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Full Length Research Paper

Antihyperglycemic and antioxidant activity of *Viscum album* extract

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The antihyperglycemic and antioxidant effects of water extract of local *Viscum album in* alloxanized-rats were investigated. This study performed during 2009 in Babol University of medical sciences (Mazandaran Province, Iran). *V. album* leaves growing on oaks collected and extracted with hot water. The 90 animals that were used in this investigation were male Wistar rats. 60 rats were gavaged with 500 and 1000 mg/kg/day of *V. album* extract. One hour after final feeding, freshly prepared alloxan injected subcutaneously. Then blood glucose level was measured according to glucose oxidase method. The antioxidant activity of serum was determined by FRAP assay and serum insulin level was measured with ELISA. The administration of *V. album* extract (500 and 1000 mg/kg/day) significantly reduced the increase in serum glucose concentration in alloxan-hyperglycemic rats. Both the extracts from *V. album* enhance the serum insulin level as compared to control rats. Serum antioxidant activity in low dose of extract was significantly higher at 48 and 72 h after alloxan injection. Serum antioxidant activity in the high dose was significantly higher at 24, 48 and 72 h. This study demonstrated that *V. album* extract reduced the blood glucose and increases the antioxidant power of alloxanized-rats. Much more work is clearly needed before phytotherapy for diabetes can be advanced to the clinic.

Key words: *Viscum album*, antihyperglycemic, antioxidant, alloxan.

INTRODUCTION

Mistletoe (*Viscum album*) a common evergreen semi parasite of woody plants, has been used for various medicinal purposes from ancient times (Park et al., 1998). *V. album* has been reported to possess a number of therapeutic applications in folk medicines in curing or managing of a wide range of diseases such as diabetes mellitus, chronic cramps, stroke, stomach problems, heart palpitations, to lower blood pressure, difficulties in breathing and hot flushing in menopause (Ohiri, 2003). *V. album* leaves was reported to exert a beneficial effect to alleviate the symptoms of diabetes in local medicines (Gray and Flatt, 1999; Orhan et al., 2005). Presence of various glycosides, alkaloids, viscotoxins, phenylpropanoids, tannins, lignins and sugars has been reported in the mistletoe collected from different host plants (Orhan et al., 2005). Diabetes is prevalent systemic disease affecting a significant proportion of the population worldwide (Bonow and Gheorghiade, 2004).

Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidants (Rahimi et al., 2005). *V. album* has radical scavenging activity and protective effect against hydroperoxide generation (Onay-Ucar et al., 2006). There exist little scientific literature on the antihyperglycemic effect and antioxidant capacity of *V. album* extract in the diabetic patients. The present study therefore, is aimed at investigating the scientific basis to the *V. album* leaves extract in preventing the destruction

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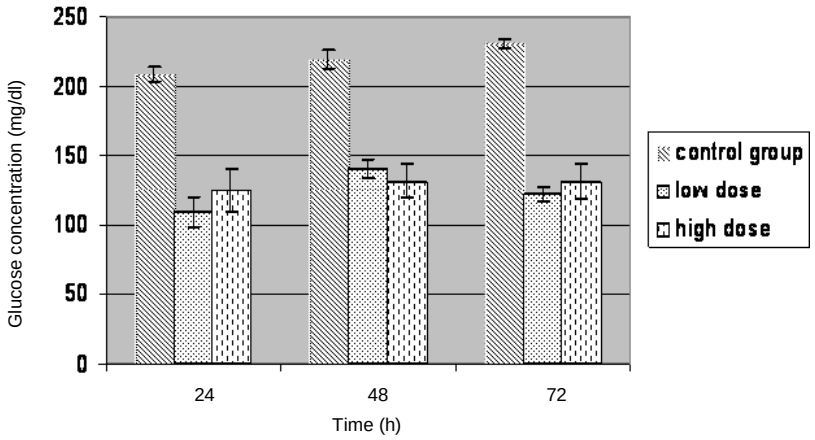

Figure 1. Changes of fasting serum glucose (mg/dl) in control, low dose (500 mg/kg/d *V. album*) and high dose (1000 mg/kg/d *V. album*) treated rats at 24, 48 and 72 h after alloxan injection .Values are represented as mean ± S.E.M. of ten rats in each time.

of pancreatic beta cells.

MATERIALS AND METHODS

This study performed during 2009 in Babol University of medical sciences (Mazandaran Province, Iran). Animals were used in this investigation were male Wistar rats (180 to 220 g), obtained 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. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care.

Extract preparation

V. album leaves growing on oaks collected from Babol (north of Iran) in December. The plant material was identified and authenticated by the Babol department of Agricultural Sciences and Natural Resources. The fresh leaves were chopped into small pieces, dried under shade and then powdered by blender for extraction. A portion of the material (500 g) was extracted with hot water (80°C) by stirring for 4 h and evaporated to dryness under reduced pressure.

