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Full Length Research Paper

To study the effects of *Securigera securidaca (L.)* seed against alloxan-induced hyperglycemia

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In the present study, the anti-hyperglycemic and antioxidant potential of the S*ecurigera* securidaca (L.) seed suspension in alloxan-induced hyperglycemic rats was investigated. The male Wistar rats (180 to 220 g) were divided in three groups and treated orally for 4 days as follow: group I, the control, was fed 10 ml/kg/day of distilled water, groups II and III were gavaged 2 and 4 g/kg/day of *S. securidaca (L.)* seed suspension, respectively. One hour after final feeding, alloxan was injected subcutaneously (70 mg/kg B.W). At the end of the experimental period (after 24, 48 or 72 h of alloxan injection), antioxidant activity, fasting glucose and insulin levels were determined. The administration of suspension (2 g/kg/d or 4 g/kg/day) significantly reduced elevated serum glucose concentration induced by alloxan and that was prominent following 24, 48 and 72 h of alloxan injection. Serum antioxidant activity in the low (2 g/kg/d) and high (4 g/kg/d) doses of suspension treated rats was significantly higher at 24, 48 and 72 h after alloxan injection as compared to control rats. These results indicate protective effect of *S. securidaca (L.)* suspension against alloxan-induced hyperglycemia and oxidative stress in rats.

Key words: Hyperglycemia, alloxan, *Securigera securidaca*, glucose, antioxidant activity.

INTRODUCTION

Diabetes is prevalent systemic disease affecting an extent proportion of the population worldwide (Rahimi et al., 2005). It is characterized by absolute or relative deficiencies in insulin secretion and / or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential and pancreatic B-cell death induced by oxidative stress plays an important role in the pathogenesis of diabetes mellitus (Ho and Bray, 1999; Scott and King, 2004; Shahaboddin et al, 2011a).

In recent years, antioxidants derived from natural resources, mainly from medicinal plants, have been extensively used to prevent oxidative damage, various foods and herbal extracts have been used to aid diabetic glycemic control in traditional folk wisdom (Al-Rowais,2002; Yeh et al., 2003; Shahaboddin et al, 2011b).

Securigera securidaca (L.) seeds have different activities such as antiepileptic, marked chronotropic, diuretic, hypokalaemic activities and hypoglycemic effect (al-Hachim and Maki, 1969; Ali et al., 1998; Hosseinzadeh et al., 2002). Phytochemical analysis has shown that the *S. securidaca (L.)* seed extracts are rich in flavonoids and so they have antioxidant properties (Hosseinzadeh et al., 2002). However, not much scientific literature data of the anti-hyperglycemic effect and antioxidant activities of *S. securidaca (L.)* seed. In the present study, we investigated the anti- hyperglycemic and antioxidant potential of the *S. securidaca (L.)* seed suspension in alloxan-induced hyperglycemic rats.

