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Full Length Research Paper

Hypolipidemic and hypoglycemic effects of aerial part of *Cynara scolymus* in streptozotocin-induced diabetic rats

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The aim of this study was to assess the effect of artichoke (*Cynara scolymus*) leaf aqueous extract (ALE) on streptozotocin (STZ)-induced diabetic rats. ALE (200 and 400 mg/kg body weight) was administered to STZ-induced diabetic rats and fasting blood glucose, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C), atherogenic index, lipid peroxidation (LPO), red blood cell (RBC) superoxide dismutase (SOD) activity and plasma antioxidant capacity were measured. The oral administration of ALE for 21 days significantly reduced TC, TG, LDL-C, VLDL-C and hyperglycemia in treated diabetic rats as compared to diabetic control group. ALE also markedly ameliorated the level of plasma malondialdehyde (MDA) and increased plasma antioxidant capacity of treated diabetic group. The results clearly indicate the beneficial reducing effects of ALE on serum TC, TG, LDL-C, VLDL-C, glucose levels and plasma MDA level in STZ-treated rats.

Key words: Hypoglycemic, hypolipidemic, diabetes, *Cynara scolymus*, superoxide dismutase, lipid peroxidation.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by disorders in metabolism of carbohydrates, lipids and several essential trace elements (Kazi et al., 2008). The use of lipid lowering diets and drugs are common for treating of hyperlipidemia in diabetes (Moon and Kashyap, 2004). Since, herbal drugs are generally out of toxic effect, using traditional and complementary medicine is developing for treatment of different diseases (Kameswara et al., 2003). Several reported studies screened some plants having folk medicine reputation for antidiabetic potency (Gupta et al., 2004; Vats et al., 2002) but there was no scientific publication about the artichoke on STZ-induced diabetic rats.

Medicinal plants especially artichoke (*Cynara scolymus* L.) leaves have long been used effectively for treating a variety of diseases in the world. Artichoke is full of natural antioxidants and contains caffeoylquinic acid derivatives (cynarin and chlorogenic acids) and flavonoids such as luteolin and apigenin (Llorach et al., 2002; Wang et al., 2003). Artichoke has been reported to significantly reduce serum cholesterol in hypercholesterolemic subjects (Joy and Haber, 2007) and declined the production of reactive oxygen species (ROS), lipid peroxidation and the oxidation of low-density lipoproteins *in vitro* experiments (Zapolska-Downar et al., 2002). There are few reports on anti-diabetic activity of *C. scolymus*. Therefore, this study was an attempt to assess the effect of *C. scolymus* aqueous extract on some biochemical factors such as lipid profiles and LPO, serum glucose, HbA1c, SOD activity of RBC, and antioxidant

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capacity in STZ-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma (Sigma Chemical Co., USA). superoxide dismutase (SOD) linked immunosorbant assay (ELISA) kit purchased from Cayman Chemical Co. (U.S.A), Hb A_{1c} kit purchased from Biosystem Co. (Spain), 2-thiobarbituric acid (TBA), ferric chloride, ethylenediaminetetra acetic acid (EDTA), sodium dodecyl sulfate (SDS) obtained from Merck (Germany). All the other chemicals used were of analytical grade.

Preparation of artichoke leaf aqueous extract

The artichoke used in our study was obtained from Isfahan Agricultural Research Center (Iran). The artichoke leaf extract (ALE) was prepared by adding 1000 ml of distilled water to 100 g of leaf powder and kept at 60°C for 60 min. The aqueous extracts were filtered and the filtrate was evaporated to dryness at 50°C. The residue was stored at 0 to 4°C for subsequent experiments. The fraction was dissolved in distilled water just prior to experimentation.

Induction of diabetes

Male Wistar rats, 200 to 250 g, were used for this experiment and kept in cages under standard laboratory conditions (12 h light/12 h darkness, 21 ± 2°C). Diabetes was induced by a single intraperitoneal injection (i.p) of freshly prepared STZ (60 mg/kg body weight) in ice-cold physiological saline (Bagri et al., 2009). The animals were considered diabetic, if their blood glucose values were above 300 mg/dl on the 5^{th} day of STZ injection and accompanied by polydipsia and polyuria (Bagri et al., 2009; Baydas et al., 2003). The animals were given standard pellets diet and water *ad libitum* throughout the experimental period.

