

Full Length Research Paper

Antidiabetic antioxidant effect of *Panax ginseng*

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This study was done to investigate the biochemical effect of *Panax ginseng* C.A. Meyer on the diabetic rats. Sixty male albino rats were divided into four groups (fifteen for each group): Normal control, normal treated, diabetic and treated diabetic groups. Blood glucose, serum insulin, serum lipids (cholesterol, triglycerides, HDL, LDL and VLDL-cholesterol) were estimated. Also, serum interleukin - 6 (IL- 6) and tumor necrosis factor alpha (TNF - α) were determined. Liver oxidant malondialdehyde (MDA), liver antioxidants {glutathione peroxidase (GPX) and superoxide dismutase (SOD)} were determined. The mean values of blood glucose, cholesterol, triglycerides, LDL and VLDL- cholesterol showed significant decrease in treated diabetic group as compared to their values in the diabetic group. Also, serum IL - 6 and TNF - α levels were improved after treatment with ginseng. Liver antioxidants were decreased in the diabetic group while they were significantly increased after treatment with ginseng. We concluded that *P. ginseng* C.A. Meyer could be used safely as antidiabetic antioxidant agent.

Key words: Ginseng, diabetes, antioxidant, IL - 6, TNF - α .

INTRODUCTION

Diabetes mellitus is a complex disease associated with peripheral and central complications. These complications include retinopathy, nephropathy and neuropathy. Several investigations have confirmed the role of oxidative stress in developmental diabetic-mediated disorders, possibly via the formation of free radicals (Manna et al., 2009).

Herbs have been used for medicinal purposes for centuries (Craig, 1999). One of the most widely used herbs is *Panax ginseng*; it is valuable in traditional medicine in many countries (Karaca et al., 2010). It is the most efficacious for immune stimulation and the prevention of diabetes (Liu et al., 2003). It has been used to treat a wide variety of diseases including anaemia, gastritis, blood pressure abnormalities together with its role to decrease blood coagulation, cholesterol and sugar levels (Cho et al., 2006). Considering the potential effects of ginseng in decreasing hyperglucoseaemia, we investigated whether administration of ginseng root extract had any protective effect against oxidative stress and whether it could ameliorate serum glucose, total cholesterol and triglycerides levels in rats with streptozotocin (STZ)- induced diabetes.

MATERIALS AND METHODS

Experimental animals

Male albino rats weighing 80 – 120 g were obtained from the animal house of the national research centre (NRC), Giza, Egypt. The animal were housed in individual suspended stainless steel cages at $22 \pm 2^\circ\text{C}$ with a 12 h light/dark cycle and allowed to acclimatize for a period of 15 days to the experiment and rats were allowed free access food and water.

Chemicals

Streptozotocin (STZ) was purchased from Sigma Chemical Company. Root powder of Korean ginseng (*Panax Ginseng* C.A. Meyer).

Induction of diabetes

Streptozotocin (STZ) was dissolved in 50 mM sodium citrate solution (pH 4.5) containing 150 mM sodium chloride. The solution (6 mg/0.5 ml/100 g body weight) was subcutaneously administered in rats (Uchiyama and Yamaguchi, 2003).

Experimental design

Sixty male rats were divided into four groups, 15 rats for each, and were classified as follow:

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Group 1: Normal control group.

Group 2: Normal treated group-normal rats received 22.5 mg ginseng/ rat/ day orally for 45 days.

Group 3: Diabetic rats.

Group 4: Diabetic treated group - Diabetic rats received 22.5 mg ginseng/ rat/ day orally for 45 days.

The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of NRC.

Samples collection

After that animals were kept fasting for 12 h, the animals were anesthetized and the blood was withdrawn from the retro-orbital venous plexus using heparinized capillary tubes. Blood samples were collected, left to clot, and then centrifuged at 3000 r.p.m for 15 min to separate the serum. Freshly prepared serum was used for determination of blood glucose according to Trinder (1969) and the rest of serum was divided into aliquots and stored at - 20°C for biochemical assays. Liver tissues were removed quickly and placed in iced normal saline, the tissues were cut into small pieces and homogenized in cold buffer (0.5 g of Na₂HPO₄ and 0.7 g of NaH₂PO₄/ 500 ml deionized water per g tissue), then centrifuged, the supernatant was used for oxidant/ antioxidant parameters estimation (Manna et al., 2005).

