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Antihyperglycemic Effect of Asafoetida (*Ferula assafoetida* Oleo-Gum-Resin) in Streptozotocin-induced Diabetic Rats

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Abstract: Asafoetida, an oleo-gum-resin obtained from the roots of *Ferula assafoetida,* is used in traditional medicine to treat various diseases including, asthma, epilepsy, gastrointestinal disorders and influenza. The aim of the current study was to evaluate the hypoglycemic activity of the asafoetida extract in streptozotocin-induced diabetic rats. Male Wistar rats were randomly divided into 5 groups, including: control, diabetic and diabetics treated with the asafoetida extract at doses of 50, 100 and 300 mg/kg. The animals were rendered diabetic by a single intraperitoneal injection of 60 mg/kg streptozotocin. Diabetic rats received the asafoetida extract daily in drinking water for 4 weeks. The blood glucose and lipids were spectrophotometrically measured in all groups at weeks 0 (before diabetes induction), 2 and 4. Diabetic rats showed an elevated serum glucose level over those of control rats at weeks 2 and 4 ($P<0.05$) and treatment of diabetic rats with the asafoetida extract at dose of 50 mg/kg significantly lowered the serum glucose concentration in comparison to diabetic rats. Regarding serum lipids, diabetes induction for 4 weeks did not change the triglyceride, total cholesterol and HDL-cholesterol concentrations in diabetic rats compared to controls. In conclusion, the asafoetida extract administration at dose of 50 mg/kg for 4 weeks shown the hypoglycemic activity in streptozotocin-diabetic rats during 2nd week and at the end of 4th week of treatment period. This effect can be explained at least in part by the presence of the phenolic acids (ferulic acid) and tannins in the extract.

Key words: Asafoetida · Streptozotocin · Diabetes · Hyperglycemia · Hyperlipidemia · Rat

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting approximately 5% of the population [1]. Chronic hyperglycemia in diabetes is associated with long term damages, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves and cardiovascular system [2]. Besides hyperglycemia, several other factors such as dyslipidemia or hyperlipidemia are also involved in the development of cardiovascular complications in diabetes which are the major causes of morbidity and mortality [3,4]. Currently available therapy for diabetes include insulin and various oral anti-diabetic agents such as sulfonylureas, metformin, thiazolidinediones and α-glucosidase inhibitors. Each of the above oral agents, along with its therapeutic effects, suffers from a number of serious adverse effects [5,6]. This concern has led to an increase demand for natural products with antidiabetic activity having fewer side effects, easily access and low costs [7-12].

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Asafoetida is an oleo-gum-resin obtained from the extract of the roots of the *Ferula assafoetida*. The oleo-gum-resin asafoetida is called "Anghouzeh", "Khorakoma" and "Anguzakoma" in Iran. The plant, which belongs to the Apiaceae family, is an herbaceous perennial with an unpleasant sulfurous odor that grows to about 2m in height [13]. Asafoetida consists of three main fractions, including resin (40-64%), gum (25%) and essential oil (10-17%) [14]. The resin fraction contains ferulic acid and its esters, coumarins, sesquiterpene coumarins and other terpenoids. The gum includes glucose, galactose, l-arabinose, rhamnose, glucuronic acid, polysaccharides and glycoproteins and the volatile fraction contains sulfur-containing compounds, monoterpenes and other volatile terpenoids [13].

Asafoetida has been used as a spice and a folk phytomedicine for centuries. It is traditionally used for the treatment of different diseases, such as asthma, epilepsy, stomach ache, flatulence, intestinal parasites, weak digestion and influenza [14-17]. Recent pharmacological and biological studies have also shown several activities, such as antioxidant [18], antiviral [17], antifungal [19-21], cancer chemopreventive [22,23], anti-diabetic [24], antispasmodic [25] hypotensive [25] and molluscicide [26] from this oleo-gum-resin.

The present study was undertaken to further study the anti-diabetic effect of asafoetida in a streptozotocin-induced diabetic model.

MATERIALS AND METHODS

Animals: Male Wistar rats, weighing 280-320g were housed in an air-conditioned colony room at 23 ± 2°C on a standard pellet diet and tap water at libitum. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the study was approved by Mashhad University of Medical Sciences.

