Hypoglycemic Effect of Ethanolic Extract of *Carum carvi* L. Seeds in Normal and Streptozotocin-induced Diabetic Rats

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Abstract

Background: Herbal medicine has been used for many years by different cultures around the world for the treatment of diabetes.

Objective: The hypoglycemic effect of caraway ethanolic extract (*Carum carvi* L.) was investigated in normal and streptozotocin-induced diabetic rats.

Methods: Intraperitoneal administration of caraway ethanolic extract seeds (0.1, 0.2, 0.4 and 0.6 g/kg body wt.) on the level of serum glucose and insulin in normal and streptozotocin-induced diabetic rats were evaluated. Before administration and 1, 3 and 5 h after administrations of the ethanolic extract, blood samples were drawn from the retro-orbital sinus. Serum glucose and insulin levels were determined.

Results: The results showed that the caraway ethanolic extract seeds at doses 0.2, 0.4 and 0.6 g/kg body wt. significantly decreased serum glucose and insulin in diabetic rats in 3 and 5 h but not in healthy rats.

Conclusions: It could be proofed that the traditional use of caraway as a hypoglycemic agent is justified and that extract from this plant show a dose-dependent hypoglycemic activity.

Keywords: Caraway, C*arum carvi* L., Streptozotocin, Diabetes, Rat

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Introduction

Diabetes is one of the most challenging diseases facing health care professionals today. Its increasing prevalence puts a large burden on society and the public health sector [1]. Type 1 diabetes is characterized by an absolute deficiency of insulin secretion, associated with auto-immune destruction of pancreatic β-cells, and this disease is more likely to occur in relatives of an affected person [2]. Type 2 diabetes, which accounts for more than 90% of cases, is caused by a combination of resistance to insulin action and impaired insulin secretion [3]. Plants have been reported as an exemplary source of drugs, and many of the currently available drugs have been derived directly or indirectly from them. In recent years, there has been renewed interest in plant medicine [4-6] for the treatment against different diseases as herbal drugs are generally out of toxic effect [7,8] reported from research work conducted on experimental model animal. Although in human, whether there is any toxic effect are not investigated. It is reported that about 800 plants may possess anti-diabetic potential [9]. Isolated studies screened various plants having "folk medicine reputation" by biochemical test for this antidiabetogenic effect [10].

The dried ripe fruits of *Carum carvi* L. Apiaceae (Umbelliferae) (caraway) are used in folk medicine as a carminative, found to be effective against spasmodic gastrointestinal complaints, flatulence, irritable stomach, indigestion, lack of appetite, and dyspepsia in adults [11-13], and in relieving flatulent colic of infants [14]. In Moroccan traditional medicine, an aqueous extract of caraway is used as an aperitif, tranquilizer, carminative, diuretic, emenagogue, galactagogue, spasmolytic, gastric stimulant and as an aphrodisiac [15]. The plant extract and the volatile oils from *Carum carvi* have also been used as an antiulcerogenic agent [16]. Furthermore, experimental studies have shown its antitumor [17], antiproliferative [18], antihyperglycemic [19], and antimicrobial [20] activities. So, the purpose of this research was to experimentally assess the hypoglycemic effect of caraway alcoholic extract used in normal and streptozotocin-induced diabetic rats.

Materials and Methods

Plant material

Caraway seeds purchased from a retail food store (Tehran, Iran) in June 2009. The seeds were ground with a blender, and the powder was kept in nylon bags in a deep freezer (−20°C) until the time of experiments. Ground seeds (about 60 g) were submitted to extraction with 300 ml ethanol (80%) in a Soxhlet apparatus for 72 h. After extraction, the solvent was filtered and then evaporated under reduced pressure by Rotavapor. The obtained dried caraway alcoholic extract was stored at −20°C until usage.

