

The Effect of *Daucus carota* Seeds Extract on Lipid Profile, LFT and Kidney Function Indicators in Streptozocin-Induced Diabetic Rats

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Abstract

Daucus carota ssp. sativum (Apiaceae) seeds include antioxidant agents, which can improve diabetes mellitus complications. This study was designed to investigate the effect of the methanol extract of *D. carota* (wild carrot) seeds on the serum levels of lipids and biochemical indices of kidney and liver function in streptozocin-induced diabetic rats. Diabetes mellitus (type I) was induced using intraperitoneal injection of streptozotocin (65 mg/kg). Fasting blood samples were collected one week later and rats with their serum glucose level exceeding 300 mg/dl were considered diabetics. These diabetic animals were divided into 5 groups and received various doses of *D. carota* seeds extract (100, 200 and 300 mg/kg body wt.), glibenclamide (600 µg/kg) and distilled water (0.5 ml) for 6 days using gavage. After treatment, fasting blood samples were collected again and total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate amino transferase (AST), alanine aminotransferase (ALT), high density lipoprotein cholesterol (HDL-C) and Low density lipoprotein cholesterol (LDL-C) levels were determined using spectrophotometry technique by respective kits. Administration of *D. carota* seeds extract in diabetic rats for six days, at all doses significantly decreased serum levels of total cholesterol, triglycerides and creatinine. Furthermore, oral administration of extract (200 and 300 mg/kg) significantly decreased serum levels of LDL-C, AST and urea. Also, extract (300 mg/kg) decreased ALT serum levels ($P < 0.05$). Thus *D. carota* seeds extract exerted antihyperlipidemic properties with no adverse effect on liver and kidney function.

Keywords

Daucus carota Seeds, Diabetes Mellitus, Lipids, Kidney, Liver Enzymes

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1. Introduction

Diabetes is a major health problem, affecting 3% of the population worldwide. Clinical trials strongly support the notion that hyperglycemia is the principal cause of diabetes complications. Modern therapies are too costly to be practical for the majority of diabetes suffer especially in developing countries where resources are meager. Medicinal plants are frequently considered to be less toxic and freer from side effects than synthetic ones. It is interesting to note that 25% of modern medicines are derived from plants that were used

traditionally. *Daucus carota* Linn. (Apiaceae) is a biennial plant used as a vegetable throughout the world. Different parts of this plant have been administered in traditional medicine for the treatment of many diseases such as kidney dysfunction, asthma, worm infections and inflammation. Carrot seed which in proper botanical language is carrot fruit, can be abortifacient, diuretic, antinociceptive and anti-inflammatory [1]. An essential oil obtained from seeds is used as a food flavoring. *Daucus carota* seeds exhibit

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antifertility [2], antisteroidogenic (in females) [3] and antibacterial properties [4]. The essential oil from seeds are reported to possess analgesic and anti-inflammatory activities [5]. Chromatographic assays show that the methanol extract of *D. carota* seeds include antioxidant agents such as *luteolin* and flavons [4]. It has been determined that glucose autooxidation generate reactive oxygen species (ROS) lead to chronic oxidative stress and toxic effects upon various organs including retinopathy, nephropathy, neuropathy and vascular disorders [6-8].

The purpose of this research was experimentally to assess the antidiabetic and antihyperlipidemic effects of *D. carota* ssp. *sativum* (wild carrot) seeds extract and to compare this with glibenclamide as a reference drug. Also, biochemical indices of kidney (urea, uric acid, creatinine) and liver (AST, ALT) function was investigated in diabetic treated rats.

2. Methods

Animals: Adult male Wistar rats weighing 200-250 g were obtained from the animal house of Pasteur Institute, Tehran, Iran. The animals were accommodated under control of environmental conditions such as temperature ($22 \pm 2^\circ\text{C}$), relative humidity (50-60%) and a 12 h light/dark cycle. Rats were fed with standard diet and allowed food and water *ad libitum*. The committee for the ethical use of animals approved our experimental protocol.

Plant material preparation and Extraction: The seeds of *D. carota* ssp. *sativum* (wild carrot) were obtained from a local herbal store (Kerman, Iran), Which was taxonomically identified and approved by Botany Section in the Biology Department of Shahid Bahonar university of Kerman (voucher number: 40643, deposited in: Herbarium of Tehran University, director: Dr F. Attar). Dried and ground seeds of plant (100 g) were macerated in methanol for 48 hours then were submitted to extraction with methanol by Soxhlation. After extraction, methanol was evaporated by rotary evaporator at $40-50^\circ\text{C}$ then dried to a powder using a freeze dryer at -50°C . The yield of seed extraction was about 7%. The powder was resuspended in distilled water before use.