Experimental design and administration of ALE

The rats were randomly divided into 4 groups (n= 6/ group) as follows. The artichoke leaf extract (200 and 400 mg/kg) were administered orally via gavage in aqueous solution once per day.

Group I: Normal control rats, received ice-cold physiological saline (1 ml/kg, body weight).
Group II: Diabetic control rats, received STZ in single dose (60 mg/kg, body weight).
Group III: ALE treated diabetic rats, received ALE (200 mg/kg/day, p.o.) 5 days after STZ treatment for 21 days.
Group IV: ALE treated diabetic rats, received ALE (400 mg/kg/day, p.o.) 5 days after STZ treatment for 21 days.

After 21 days of the administration period, blood samples were obtained through cardiac puncture for biochemical estimations, and then the serum and plasma were immediately separated from the blood samples by centrifugation. All animal procedures were performed with regard to Iranian animal ethics society and local university rules.

Analytical procedures

Serum TC, TG and HDL-C were determined by enzymatic method (Pars Azmun kit, Iran) with JENWAY spectrophotometer (model 6105, England). LDL-C and VLDL-C were calculated with Fridewald formula (Friedewald et al., 1972). The plasma MDA level was measured as LPO by the thiobarbituric acid reactive substances (TBARS) method described by Ohkawa et al. (1979). Briefly, to 100 µl plasma or standard, 100 µl sodium dodecyl sulfate (8.1%) and 2.5 ml TBA/buffer (prepared by dissolving of 0.53% thiobarbituric acid in 20% acetic acid as adjusted to pH 3.5 with NaOH) were added. The tubes were covered with caps and incubated at 95°C for 60 min. The reaction was stopped by placing tubes on ice followed by centrifugation at 4000 rpm for 10 min. The optical density of pink color developed in the supernatant was measured against distilled water as blank at 532 nm by JENWAY spectrophotometer (model 6105, England).

The measurements were done in duplicates and the results were expressed in µmol/L. MDA standards were prepared from 1, 1, 3, 3-tetraethoxypropane. Antioxidant power of plasma was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} with ferric reducing ability of plasma (FRAP) test (Benzie and Strain, 1996). The reagents included 300 mM acetate buffer (3.1 g of sodium acetate $3H_2O$ + 16 ml of glacial acetic acid made up to 1 L with distilled H_2O, pH=3.6), 10 mM TPTZ (trypyridyl-s-triazine) in 40 mM HCl and a 20 mM $FeCl_3$. Fresh working FRAP reagent was provided by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml $FeCl_3$. Fifty five microliter of plasma was added to 1.5 ml of freshly prepared working FRAP reagent and incubated at 37°C. After 10 min, the complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593 nm. $FeSO_4$ was used as a standard of FRAP assay at a concentration range between 100 and 1000 µM. The results are expressed as µmol/L. SOD of RBC was measured by ELISA kit (Cayman Chemical Co. U.S.A) with Kayto microplate reader (model RT 21000, China), in accordance with the instructions of the manufacture. HbA_{1c} was determined by Biosystem kit (Spain), in accordance with the instructions of the manufacturer.

Statistical analysis

Data were expressed as mean ± SD. The data were analyzed by SPSS software (version 11.5). For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by LSD post hoc test for multiple comparison. Differences were considered significant at $P < 0.05$ level.

RESULTS

Effect of ALE on hyperglycemia

Figure 1 shows the levels of glucose in normal and diabetic animals at the end of the experiment. There was a significant elevation ($P < 0.001$) in serum glucose level of the STZ-induced diabetic rats (Group II) when compared with normal control rats (Group I). The administration of ALE significantly ($P < 0.001$) decreased glucose level in treated diabetic rats (Groups III and IV) as compared with diabetic control rats (Group II). Thus, the level of blood glucose returned to near normal range in treated diabetic rats (Groups III and IV).