MATERIALS AND METHODS

S. securidaca (L.) seeds were purchased from local market

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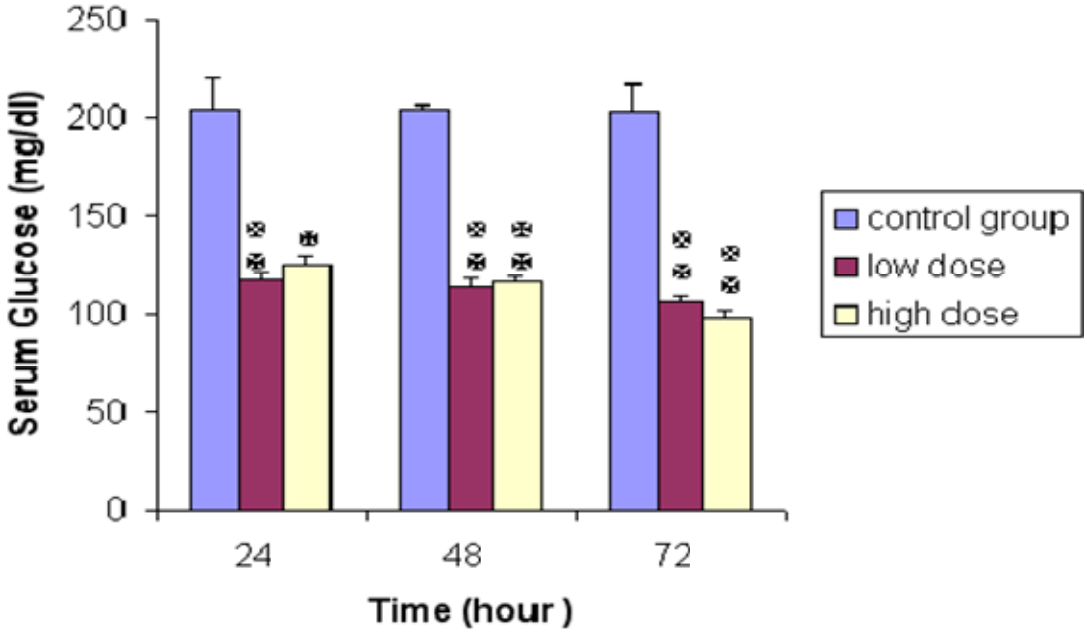

Figure 1. Changes of fasting serum glucose (mg/dl) in S. securidaca treated rats at 24, 48, and 72 h after alloxan injection. serum glucose (mg/dl) in control, low dose (2 g/kg/d S. *securidaca*) and high dose (4 g/kg/d S.*securidaca*) groups. Data are represented as mean ± S.E.M. of ten rats in each time. Significant difference: Ξ $p<0.01$, Ξ Ξ $p<0.001$ in each time compared to control group.

(Babol-Iran). The seeds were milled and spread in distilled water. Wistar rats (180 to 220 g) were purchased from Pasteur Institute (Tehran, Iran).The animals were given standard rat chow diet and water ad libitum. The research committee of the Babol University of Medical Sciences approved this study.

Animals were divided in three groups and treated orally for 4 days as follow: group I, the control, was fed 10 ml/kg/day of distilled water, groups II and III were intra-gastric gavaged (i.g.) 2 and 4 g/kg/day of *S. securidaca (L.)* seed suspension, respectively.

One hour after final feeding, freshly prepared alloxan monohydrate (Sigma) dissolved in normal saline was injected subcutaneously (s.c.) (70 mg/kg B.W) to these animals. At the end of the experimental period (after 24, 48 or 72 h of alloxan injection), the animals were fasted overnight and then sacrificed by decapitation, blood was collected and serum separated. Sera were stored at -20°C until further analysis.

Total antioxidant activity was measured by the FRAP assay (Benzie and Strain, 1996). This method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 ml of a 10 mmol/ L, TPTZ (2, 4, 6 – tripyridyl-s-triazine, sigma) solution in 40 m mol/ L HCl plus 2.5 ml of 20 mmol/ L $FeCl_3$ and 25 ml of 0.3 mol/ L acetate buffer, pH 3.6 and was prepared freshly and warmed at 37°C.

Working FRAP reagent (1.5 ml) was mixed with 50 µl serum or standard in a test tube. After exactly 10 min at 37°C, the absorbance at 593 nm was read against blank. The 1 mmol/ L Fe SO_4 was used as the standard solution. The final result was expressed as the concentration of antioxidant having a ferric reducing ability equivalent to that of 1mmol/ L Fe SO_4. A standard enzymatic method using glucose oxidase from commercially available kits (Pars Azmon) was performed for determination of glucose. Serum insulin level was determined with an enzyme-linked immunosorbant assay (ELISA) kit (Mercodia-Sweden). The data were expressed as mean ± S.E.M, analyzed by one-way ANOVA test followed by Tukey's multiple-comparison post hoc test. $P< 0.05$ was considered statistically significant.

