our data regarding mRNA expression of IRs come in accordance with the study of (Cheon et al., 2015) which reported the increase IR mRNA expression in old-aged ob/ob mice received *Panax ginseng* for 16 weeks. In this direction, our data proved the ability of ginseng to improve insulin sensitivity in diabetic rats. There were many studies that have demonstrated the effect of ginseng on GLUTs, it have been reported that ginseng induced translocation of GLUT4 (Tabandeh et al., 2015; Ota and Ulrih, 2017), up-regulation of GLUT4 in muscles of diabetic rats (Lai et al., 2006; Kang et al., 2017), high expression levels of GLUT1 and GLUT4 in myotubes of diabetic rats (Seo et al., 2016), increase the expression of GLUT1 and GLUT4 in livers of diabetic mice (Jung and Kang, 2013; Xie et al., 2015), , and up-regulation of GLUT4 in adipose tissue of diabetic rats (Kim and Kim, 2012). Regarding GLUT2 mRNA expression levels our data come in the same line of (Kang et al., 2017) which demonstrated the high expression levels of hepatic GLUT2 hand by hand with high phosphorylation of AMPK in diabetic mice received ginseng for 4 weeks. Similar data have been come with the same line of our observations regarding high expression levels of GLUT2 in different cell lines/tissues of diabetic models received ginseng (Ohnishi et al., 1996; Gu et al., 2013; Liu et al., 2013). In general, the ability of ginseng to increase the expression levels of GLUTs and especially GLUT2 explained its ability to increase the uptake of glucose by insulin targeted-cells especially liver cells. The matter which translated to lower blood glucose in diabetic models received ginseng. Also we showed that *Panax ginseng* induced a significant increase in activities of hepatic GK (6.4 times) and GS (1.36 times) of diabetic rats received ginseng for 30 days if compared with non-treated rats. Their activities also non significantly increased in non-diabetic rats received ginseng when compared with control. It well-known that hepatic GK activity and expression are decreased in DM (Song et al., 2017), in the same way, we observed the lowest activity of GK in diabetic non-treated rats. It was also known that glycogen storage is impaired in livers of diabetic models due to impairment of GS (Ros et al., 2011) the matter which leads to hyperglycemia. Many targets which have been used to lower blood glucose in diabetic models targeted the activation of the two enzymes. The activation of AMPK has lead to an increase in GK and GS activities (Chen et al., 2018)). In our study, ginseng activated both GK and GS in diabetic rats. Ginseng has to increase GK expression in adipocytes (Lee et al., 2017a) and induced the expression of GK in pancreatic cells (Park et al., 2008), in the same line, ginseng have to increase hepatic or muscles glycogen deposition (Xie et al., 2015; Xu and Dou, 2016) through inhibition of Glycogen synthase kinase (GSK) in hepatocytes (Kho et al., 2016;

Table 1
Fasting blood glucose and serum insulin levels in experimental rats.

Groups	Glucose (mg/dL)	Insulin (μIU/mL)
Control	80.5 ± 14.3[c]	13.8 ± 0.51[c]
Normal + ginseng	78.3 ± 24[c]	13.2 ± 0.38[c]
Diabetic	298 ± 28.17[a]	7.9 ± 0.11[a]
Diabetic + ginseng	167.9 ± 3.4[b]	11.2 ± 0.23[b]

Means carrying different superscripts are significant at $P < 0.05$.

Table 2
Liver glucokinase and glycogen synthase activities in experimental rats.

Groups	Glucokinase (U/g liver tissue)	Glycogen synthase (mU/mg protein)
Control	1.21 ± 0.02[a]	3.7 ± 0.08[a]
Normal + ginseng	1.43 ± 24[a]	3.5 ± 0.38[a]
Diabetic	0.16 ± 0.008[b]	1.98 ± 0.1[c]
Diabetic + ginseng	1.03 ± 0.05[a]	2.7 ± 0.22[b]

Means carrying different superscripts are significant at $P < 0.05$.

Table 3
Serum lipids profile levels in experimental rats.

Groups	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-cholesterol (mg/dL)	LDL- cholesterol (mg/dL)
Control	87.2 ± 1.35[c]	83.4 ± 2.51[b]	46.18 ± 2.93[b]	23.34 ± 1.59[c]
Normal + ginseng	81.9 ± 1.51[c]	82.2 ± 3.25[b]	48.87 ± 1.52[b]	15.9 ± 1.44[d]
Diabetic	171.8 ± 2.1[a]	119 ± 0.11[a]	38.3 ± 2.02[a]	99.5 ± 2.92[a]
Diabetic + ginseng	111.3 ± 2.83[b]	85 ± 1.92[b]	48.1 ± 1.56[b]	45.2 ± 2.17[b]

Means carrying different superscripts are significant at P < 0.05.

Table 4
mRNA expression levels of examined proteins in experimental rats.