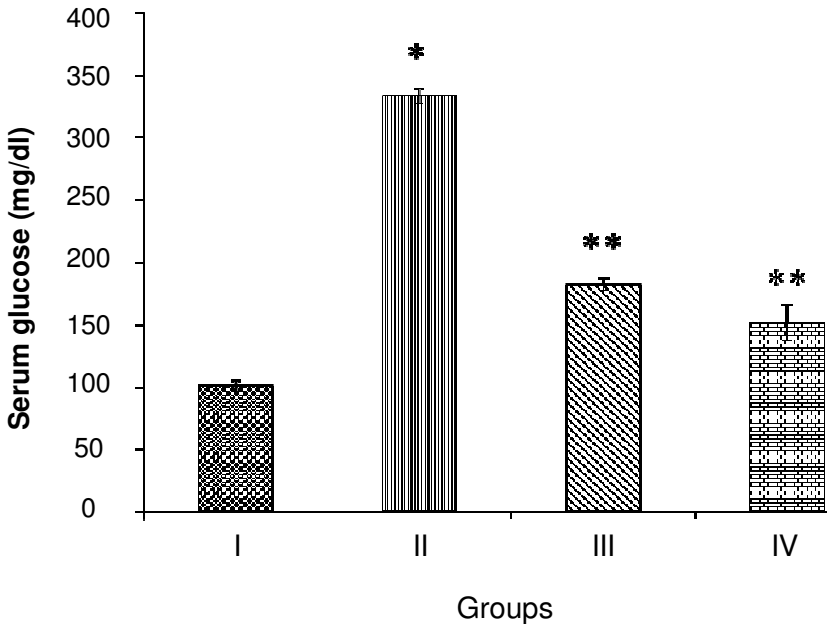

Figure 1. Effect of ALE on serum glucose in diabetic rats. (I) Normal control; (II) diabetic control; (III) ALE treated at 200 mg/kg/body; (IV) ALE treated at 400 mg/kg/body. The data are expressed as mean ± S.D. (n = 6). *P < 0.001 compared with the corresponding value for normal control animals. **P < 0.001 compared with the corresponding value for diabetic control animals.

Effect of ALE on hyperlipidemia

Table 1 shows the serum levels of TC, TG, LDL-C, VLDL-C, HDL-C and atherogenic index (TC/HDL-C, LDL/HDL-C) in normal and experimental animals in each group. In STZ-induced diabetic rats the serum levels of TC, TG, LDL-C, VLDL-C were significantly increased (P < 0.001) whereas, there was a reduction in HDL-C levels as compared to the normal control rats. Also, there was a significant (P < 0.001) reduction in TC, TG, LDL-C, VLDL-C and atherogenic index of diabetic rats treated with ALE (Groups III and IV).

Effect of ALE on SOD

SOD activity in RBC is shown in Figure 2. There was a significant decrease (about 54%, P < 0.001) in SOD activity of RBC in STZ-induced diabetic rats compared with control rats (Group I). SOD activity was significantly increased (P < 0.001) in treated diabetic rats with ALE (Groups III and IV) as compared to the diabetic control rats (Group II). There was no dose-dependent increase in SOD activity following the administration of ALE at different concentrations.

Effect of ALE on LPO

The levels of TBARS in blood plasma of the different groups of rats are shown in Figure 3. There was a significant (P < 0.001) elevation in TBARS of diabetic rats as compared with normal rats. The feeding of diabetic rats with ALE (Groups III and IV) for 21 days resulted in significant reduction (P < 0.001) in the levels of TBARS in plasma, as compared to the diabetic control rats (Group II).

Effect of ALE on plasma antioxidant power

Plasma antioxidant power significantly declined (P< 0.05) in diabetic group in comparison to normal control (Figure 4). Plasma antioxidant power significantly increased after treatment with ALE (P < 0.001) with respect to the diabetic control group. The elevation of plasma antioxidant power for ALE was not dose dependent.