Experimental design: Diabetes was induced by a single injection of Streptozocin (STZ) (65 mg/kg, i.p). A week after injection of STZ, rats were anaesthetized briefly by diethylether and fasting blood samples (Food was removed from cages 12 h before blood sampling) were collected from eye cavernosa sinus [9]. Diabetes was confirmed in STZ administered rats by showing fasting blood glucose above 300 mg/dL. Diabetic rats (n=30) were divided into 5 groups randomly and received 0.5 mL of distilled water, glibenclamide (600 $\mu\text{g}/\text{kg}$ body wt.), *D. carota* seeds extract (100, 200 and 300 mg/kg body wt.) respectively, using an intragastric tube daily for six days. At the end of treatment, anaesthetized, fasted animals were sacrificed by decapitation. Blood samples were immediately collected into tubes. Serum of above groups and a control group (normal) was extracted by centrifugation and subsequently utilized for measurements.

Biochemical assays: Serum total cholesterol, triglycerides, urea, uric acid, creatinine, aspartate amino transferase (AST), alanine aminotransferase (ALT), high density lipoprotein cholesterol (HDL-C) were measured by an automatic biochemistry analyzer using respective kits. LDL-C was calculated by Freidwald equation [10].

Statistical analysis: Statistical analysis was carried out between groups using one-way ANOVA followed by the Tukeys *post hoc* test through SPSS version 16 software. The criterion for statistical significance was $p < 0.05$. All data are presented as mean \pm standard deviation.

3. Results

There was a statistical significant elevation in serum total cholesterol, triglycerides, LDL-C, urea, uric acid, creatinine, ALT and AST in the diabetic rats, whereas HDL-C decreased significantly (Tables 1, 2). Our findings showed that in diabetic rats treated with all doses of methanol extract of *D. carota* for six days, serum level of cholesterol and triglycerides decreased, although LDL-C decreased only at higher doses (200 and 300 mg/kg) (Table 1).

Table 1. Mean values of serum triglycerides, total cholesterol, HDL-C and LDL-C in all groups.

Group	Triglycerides (mg/dL)	Total cholesterol (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Control	87 \pm 4.3	77.2 \pm 2.6	41 \pm 1.7	18.8 \pm 4.9
Diabetic	124 \pm 7.07 ^{a**}	107.8 \pm 4.5 ^{a**}	30.8 \pm 1.8 ^{a*}	52.2 \pm 5.7 ^{a***}
Diabetic+extract (100mg/kg)	66.6 \pm 5.7 ^{b***}	83.8 \pm 4.4 ^{b*}	27.6 \pm 6.4	42.1 \pm 8.1
Diabetic+extract (200mg/kg)	72 \pm 2.5 ^{b***}	73 \pm 6.2 ^{b***}	30.4 \pm 5.5	28.2 \pm 6.14 ^{b**}
Diabetic+extract (300mg/kg)	70 \pm 3.5 ^{b***}	69.8 \pm 5.1 ^{b***}	33.6 \pm 5.9	22.8 \pm 7.4 ^{b***}
Diabetic+glibenclamide	73.4 \pm 3.3 ^{b***}	82.6 \pm 4 ^{b*}	39.6 \pm 6.1	28.4 \pm 6.6 ^{b**}

Values are mean \pm S.E.M for six rats. a: vs. Control group. b: vs. Diabetic group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Also results showed that the methanol extract of *D. carota* at 300 mg/kg decreased ALT and at 200 and 300 mg/kg

decreased AST serum levels. Furthermore, the oral administration of extract (200 and 300 mg/kg) significantly

decreased urea and in all doses decreased creatinine serum levels (Table 2).

Table 2. Mean values of serum urea, uric acid, creatinine, AST and ALT in all groups.

Group	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)	AST (IU/L)	ALT (IU/L)
Control	39.2 ± 0.6	0.98 ± 0.1	0.9 ± 0.05	141 ± 6.4	76.6 ± 5.2
Diabetic	115.2 ± 4.8 ^{a***}	2.9 ± 0.07 ^{a*}	1.6 ± 0.1 ^{a*}	250 ± 3 ^{a***}	183 ± 4.9 ^{a***}
Diabetic+extract (100mg/kg)	89 ± 12	2.5 ± 0.27	0.8 ± 0.1 ^{b**}	190 ± 17	174.6 ± 7.7
Diabetic+extract (200mg/kg)	78.2 ± 9.7 ^{b*}	3.4 ± 0.5	0.89 ± 0.07 ^{b*}	184 ± 5 ^{b*}	164 ± 16.9
Diabetic+extract (300mg/kg)	77.6 ± 5.6 ^{b*}	3.4 ± 0.8	0.92 ± 0.08 ^{b*}	190 ± 6 ^{b*}	124 ± 12.8 ^{b**}
Diabetic+glibenclamide	115 ± 8.6	2.54 ± 0.3	1.6 ± 0.29	174 ± 24 ^{b*}	204 ± 12