RESULTS

Effects of *S. securidaca (L.)* seed suspension on serum glucose and insulin is shown in (Figures 1 and 2). The administration of suspension (2 g/kg/d or 4 g/kg/day) significantly reduced the increase in serum glucose concentration (mg/dl))induced by alloxan (in the low dose: 117.2±4.6 at 24 h,114±4.03 at 48 h,106.1±2.7 at 72 h, in the high dose 125.5±3.9 at 24 h,116.7±3.1 at 48 h, 98.1±3.9 at 72 h versus 204.1±15.9 at 24 h, 204±0.2 at 48 h, 203.1±13.8 at 72 h in control groups) and increased in serum insulin levels ng/ml (in the low dose: 6.6±0.2 at 24 h, 7.2±0.31 at 48 h, 6.3± 0.46 at 72 h, in the high dose: 7.7±0.34 at 24 h, 7.8±0.40 at 48 h, 7.4±.040 at 72 h versus 5.1±0.22 at 24 h, 4.4±0.35 at 48 h, 4.4±0.21 at 72 h in control groups), that was prominent following 24, 48 and 72 h of alloxan injection. Serum antioxidant activity (µM) in the low (2 g/kg/d) and high (4 g/kg/d) doses of suspension treated rats was significantly higher at 24, 48 and 72 h after alloxan injection as compared to control rats (in the low dose: 1182.5±141.02 at 24 h, 1045±19.4 at 48 h, 979.5.1±30.4 at 72 h, in the high dose 1224.2±43.4 at 24 h, 1420±55.4 at 48 h, 1074±29.2 at 72 h versus 682.2±45.5 at 24 h, 699.4±35.5 at 48 h, 716±27.6 at 72 h in control groups) (Figure 3). The decrease of serum glucose level was more prominent at

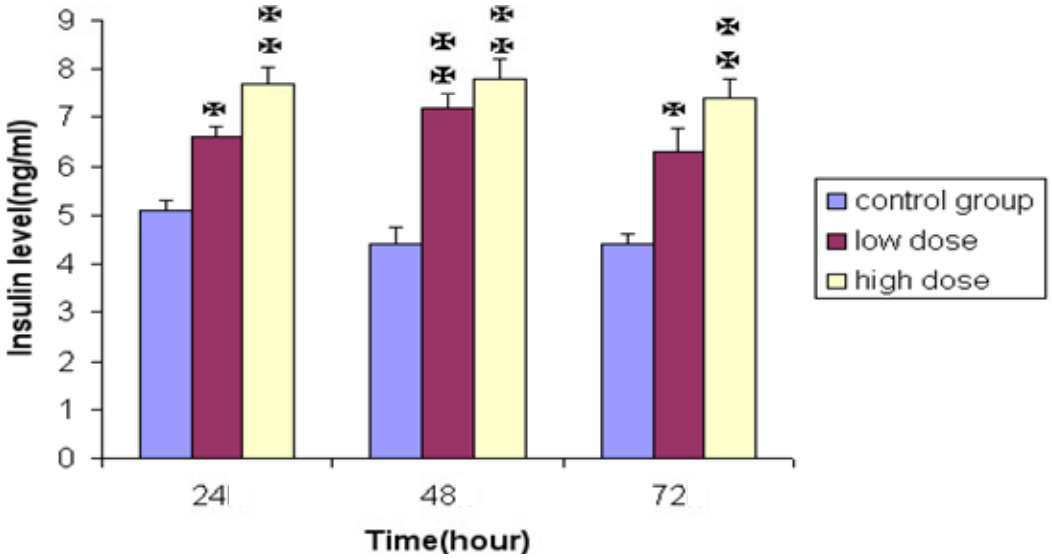

Figure 2. Changes of serum insulin levels (ng/ml) in *S. securidaca* treated rats at 24, 48, and 72 h after alloxan injection. Changes of serum insulin levels (ng/ml) with pretreatment of S.*securidaca* . Data are represented as mean ± S.E.M. of ten rats in each time. Significant difference: Ξ p<0.01, Ξ Ξ p<0.001 in each time compared to control group.