DISCUSSION

In modern medicine insulin therapy is used for the management of diabetes mellitus but there are several side effects such as insulin resistance, anorexia nervosa, brain atrophy and fatty liver (Piedrola et al., 2001; Yaryura-Tobias et al., 2001). Also, chronic treatment with sulfonylurea and biguanides is associated with some side effects (Rang et al., 1991). Therefore, herbal medicine development against the non-communicable disease like

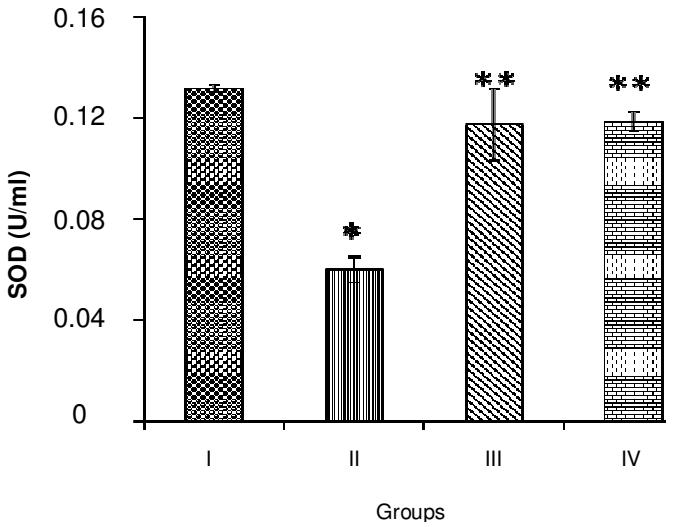

Figure 2. Effect of ALE on SOD activity in diabetic rats. (I) Normal control; (II) diabetic control; (III) ALE treated at 200 mg/kg/body; (IV) ALE treated at 400 mg/kg/body. The data are expressed as mean ± S.D. (n = 6). *P < 0.001 compared with the corresponding value for normal control animals. **P < 0.001 compared with the corresponding value for diabetic control animals.

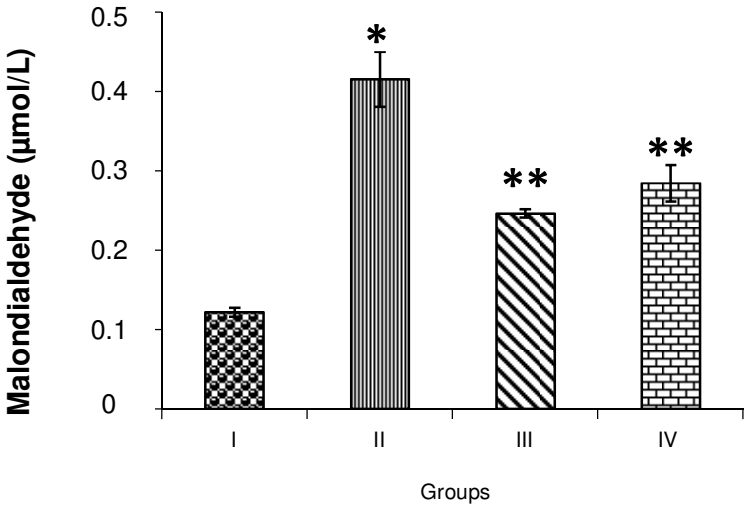

Figure 3. Effect of ALE on plasma malondialdehyde level in diabetic rats. (I) Normal control; (II) diabetic control; (III) ALE treated at 200 mg/kg/body; (IV) ALE treated at 400 mg/kg/body. The data are expressed as mean ± S.D. (n = 6). *P < 0.001 compared with the corresponding value for normal control animals. **P < 0.001 compared with the corresponding value for diabetic control animals.

diabetes is one of the trust areas of research for finding natural drugs with hypoglycemic activity. Herbal drugs have less toxicity with fewer side effects compared with synthetic drugs (Kameswara et al., 2003). In the present work, we examined the effects of ALE on serum glucose, dyslipidemia, HbA_1c, SOD activity of RBC, lipid peroxidation and antioxidant capacity in STZ-induced diabetic rats.

Our results showed that STZ-treated rats had markedly increased serum glucose, triglyceride, and total cholesterol levels. Hyperglycemia is considered as the pathogenesis of diabetes and diabetic complications such as oxidative stress and the formation of advanced glycation end products (Rolo and Palmeira, 2006).