Values are mean ± S.E.M for six rats. a : vs. Control group. b: vs. Diabetic group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4. Discussion

A few reports are available on the antihyperlipidemic effect of *D. carota* seeds extract. In agreement with these results, Vasudevan *et al.* (2006) determined that the ethanolic extract of *D. carota* seeds reduced cholesterol level in young and aged mice [1]. Other recent studies show that administration of *D. carota* seeds extract for 3 days significantly decreased serum glucose, cholesterol, triglycerides, LDL and VLDL serum levels [11]. Also, in diabetic rats treated with this extract for 6 days, serum glucose significantly decreased and insulin serum level increased and it has been showed that extract can improve pancreas asinus and islets [12]. Oxidative stress is an important aspect of glucose toxicity in pancreatic beta cells which contributes to progressive β -cell failure. Elevated level of glucose in diabetes is accompanied with lipid peroxidation and tissue injury which observed in diabetes. Oxidative stress is mainly due to an increased reactive oxygen species (ROS), lipid peroxidation products (malondialdehyde) and a significant reduction of antioxidant defense system and it plays a significant role in incidence of degenerative diseases [13,14]. Also it is determined that antioxidants play an important role in protection of cells from toxic agent effects [15]. Antioxidant therapy acts as a protection against oxidative stress. Herbal products can improve antioxidant status so improve diabetes complications [14,16]. Recent studies show that plants contain many antioxidant agents like flavonoides which exert a protection against beta cell impairment due to oxidative stress. The flavonoids are the most prominent and the most important plants' antioxidants [17]. The antioxidative activities of flavonoids are multifaceted. Most flavonoids possess the ability to scavenge free radicals by acting as hydrogen and electron donors. Some flavonoids can also act as antioxidant agents by direct reaction with radicals to form less reactive products, and some species possess a capacity to chelate transition elements [18]. Some flavonoids are also strong inhibitors of certain metabolic enzymes in the body that generate free radical products such as a cyclooxygenase, lipooxygenase, monoamine oxidase, xanthin oxidase, inducible nitric oxide synthase [17,19]. Reports have shown

that flavonoides could exert a protection on beta cell function by lowering the level of lipid and blood glucose as well as increasing insulin sensitivity [20].

The major constituents isolated from the methanol extract of *D. carota* seeds by reverse phase preparative high performance chromatography were three flavones that named luteolin, luteolin 3-O- β -D-glucopyranoside and luteolin 4-O- β -D-glucopyranoside [14]. Among these three flavones, luteolin showed the highest degree of free radical scavenging activity [14]. Phytochemical examination of *D. carota* seeds extract resulted in the isolation of apigenin 4-O- β -D-glucoside, kaempferol 3-O- β -D-glucoside and a new flavone glycoside which was characterized as apigenin 7-O- β -D-galactopyranosyl-(1-->4)-O- β -D-mannopyranoside [21]. It has been shown that apigenin as a flavonoid attenuated cell damage in pancreatic beta cell via oxidative stress [22]. An increase in ALT and AST enzymes reflects liver damage. Inflammatory hepatocellular disorders cause extremely elevated transaminase levels [23]. Apigenin exhibit good hepatoprotective effect against acute hepatotoxicity in mice [24] and in rats treated for 7 days with apigenin serum ALT and AST and hepatic MDA decreased but GSH (reduced glutathione) content increased significantly [25]. Elevation of important markers such as urea and creatinine in serum are related to renal dysfunction in diabetic hyperglycemia [26,27]. Our findings indicated that uric acid serum level increased in diabetic rats. This may be due to a metabolic disturbance in diabetes because of high activity of xanthine oxidase [28]. Furthermore, protein glycation in diabetes may lead to muscle wasting and an increased release of purine that is the main source of uric acid [29]. It has been reported that apigenin significantly decreased creatinine level [30]

5. Conclusion

Consequently, antidiabetic and antihyperlipidemic effects of *D. carota* seeds extract observed in this study could be attributed to the antioxidant ingredients including apigenin and luteolin, although the role of other factors cannot be unnoticed.

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