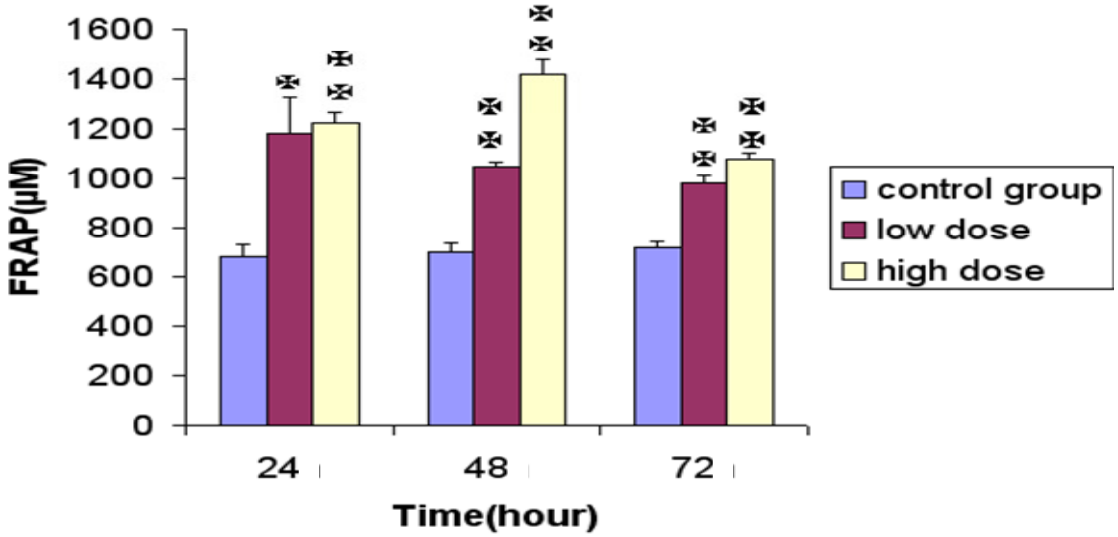

Figure 3. Changes of FRAP assay (µM) in *S.securidaca* treated rats at 24, 48, and 72 h after alloxan injection. Changes FRAP assay (µM) in S. *securidaca* treated rats. Data are represented as mean ± S.E.M. of ten rats in each time. Significant difference: Ξ p<0.01, Ξ Ξ p<0.001 in each time compared to control group.

72 h after alloxan injection in the high dose group. The antioxidant level was more prominent increased at 48 h after receiving alloxan in the high dose group.

DISCUSSION

This study demonstrated that *S. securidaca (L.)* seed suspensions reduced the blood glucose and increase in the antioxidant power of alloxanized rats.

A previous report has demonstrated that aqueous and alcoholic extracts of *S. securidaca* reduced the plasma glucose in alloxan-diabetic mice. The mechanism of hypoglycemic action of the seed is different from sulfonylurea agents (Hosseinzadeh et al., 2002). The chloroform extract of *S. securidaca* seed after oral

administration can cause hypoglycemic effect through either inducing insulin-like effects or increasing insulin release (Zahedi-asl et al., 2005).

The role of free radicals on B-cell damage is completely documented (Dennery, 2006). On the other hand antioxidant constituents can alleviate the oxidative damage on the cells. Some investigations have established that treatment by herbs containing antioxidants such as flavonoids could significantly protect the B-cell against free radicals (Gurib-Fakim, 2006; Havsteen, 2002; Rahimi et al., 2005).

Phytochemical results have demonstrated that the *S. securidaca* seed extracts are rich in flavonoid (Hosseinzadeh et al., 2002). One of the more prominent properties of the flavonoids are their excellent radical scavenging ability (Havsteen, 2002). It is possible that the antihyperglycemic effect and increase in antioxidant power are related to this component.

In this study, hypoglycemic and antioxidant activities were long-term compared with control. This profile suggests that the protection induced by seed could be permanent. However, it is necessary to confirm this hypothesis by additional investigations.

Conclusions

The present study showed that *S. securidaca (L.)* seed suspension significantly reduced blood glucose, increased serum insulin, and total antioxidant activity in the alloxan-induced hyperglycemic rats, at 24, 48 and 72 h after alloxan injection. *S. securidaca (L.)* suspension has a protective effect against alloxan-induced hyperglycemia and oxidative stress in rats.

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