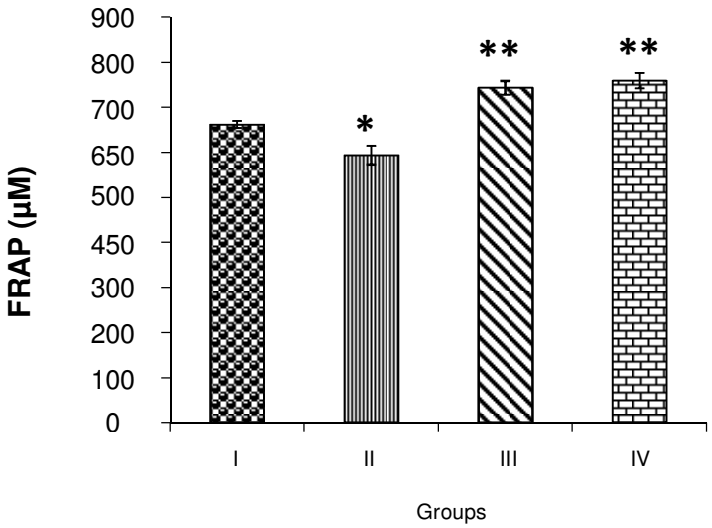

Figure 4. Effect of ALE on plasma antioxidant power in diabetic rats. (I) Normal control; (II) diabetic control; (III) ALE treated at 200 mg/kg/body; (IV) ALE treated at 400 mg/kg/body. The data are expressed as mean ± S.D. (n = 6). *P < 0.001 compared with the corresponding value for normal control animals. **P < 0.001 compared with the corresponding value for diabetic control animals.

Table 1. Effect of ALE on TC, TG, LDL-C, HDL-C, VLDL-C and atherogenic index (TC/HDL-C, LDL/HDL-C) in diabetic rats.

Parameters	Control	Diabetic control	Diabetic + ALE (200 mg/kg)	Diabetic +ALE (400 mg/kg)
TC (mg/dl)	83.27±4.41	123.38±7.99*	85.06±7.20**	77.75±10.21**
TG (mg/dl)	49.45±4.72	59.78±3.17*	47.66±5.46**	37.60±2.89**
LDL-C (mg/dl)	28.85±4.62	77.01±8.67*	41.03±4.06**	29.31±9.94**
HDL-C (mg/dl)	44.53±2.64	34.42±1.72*	36.50±1.11**	40.32±2.06**
VLDL-C (mg/dl)	9.90±0.94	11.95±0.63*	9.53±1.10**	7.50±0.57**
TC/HDL-C (units)	1.87±0.14	3.59±0.34*	2.38±0.10**	1.92±0.29**
LDL/HDL-C (units)	0.82±0.13	2.24±0.33*	1.12±0.08**	0.73±0.27**

* P < 0.001 compared with the corresponding value for control animals. ** P < 0.001 compared with the corresponding value for diabetic control animals. The data are expressed in mean ± S.D.; n = 6 in each group.

Hypertriglyceridemia and hypercholesterolemia are associated with abnormalities of lipoprotein levels in the blood, and STZ increased the levels of chylomicron, VLDL, and LDL in the blood, and decreased the HDL level (Gylling et al., 2004). Also, dyslipidemia is one of the major cardiovascular risk factors. It has been suggested that insulin deficiency in DM is associated with a variety of abnormalities in metabolic and regulatory processes, which causes the accumulation of lipids such as TC and TG in diabetic patients (Goldberg, 1981). As shown in Table 1 and Figure 1, the levels of serum glucose, total cholesterol and triglycerides in the STZ-treated rats markedly increased compared to normal control rats. However, the elevated serum glucose, triglyceride and total cholesterol levels significantly reduced by the oral administration of artichoke (200 and 400 mg/kg) in a dose-dependent manner. Moreover, LDL-C /HDL-C and TC/HDL-C ratios are markers of dyslipidemia and atherogenic ratio (Elisaf et al., 1995). This ratio has increased both in STZ-treated rats, and after the supplementation of ALE, these ratios significantly decreased and resettled towards the control level in the serum of treated diabetic rats (Groups III and IV). ALE supplementation also resulted in the significant attenuation in the level of LDL-C and HDL-C in serum, toward the control level which again strengthen the hypolipidemic effect of this extract. Other reported investigations are consistent with our results (Nazni et al., 2006).