Groups	AMPK	IRA	GLUT2
Control	1.05 ± 0.20[a]	1.07 ± 0.21[a]	1.11 ± 0.28[a]
Normal + ginseng	1.02 ± 0.28[a]	1.03 ± 14[a]	1.05 ± 0.18[a]
Diabetic	0.45 ± 0.26[b]	0.36 ± 0.08[b]	0.48 ± 0.1[b]
Diabetic + ginseng	0.96 ± 0.11[a]	0.87 ± 0.05[a]	0.91 ± 0.22[a]

Means carrying different superscripts are significant at P < 0.05.

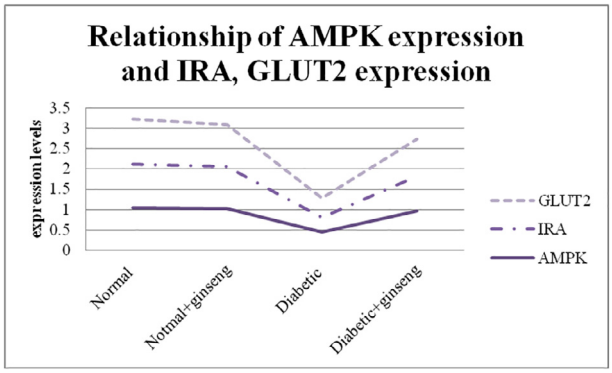

Fig. 1. Relationship between AMPK expression and IRA and GLUT2 expression in hepatocytes.

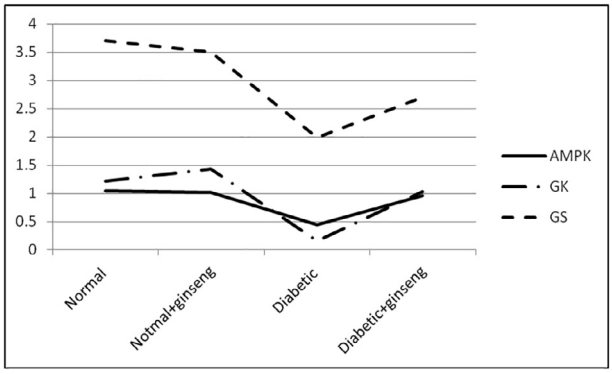

Fig. 2. Correlation between AMPK expression and GK and GS activities in diabetic rats.

Zhang et al., 2016; Shi et al., 2018). Others conclude that ginseng have to suppress the expression of GS (Seo et al., 2016). Although, there were a lot of data about the molecular effect of ginseng on GK and GS expressions, to the best of our knowledge, we are the first record for the assessment of direct effect of *Panax ginseng* on the enzymatic activities of hepatic GK and GS. On the contrary of our data, authors reported the inhibitory effect of *Panax ginseng* on ovarian Hexokinase (Li et al., 2015), while the study of (Chung et al., 2001) on hepatic Hexokinase agreed with us. The ability of ginseng to activate both GK and GS activities indicates how it reduces blood glucose in diabetic rats. It does so through activating both glucose oxidation (glycolysis) and increase glycogen deposition (glycogenesis) through AMPK activation pathway (Fig. 2). Also lipids profiles tended to be reduced in diabetic rats received ginseng. Usually DM is characterized by an impairment of lipids metabolism (Cantuaria et al., 2018). The progress of DM without glycemic control by the way will induce distorted blood lipids profile (dyslipidemia) (Levitt Katz et al., 2018). We tested the ability of *Panax ginseng* to correct this impairment. In the present study, ginseng increased the levels of HDL-c and decreased TC, TGs, and LDL-c. Our results come in accordance with the results obtained from studying the effect of different types of ginseng on dyslipidemic models. It has been reported that ginseng can reduce blood TGs (Lee et al., 2017b), plasma TGs and cholesterol, LDL-c (Cheon et al., 2015; Gui et al., 2016; Kim et al., 2016; Lee et al., 2016; Chen et al., 2017; Kang et al., 2017; Shin and Yoon, 2018). Although, others found that there was no significant change in HDL-c levels between treated and non-treated models (Gui et al., 2016), we reported an increase in serum HDL-c in diabetic rats received ginseng. It has been reported that activation of AMPK will targeted for all lipid metabolism processes leading to oxidation (Sharma et al., 2018) and so will reduce blood lipids. We relate the activity of ginseng to lower high lipid levels in diabetic rats to its ability to activate AMPK. The overall observation indicated that *Panax ginseng* can modulates the dyslipidemias originated from DM.

5. Conclusion

In conclusion, the study pointed to the ability of *Panax ginseng* to reduce blood glucose in diabetic rats through activation of AMPK pathways, increase insulin secretion and tolerance, increase glucose uptake and oxidation, and increase glycogen deposition in liver cells.

6. Conflicts of interest

The authors declare no conflict of interest.

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