Animals

Male Wistar rats initially weighing 200 to 250 g were used. The animals were housed in groups of 5 per cage with free access to standard laboratory chow (35% carbohydrates, 25% proteins, 7% lipids, and 3% vitamins) and tap water. The diet was purchased from Pars-Dam food service, Tehran, Iran. The animal room was maintained at 22 ± 2°C with timed lighting on from 7 AM to 19 PM and relative air humidity of 40 to 60%.

Preparation of diabetic rat

The animals were injected with streptozotocin (70 mg/kg, i.p.). Five days after injection, the rats with fasting blood glucose

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higher than 300 mg/dl were used for the experiments. The food was removed 12 h before blood sampling. Six rats were used in each experiment. Each animal was used once only in all of experiments. The food was removed from cages 12 h before testing.

Experimental design

In the present experiment, 60 rats (30 diabetic, 30 normal rats) were used. The rats were divided into ten groups. Six rats were used in each group.

Group 1: Normal control rats were administrated saline.

Groups 2-5: Normal rats were administrated caraway ethanolic extract (0.1, 0.2, 0.4 and 0.6 g/kg body wt.) intraperitoneally.

Group 6: Diabetic control rats were administrated saline.

Groups 7 - 10: Diabetic rats were administrated caraway ethanolic extract (0.1, 0.2, 0.4 and 0.6 g/kg body wt.) intraperitoneally.

Blood sampling and Biochemical assays

Before administration and 1, 3 and 5 h after administrations of the ethanolic extract, rats were anesthetized with sodium pentothal (intraperitoneally), and 1 ml of blood was withdrawn through the retro-orbital plexus using a glass capillary (Micro Hematocrit Capillaries, Mucaps) and collected in polystyrene tubes without the anticoagulant [21]. Serum was separated by centrifugation at 3000 rpm for 10 min. Samples were stored at −20 °C until assayed. Blood glucose levels were determined by the glucose oxidase method [22]. Plasma insulin was determined by using a rat insulin radioimmunoassay kit (DiaSorin, Saluggia, Italy), in a gamma counter (Peckard, USA) based on the method of Ram et al. [23]. Serum glucose and insulin levels were considered as control values before administration.

Statistical analysis

All the data were expressed as mean ± S.E.M. Statistical analysis was carried out using one-way ANOVA followed by Tukey post hoc test. The criterion for statistical significance was $p<0.05$.

Results

There was a significant elevation in serum glucose while the serum insulin level significantly decreased in the diabetic rats. Intraperitoneal administration of caraway ethanolic extract seeds produces a significant decrease in serum glucose level only in streptozotocin-induced diabetic rats (Fig. 1) but not in healthy fasted rats (Fig. 2). Intraperitoneal administration of caraway ethanolic extract seeds significantly increased serum insulin levels in diabetic (Fig. 3) but not in healthy rats (Fig. 4).

Discussion

The results demonstrated that the ethanolic extract of caraway seeds exerted a significant and potent anti-hyperglycemic activity in STZ-diabetic rats. This strong effect was first demonstrated in this study realized in diabetic rats, an experimental model of type 1 diabetes mellitus [24]. It is now well established that STZ selectively destroys the pancreatic cells and produces hyperglycemia [25], which is evidenced by the decreased level of plasma insulin. STZ is commonly used in chemically induced diabetic animal model. The timing of STZ injection is important and will affect the type of diabetes that subsequently develops. If STZ is injected to adult animals (i.e. 3 months or older), type 1 diabetes results. However, if