ALE contains bioactive and flavonoid compounds such

as caffeoylquinic acids and luteolin glucosides. As it is known, cynarin is a major dicaffeoylquinic acid and chlorogenic acid is the main monocaffeoylquinic acid, whereas luteolin-7-O glucoside is the major flavonoid (Llorach et al., 2002; Wang et al., 2003). Both caffeoylquinic acids and flavonoids present in ALE are considered to be responsible for its antiatherogenic actions (Gebhardt, 1997; Llorach et al., 2002; Pittler et al., 2002; Wang et al., 2003). Also, luteolin is thought to be one of the constituents of ALE which inhibits *de novo* synthesis of cholesterol (Gebhardt, 2002). Therefore, in the current study the reduction of atherogenic parameter, triglyceride and total cholesterol levels in treated groups with ALE are due to bioactive and flavonoid compounds of ALE. These results indicate that ALE has a lipid-lowering effect on the diabetic rats.

Lipid peroxide is considered as cellular injury in humans, and is used an indicator of oxidative stress in cells and tissues. MDA, lipid peroxide derived from polyunsaturated fatty acids, is used as an indicator of oxidative stress and lipid peroxidation. In diabetes, oxidative stress influences important risk factors such as lipid and glucose metabolism. Moreover, in diabetes, high blood glucose level leads to glucose auto-oxidation and increases oxygen free radicals (Yadav et al., 1997). Diet, especially fruit and vegetables, plays a critical role as a chemopreventive agent against various diseases through their biological active substances such as antioxidants (Davi et al., 2005; van der Schouw et al., 2005). Figure 3 shows serum levels of MDA markedly increased in diabetic rats, and it was significantly reduced by oral ALE administration compared to diabetic rats. Recently, the use of the antioxidant therapy has been reported to have various medicinal properties, including the prevention and the treatment of diabetes and liver diseases (Medina and Moreno-Otero, 2005; Ros et al., 2004). ALE is known to have antioxidant potential. Many *in vitro* studies have demonstrated that the antioxidant potential of ALE is due to radical scavenging and metal ion chelating effect of its constituents such as cynarin, chlorogenic acid and flavonoids (Brown and Rice-Evans, 1998; Pérez-Garcia et al., 2000).

Also, the results in this study suggest that ALE can alleviate oxidative stress under diabetic pathological conditions through the inhibition of lipid peroxidation. Diabetes is associated with the reduction of antioxidant potential and an increase in the formation of free radicals. This situation disturbs the normal balance present in cells between radical formation and protection against them (Naziro and Butterworth, 2005). Therefore, an imbalance of oxidant/antioxidant defence systems leads to alterations in the activity of antioxidant enzymes such as SOD (Maritim et al., 2003). SOD plays a critical role in oxygen defence metabolism by converting superoxide to hydrogen peroxide (Lin et al., 2005; Vincent et al., 2004). Figures 2 and 4 show that red blood cell SOD activity and plasma antioxidant power markedly decreased in diabetic rats (Group II), whereas oral ALE administration significantly increased these parameters in treated rats (Groups III and IV) with respect to diabetic rats (Group II). The decreased natural cellular antioxidant enzyme SOD in control diabetic rats may be due to the formation of reactive oxygen species (ROS) such as superoxide (O^{-2}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) that reduce the activity of SOD as reported by other investigators (Kaleem et al., 2006; Vincent et al., 2004).

Conclusion

The findings of this work shows a number of positive effects of ALE on rats with STZ- induced disturbances in lipoprotein profile, antioxidant status, and glucose tolerance. Therefore, ALE is useful in the control of diabetes, abnormalities in lipid profiles and oxidative stress by activation of SOD activity.

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