Biochemical parameters

Serum cholesterol and triglycerides were estimated by the method according to Richmond (1973) and Fossati and Principe (1982), respectively. HDL - cholesterol was measured by the enzymatic method according to Lopez-Virella et al. (1977) while LDL-cholesterol was calculated according to Glatter (1984). Serum insulin, Inter leukin-6 (IL - 6) and tumor necrosis factor alpha (TNF - α) were determined by an enzyme amplified sensitivity immunoassay (EASIA) according to Yalow and Bauman (1983), Le Moineo (1994) and Aukrust et al. (1994), respectively, the kits were purchased from Biosource, Belgium.

Liver glutathione peroxidase activity (GPX) was measured according to Paglia and Valentine (1967). Determination of liver SOD was passed on the method of Beauchamp and Fridovich (1971). Liver MDA was measured by the method of Uchiyama and Mihara (1978) using thiobarbituric acid (TBA) reaction. The protein content of the homogenate was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical analysis

Statistical analysis of the results were carried out using the standard computer program SPSS (V 9.04, Echsoft Corporation USA, 1998). Normally distributed results were compared using student's test. Differences among groups were evaluated using one way ANOVA. Results were expressed as mean \pm SE. P values less than 0.05 were considered to be significant.

RESULTS

In this study, the mean values of fasting blood glucose and serum insulin were insignificantly changed in the normal treated group compared to the control group which indicated the safety of *Panax ginseng*. In diabetic group, the mean value of fasting blood glucose was

significantly increased concomitant with a significant decrease in serum insulin level compared to the control group. After treatment with ginseng, the mean value levels of glucose and insulin showed significant improvement (decrease in blood glucose level and increase in the insulin level) (Table 1).

In the current study, no significant change was observed in serum lipid profile levels in the ginseng treated group compared to the control group. The mean values of serum cholesterol, triglycerides, LDL and VLDL were significantly increased in diabetic group compared to control group. All these values decreased significantly after treatment with ginseng although they are still higher than those of the control (Table 2).

In the same line, the mean values of serum IL-6 and TNF - α were significantly increased in diabetic group compared to control group while the treatment by *P. ginseng* decreases these values significantly (Table 3).

The current study showed a significant increase in liver MDA and significant decrease in antioxidant parameters in diabetic group compared to control group while they improved again after treatment with *P. ginseng* (Table 4).

DISCUSSION

P. Ginseng C.A. Meyer (ginseng) has been widely used to treat diabetes (Choe et al., 2006). Results of clinical studies demonstrated that ginseng could improve the immune response in diabetic patients (Kiefer and Pantuso, 2003).

In this study, a significant antihyperglycemic action on fasting blood glucose was observed after treatment. This finding provided a valuable evidence for past reports on antidiabetic effect of ginseng (Xie et al., 2004, 2005).

Xie et al. (2004) concluded that this antidiabetic effect of ginseng is related to ginsenosides. This conclusion is in agreement with Tomoda et al. (1985) who indicated that the main active constituents of ginseng are triterpenoid saponins, ginsenosides and polysaccharides.

Different mechanisms are involved in suppressing blood glucose levels by ginseng supplementation: modulation of glucose transport (Yamasaki et al., 1993); glucose disposal (Yokozawa et al., 1984) and insulin secretion (Waki et al., 1982). Also, some components that are found in dry ginseng such as phenolic and flavonoids are known to be responsible for hypoglycemic activity (Ragunathan and Sulochana, 1994). Our results have clearly demonstrated the anti-hypercholesterolemic efficacy of ginseng on diabetic rats.

Also, a reduction in serum triglycerides level was observed after treatment. These results were in agreement with the results of Dixit et al. (1991) who mentioned that ginseng markedly reduced serum triglycerides and cholesterol in hyperlipidemic monkeys. In addition, Hwang et al. (2008) indicated that the administration of ginseng saponins to rabbits fed high cholesterol diet decreased the serum cholesterol level.

Table 1. Fasting blood glucose and serum insulin levels in different studied groups.

Groups	Parameter	
	Glucose mg/dl	Insulin uIU/ml
Control	78.2 ± 1.22	13.8 ± 0.51
Normal treated	77.8 ± 0.31	13.2 ± 0.38
Diabetic	244.1 ± 3.45 ^a	8.9 ± 0.11 ^a
Treated diabetic	167.9 ± 3.44 ^{ab}	12.2 ± 0.23 ^{ab}

Values are expressed as mean ± SE (a) significant difference compared to control group (p<0.05), (b) significant difference compared to diabetic group (p<0.05).