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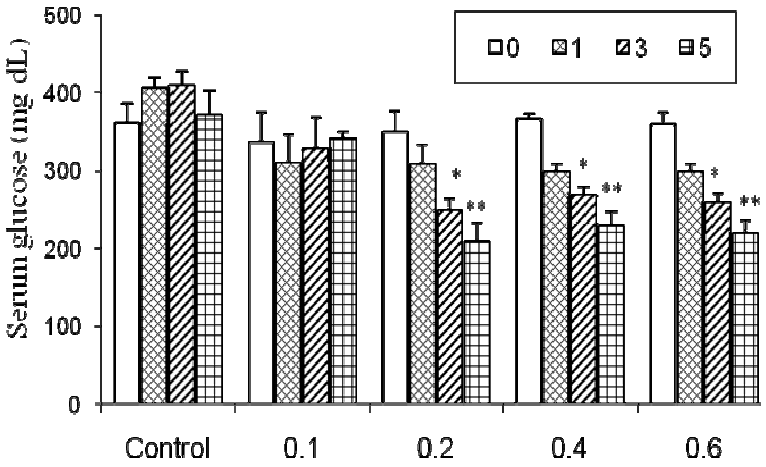

Fig. 1. Effect of intraperitoneally administration of caraway ethanolic extract seeds at doses of 0.1, 0.2, 0.4 and 0.6 g/kg body weight on serum glucose in streptozotocin-induced diabetic fasted rats for 1, 3 and 5 h after administration. Each point represents mean ± SEM for six rats. Serum glucose levels at zero time or before administrations were considered as control values. *p< 0.05, **p< 0.01 different from zero time. Control group was administered with saline as a vehicle

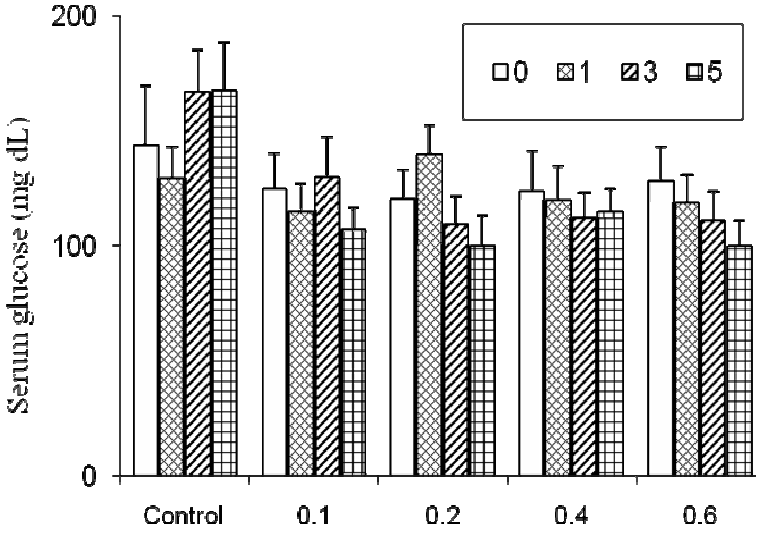

Fig. 2- Effect of intraperitoneally administration of caraway ethanolic extract seeds at doses of 0.1, 0.2, 0.4 and 0.6 g/kg body weight on serum glucose in healthy fasted rats for 1, 3 and 5 h after administration. Each point represents mean ± SEM for six rats. Serum glucose levels at zero time or before administrations were considered as control values. Control group was administered with saline as a vehicle.

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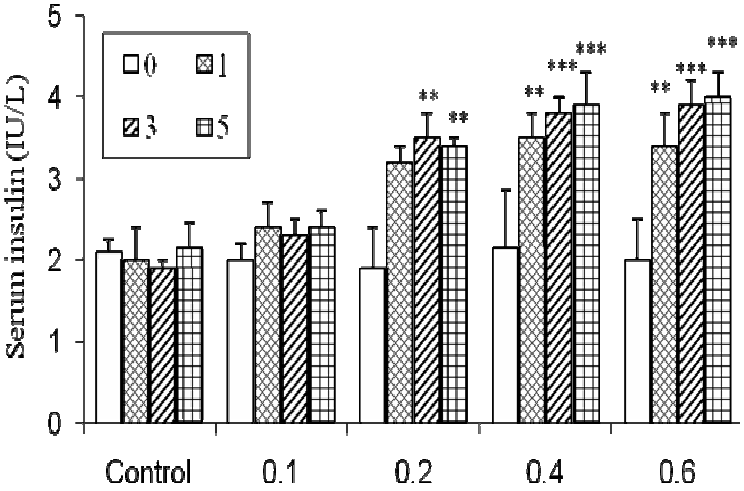