Preparation of the Extract: The oleo-gum-resin asafoetida was obtained from a local store in Mashhad and verified in Pharmacy Faculty (Mashhad, Iran). The oleo-gum resin asafoetida (10g) was powdered, dissolved in 100 ml distilled water and then filtered through filter paper. The volume of the filtered solution was increased to 100 ml with distilled water so that 1 ml of the solution was equivalent to 10 mg of starting material. The freshly prepared extracts were used in the experiments.

Experimental Protocol: The overnight fasted rats were rendered diabetic by a single intraperitoneal injection of 60 mg/kg STZ (Enzo Life Sciences, USA) freshly dissolved in cold distilled water. After 72h of the STZ injection, serum glucose concentrations was measured using a glucometer (Glucocard, Japan). Only those animals with serum glucose higher than 250 mg/dl were selected as diabetics for the following experiments. The day on which hyperglycemia had been confirmed was designated as day 0. Diabetes was also confirmed by the presence of polyphagia, polydipsia and polyuria during the experiment. The rats were randomly allocated and similarly grouped into five groups: control (n=7), diabetic (n=8), diabetics treated with the extract of asafoetida in drinking water at doses of 50 (A50, n=8), 100 (A100, n=9), 300 mg/kg (A300, n=10). The animals received the asafoetida extracts in drinking water since day 0 for 4 weeks. Changes in body weight, food consumption and water intake were regularly recorded during the experimental period.

For blood sampling, rats were fasted overnight and blood samples were obtained from retro orbital plexus before diabetes induction (week 0) and at the end of weeks 2 and 4. Blood was allowed to clot and serum separated by centrifugation at 3500 rpm for 10 min. Serum glucose and lipid levels were spectrophotometrically measured using appropriate kits (Parsazmun, Tehran) by Convergys 100 (Germany).

Statistical Analysis: The data were expressed as mean ± S.E.M. Statistical analysis was carried out using one-way ANOVA followed by Tukey post hoc test. A statistical P value less than 0.05 was considered significant.

RESULTS

As shown in Figure 1, measurements of serum glucose indicated that before diabetes induction, there were no significant differences among animals in the experimental groups. However, untreated diabetic rats showed an elevated serum glucose level over those of control rats at weeks 2 and 4 (Figure 1, P <0.05). Treatment of diabetic rats for 2 and 4 weeks with Asafoetida extract at dose of 50 mg/kg reduced the glucose concentration compared to untreated diabetic rats (Figure 1, P <0.05) and restored it to the normal levels. Treatment of diabetic rats

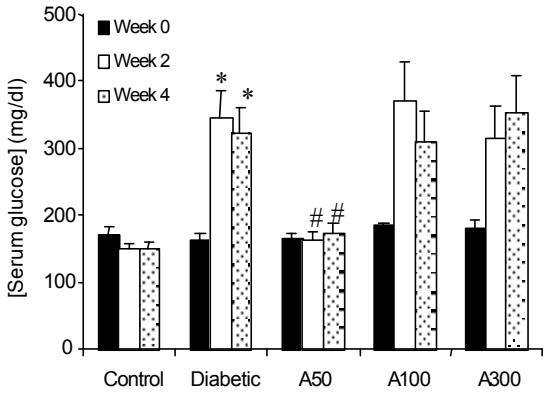

Fig. 1: Comparison of serum glucose concentrations between control (n=7), diabetic (n=8) and diabetics treated with the Asafoetida extract at doses of 50 (A50, n=8), 100 (A100, n=9) and 300 mg/kg (A300, n=10) at week 0 (before diabetes induction) and the end of weeks 2 and 4. Data were expressed as mean ± SEM, * P <0.05 *vs* to control group, # P <0.05 *vs* diabetic group.

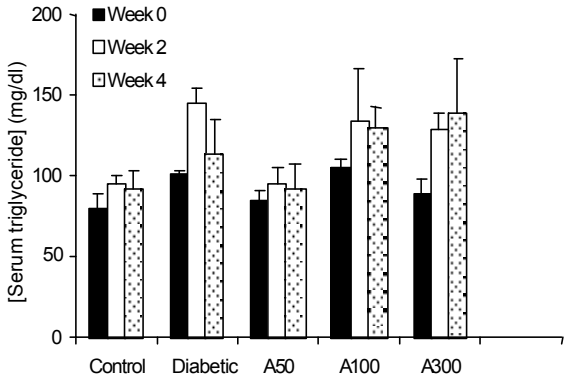

Fig. 2: Comparison of serum triglyceride concentrations between control (n=7), diabetic (n=8) and diabetics treated with the Asafoetida extract at doses of 50 (A50, n=8), 100 (A100, n=9) and 300 mg/kg (A300, n=10) at week 0 (before diabetes induction) and the end of weeks 2 and 4. Data were expressed as mean ± SEM.