Table 2. Serum lipid profile levels in different studied groups.

Groups	Parameters				
	Chol. mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control	84.2 ± 1.35	78.4 ± 2.51	46.18 ± 1.93	22.34 ± 1.9	15.68 ± 1.1
Normal treated	81.2 ± 1.51	82.5 ± 3.25	48.8 ± 1.52 ^b	15.9 ± 1.4	16.5 ± 1.2
Diabetic	161.8 ± 2.1 ^a	110 ± 0.11 ^a	40.3 ± 2.02 ^a	99.5 ± 2.9 ^a	22 ± 0.4 ^a
Treated diabetic	121.3 ± 2.8 ^{ab}	85 ± 1.9 ^{ab}	48.1 ± 1.56 ^b	55.2 ± 2.1 ^{ab}	17 ± 1.4 ^{ab}

Values are expressed as mean ± SE (a) significant difference compared to control group (p<0.05). (b) significant difference compared to diabetic group (p<0.05).

Table 3. Interleukin - 6 and tumor necrosis factor alpha in different studied groups.

Groups	Parameters	
	IL-6 Pg/ml	TNF-α Pg/ml
Control	0.44 ± 0.11	24.19 ± 5.4
Normal treated	0.41 ± 0.31	24.66 ± 3.5
Diabetic	2.9 ± 0.5 ^a	41.04 ± 5.3 ^a
Diabetic treated	2.2 ± 0.6 ^{ab}	33.10 ± 3.3 ^{ab}

Values are expressed as mean ± SE (a) significant difference compared to control group (p<0.05) (b) significant difference compared to diabetic group (p<0.05).

Table 4. Liver oxidant/ antioxidant parameters in different studied groups.

Groups	Parameters		
	Malondialdehyde nmol/mg protein	Glutathione peroxidase nmol/mg protein	Superoxide dismutase u/mg protein
Control	0.66 ± 0.12	7.94 ± 0.03	23 ± 0.8
Normal treated	0.69 ± 0.01 ^b	7.98 ± 0.05	22.3 ± 0.1
Diabetic	0.92 ± 0.09 ^a	7.23 ± 0.02 ^a	16.1 ± 0.66 ^a
Treated diabetic	0.71 ± 0.11 ^{ab}	7.87 ± 0.02 ^b	21.1 ± 0.61 ^{ab}

Values are expressed as mean ± SE (a) significant difference compared to control group (p<0.05) (b) significant difference compared to diabetic group (p<0.05).

There is increasing evidence showing that diabetes is associated with increased oxidative stress (Lee and Chung, 1999). Hyperglycemia may cause increased production of free radicals which is related to glucose auto-oxidation that has been linked to non enzymatic

glycation and glycated proteins have been shown to be a source of free radicals (Ceriello et al., 1992).

Glutathione, the primary endogenous antioxidant, has a multifaceted role in antioxidant defence and it is a direct scavenger of free radicals as well as a co-substrate for

peroxide detoxification by glutathione peroxidases (winterbourn, 1995). Malondialdehyde, an index of endogenous lipid peroxidation, often acts as a quantitative marker for the aggression suffered by tissue (Choe et al., 2006).

In the current study, it was found that *P. ginseng* improved antioxidants' levels and decreased malondialdehyde levels in the treated group when compared to the diabetic group. In agreement, Liu et al. (2003) found that ginseng extracts scavenge oxidative species, also, Surh et al. (2001) indicated that ginseng extracts attenuate lipid peroxidation. That is, it may be related to saponins which play a major role in antioxidant activities.

In addition, ginsenosides which are important components heavily present in ginseng production of powerful antioxidant activities other than radical scavenging activities by stimulating gene expression of antioxidant enzymes and enhancing their activities (Kim et al., 1996).

In the present study, the mean values of serum TNF - α and IL - 6 were increased in the diabetic group and then improved by ginseng treatment.

These results were in agreement with Kim et al. (2003) who reported that, ginseng saponins have been proposed as possible candidates in the research of therapeutic modulation of stress-related disorders, for their inhibitory effect on the level of stress induced IL - 6 in mice.

Moreover, Chun et al. (2007) indicated that ginsenosides inhibited the lipopolysaccharide induced production of TNF - α by blocking transcription factor (NF - KB) which regulates the transcription of many gene associated with inflammation.

Conclusion

P. ginseng has antihyperglycemic, anti-inflammatory, antilipidemic and antioxidant effect which may help in the treatment of diabetes and diabetic complications.

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