Fig. 3- Effect of intraperitoneally administration of caraway ethanolic extract seeds at doses of 0.1, 0.2, 0.4 and 0.6 g/kg body weight on serum insulin in streptozotocin-induced diabetic fasted rats for 1, 3 and 5 h after administration. Each point represents mean ± SEM for six rats. Serum insulin levels at zero time or before administrations were considered as control values. **p< 0.01, ***p< 0.001 different from zero time. Control group was administered with saline as a vehicle

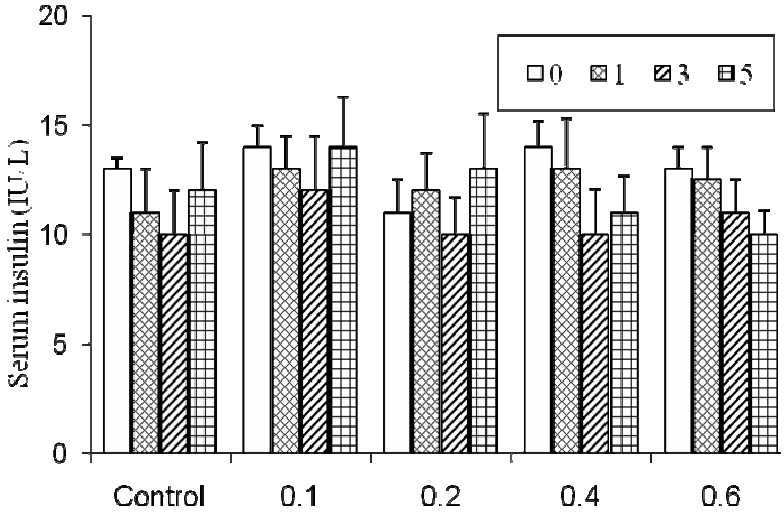

Fig. 4- Effect of intraperitoneally administration of caraway ethanolic extract seeds at doses of 0.1, 0.2, 0.4 and 0.6 g/kg body weight on serum insulin in healthy fasted rats for 1, 3 and 5 h after administration. Each point represents mean ± SEM for six rats. Serum insulin levels at zero time or before administrations were considered as control values. Control group was administered with saline as a vehicle

injected during the first week of birth while the capacity of pancreatic -cell regeneration remains in the animals, type 2 diabetes develops [25, 26].

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The known main constituents of caraway have been demonstrated to be carvone (40 – 60%), limonene, carveol, dihydrocarveol, thymol in addition to glucosides and flavonoids [27-30]. Flavonoids are considered as active principles in many medicinal plants and natural products with positive effect for human health [31].

The caraway extract seeds had increasing effect on basal plasma insulin concentrations in diabetic rats. It appears that the fruit extracts of these plants exerts a hypoglycemic effect dependently of insulin secretion. Recent-onset insulinopenia in STZ-diabetic rats was associated with glucose overproduction in the basal (hyperglycemic) state [32]. The hypoglycemic activity of this plant may therefore be due to inhibition of hepatic glucose production [32] and/or stimulation of glucose utilization by peripheral tissues, especially muscle and adipose tissue. The plant extracts could also act as inhibitors of tubular renal glucose reabsorption [33].

As a result, it may be concluded that, ethanolic extract of caraway seeds is effective in attenuate of increasing serum glucose and decreasing serum insulin resulting from the damage of STZ-induced diabetic rats. It could be proofed that the plant must be considered as excellent candidate for future studies on diabetes mellitus. In addition, further comprehensive pharmacological investigations, including experimental chronic studies, should be carried out.

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