Study design

The 90 rat were divided in three groups and treated orally 4 days as follow: group I, the control, was fed 10 ml/kg/day of distilled water; groups II and III were gavaged with 500 and 1000 mg/kg/day of *V. album* extract, respectively. One hour after final feeding, freshly prepared alloxan (Sigma) dissolved in normal saline was injected subcutaneously (70 mg/kg body weight) to this animals. At the end of the experimental period (after 24, 48 or 72 h of alloxan injection), the animals were fasted overnight and then scarified by decapitation, blood was collected and serum separated. Sera were stored at -20°C until further analysis.

FRAP assay

Total antioxidant capacity was measured by the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored from 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 mmol/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) is 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 $FeSO_4$ 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 1 mmol/L $FeSO_4$.

Glucose and insulin measurement

A standard enzymatic method using glucose oxidase from comercially 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).

Data analysis

All data are presented as mean ± S.E.M. comparison between groups was analyzed by one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

Glucose and insulin

Effects of *V. album* leave extract on serum glucose and insulin shown in Figure 1 and 2. The administration of extract (500 or 1000 mg/kg/day) significantly reduced the increase in serum glucose concentration induced by alloxan, was prominent 24, 48 and 72 h of alloxan injection. As shown in Figure 2, both the extracts from *V. album* significantly enhance the serum insulin level as

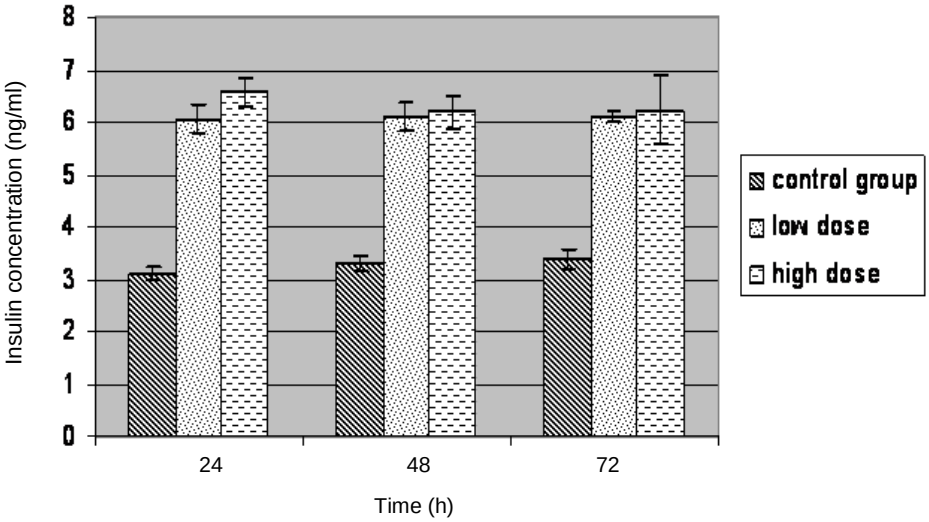

Figure 2. Changes of serum insulin level (ng/ml) in control, low dose (500 mg/kg/d *V. album*) and high dose (1000 mg/kg/d *V. album*) treated rats at 24, 48 and 72 h after alloxan injection .Values are represented as mean ± S.E.M. of ten rats in each time.

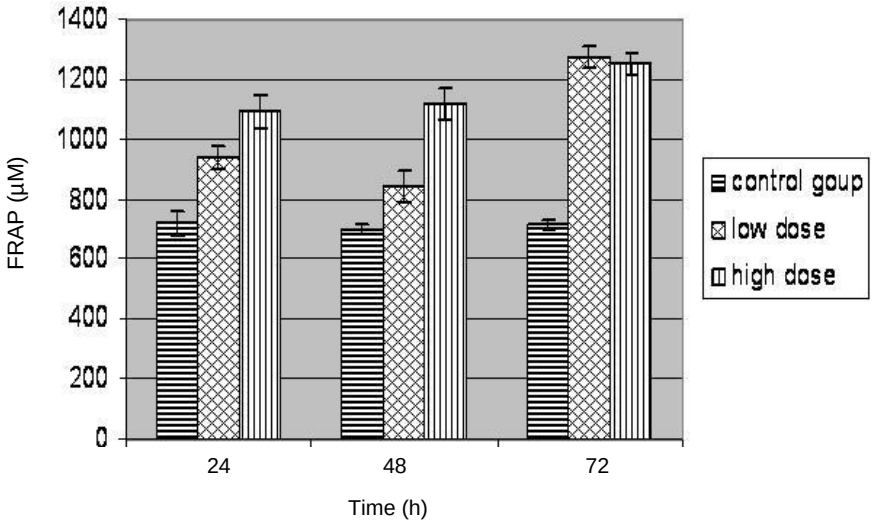

Figure 3. Changes FRAP assay (µM) in control, low dose(500 mg/kg/d *V. album*) and high dose (1000 mg/kg/d *V. album*) treated rats at 24, 48 and 72 h after alloxan injection .Values are represented as mean ± S.E.M. of rats in each time.

compared to control rats.