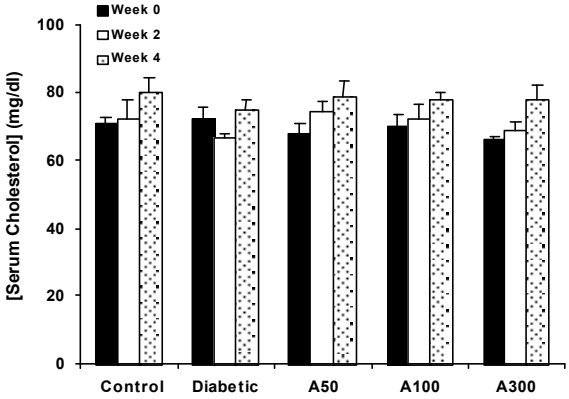

Fig. 3: Comparison of serum total cholesterol concentrations between control (n=7), diabetic (n=8) and diabetics treated with the Asafoetida extract at doses of 50 (A50, n=8), 100 (A100, n=9) and 300 mg/kg (A300, n=10) at week 0 (before diabetes induction) and the end of weeks 2 and 4. Data were expressed as mean ± SEM.

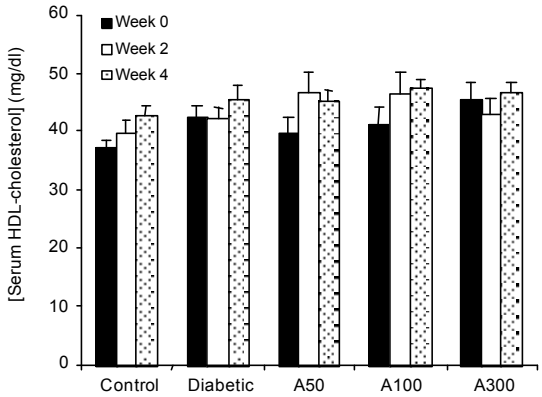

Fig. 4: Comparison of serum HDL-cholesterol concentrations between control (n=7), diabetic (n=8) and diabetics treated with the Asafoetida extract at doses of 50 (A50, n=8), 100 (A100, n=9) and 300 mg/kg (A300, n=10) at week 0 (before diabetes induction) and the end of weeks 2 and 4. Data were expressed as mean ± SEM.

with the Asafoetida extract at doses of 100 and 300 mg/kg did not significantly change the serum glucose concentration in comparison to untreated diabetic rats (Figure 1).

Regarding serum lipids, one-way ANOVA revealed that diabetes induction for 4 weeks caused no change in triglyceride concentration compared to baseline data (Figure 2) and treatment of diabetic rats with Asafoetida had also no effect on triglyceride concentration. Meanwhile, comparing Asafoetida -treated and untreated diabetic groups showed that there was no difference between the groups after 4 weeks regarding serum total cholesterol and HDL-cholesterol concentrations (Figures 3,4).

DISCUSSION

In the present study, the effects of Asafoetida on diabetes were assessed using a STZ-induced diabetic rat model. It is well known that injection of a high dose of STZ (>45 mg/kg) significantly damages the ability of pancreatic ß-cells to synthesize and secrete insulin in rats [27]. Consequently, these animals develop impaired insulin response to food ingestion and glucose loading and accordingly, impaired glucose uptake/utilization capabilities [28,29], mimicking human type 1 diabetes mellitus.

The results showed that administration of STZ to rats, as expected, resulted in hyperglycemia. STZ is taken up by pancreatic β cells via transporter GLUT2. The main cause of STZ-induced β-cell death is alkylation of DNA by the nitrosourea moiety of this compound. Because STZ is a nitric oxide donor, nitric oxide brings about the destruction of pancreatic islet cells and also STZ by itself generates reactive oxygen species, which contributes to DNA fragmentation and evokes other deleterious changes in the cells. Therefore, the synergistic action of both nitric oxide and reactive oxygen species may also contribute to DNA fragmentation and other deleterious changes [27,30].