Antioxidant capacity

Serum antioxidant activity in the low dose (500 mg/kg/day) of extract treated rats was significantly higher at 48 and 72 h after alloxan injection as compared to control rats (Figure 3). Serum antioxidant capacity in the high dose (1000 mg/kg/day) was significantly higher at 24, 48 and 72 h (Figure 3).

DISCUSSION

The present study showed that *V. album* extract significantly reduced blood glucose and increased serum insulin and total antioxidant activity in the alloxan-induced hyperglycemic rats, at 24, 48 and 72 h after alloxan injection.

Some researches demonstrated that V*. album* have

water soluble, heat resistance and insulin releasing components (Gray and Flatt, 1999). Some studies reported chronic administration of mistletoe ameliorated symptoms of polydipsia, hyperglycemia and body weight loss in severely hyperglycemic streptozocin-diabetic mice. These extracts are likely to act at an early stage of the insulin-secretary pathway before Ca^{2+} influx (Swanston-Flatt et al., 1989; Gray and Flatt, 1999). Some observations indicated that the insulin-releasing activity of mistletoe extract is not mediated by lectins. Leaves of *V. album* have been reported to contain ß-amyrin, tyramin, quercitin, syringin and flavoyedorinin A and B (Gray and Flatt, 1999) . However, the presence of these compounds in *V. album* extract and the possible involvement of these or other natural products in the insulin-releasing action remains to be established. Chemical characterization of the natural products responsible for the stimulation of insulin secretion merit further studies.

Some investigations indicated that V. album has radical scavenging activity and protective effect against hydroperoxide generation. They have shown that the antioxidant capacity of the extract could differ depending on the harvesting time of the plant as well as nature of the host tree. It seems that V. album plant living on different host trees is endowed with different antioxidant activity (Onay-Ucar et al., 2006).

Some studies reported that antidiabetic and antioxidant properties of the European mistletoe subspecies were investigated in normal, glucose loaded and STZ-diabetic rats and found to be highly dependent on the host plant species (Orhan et al., 2005) . Total antioxidant capacity by FRAP assay and serum insulin level determined in our study, that was not determined by Orhan, we used *V. album* leaves instead of whole plant materials and demonstrated protective effect against hyperglycemia. We harvest in December, a little different results exist because the biological activity of *V. album* extract could be influenced by the geographical origin, cultivar and harvest or storage time.

Mistletoe extracts were applied to a large number of cancer patients because of their modulatory effect on the natural immune system (Hajto et al., 2005). Cytotoxicity ribosome-inactivating proteins (RIP) activity, induction of apoptosis, selective binding, release of cytokines and stimulation of natural killer (NK) function, found in the *V. album* extract (Hajto et al., 2005). In mice shown that immunostimulation may be a more viable means of keeping the prediabetic patient non-diabetic than immunosuppression (Panchnadikar and Bhonde, 2003). Further experimental research is required to establish the role of immunomodulatory effect of *V. album* extract in the prevention and treatment of diabetic mellitus, however toxicity induced by *V. album* extract will be regarded.

Some studies suggest that part of the antihyperglycemic actions of *V. album* extract may be decreasing glucose absorption *in vivo* (Gallagher et al., 2003). Further studies with the most effective plants demonstrated that the antihyperglycemic activities were in part explained by the ability of water soluble plant components to increase glucose transport and metabolism in muscle and/or to simulate insulin secretion (Gray and Flatt, 1999). Decreasing gastrointestinal glucose convection and diffusion now thought to be the reason why viscous plant components have antihyperglycemic properties (Gallagher et al., 2003). Published research suggests that there is a direct relationship between a plant's ability to inhibit glucose absorption and the viscosity of the plants constituent soluble polysaccharides (Edwards et al., 1987). In our study, *V. album* extract was viscous but viscosity of extract was not determined in the present study.

Conclusion

This study demonstrated *V. album* extract reduced the blood glucose and increase the antioxidant power of alloxanized rats. Many question related to antihyperglycemic and antioxidant effect of *V. album* extract remain unanswered. Much more work is clearly needed before phytotherapy for diabetes can be advanced to clinic.

ACKNOWLEDGEMENTS

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