The present study also indicated that the extract of Asafoetida at dose of 50 mg/kg shown the hypoglycemic effect in STZ-diabetic rats during the 2 and 4-week treatment periods. However, the higher doses; 100 and 300 mg/kg had no effect on the fasting blood glucose level in diabetic rats. Jain and co-workers have also reported that the administration of Asafoetida at two doses of 100 and 200 mg/kg could not decrease the fasting blood glucose levels, rather the blood glucose was significantly increased at 3 weeks from baseline in STZ-induced diabetic rats [31]. This finding is in favour of the present results with doses of 100 and 300 mg/kg of Asafoetida extract in our experiment. However, several other studies have also investigated the effect of *Ferula assafoetida* in alloxan diabetic model. Abu-Zaitun *et al.* (2010) have found that the injection of Asafoetida extract at a dose of 0.2 g/kg for 14 days had the hypoglycemic and hyperinsulinemic effects on alloxan-diabetic rats [24]. Moreover, Helal *et al.* (2005) have also reported the hypoglycemic and hyperinsulinemic effects of Ferula assafoetida (100 mg/kg orally for 1 month) in alloxan-inducd diabetes [32].

The capacity of Asafoetida extract to decrease the elevated blood glucose to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The possible mechanism by which Asafoetida extract exerts its hypoglycemic action in diabetic rats may be due to potentiating the insulin release since the percentage fall in blood glucose levels was very significant (P <0.05) at 50 mg/kg in our experiment. This is confirmed by several studies which have reported the hyperinsulinemic as well as protective effect of Asafoetida on pancreatic beta cells in diabetic rats [24,32]. It seems that asafoetida at dose 50 mg/kg may strongly increase the insulin release which leads to the decreased level of glucose. It is likely that the higher doses could not produce the expected higher hypoglycemic effect due to the presence of some other substances in the extract, which interfere with the hypoglycemic effect.

Phytochemical analysis of Asafoetida have revealed that the major chemical constituents of the Asafoetida were polyphenols like ferulic acid and tannins [13, 30, 33, 34], that could account for the observed hypoglycemic effects of the plant extract. It has been reported that the blood glucose level in streptozotocin induced diabetic animals is reduced by ferulic acid. Administration of ferulic acid at a dose of 0.01% and 0.1% of basal diet showed it can suppress the blood glucose levels in streptozotocin induced diabetic mice. In KK-Ay mice 0.05% of ferulic acid suppressed the blood glucose level effectively [35]. Ferulic acid exerts its hypoglycemic effect by acting at different levels. First, ferulic acid lowered blood glucose in a model of db/db mice followed by a significantly increase in plasma insulin level [36]. Ferulic acid, which has been shown to have antioxidant properties [37,38], helps to neutralize the free radicals produced by streptozotocin in the pancreas and thereby decrease the toxicity of streptozotocin. This decreased oxidative stress/toxicity on the pancreas may help the beta cells to proliferate and secrete more insulin, which may have been reduced due to streptozotocin treatment. This increased insulin secretion can cause increased utilization of glucose by the extra hepatic tissues and thereby decrease the blood glucose level [39].

Second, it has been shown that polyphenol inhibited the activity of α-glucosidase which converts carbohydrates into monosaccharides which can be absorbed through the intestine and results in a high glucose level in diabetic subject [36]. Third, ferulic acid also increased the activity of glucokinase, a key enzyme in the regulation of blood glucose levels because it facilitates the phosphorylation of glucose to glucose-6-phosphate, the first step in both glycogen synthesis and glycolysis in the liver [36].

However, another major constituents in the asafoetida extract are tannins. It has been shown that the tannin-containing drugs have demonstrated antidiabetic activity [40,41]. Therefore, on the basis of the above evidences it is possible that the presence of polyphenols like ferulic acid and tannins are responsible for the observed antidiabetic activity.

CONCLUSION

This study showed that the aqueous extract of Asafoetida at a dose of 50 mg/kg shown the hypoglycemic activity in STZ-diabetic rats. This effect can be explained at least in part by the presence of the phenolic acids (ferulic acid) and tannins in the extract. Further investigations are needed to isolate the individual compounds in the extract in order to elucidate the mechanism of hypoglycemic effects of the asafoetida extract observed in this study.

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