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Original Article

Effects of Caffeic Acid on Serum Lipid Profile and Atherogenic Index in Alloxan-Induced Diabetic Rats

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Abstract

Background and Aim: Diabetes is a major public health problem worldwide. Oxidative stress is a risk factor in diabetes. The aim of this study is to evaluate the possible beneficial effect of caffeic acid (CA) on serum lipid parameters and atherogenic index in alloxan-induced male diabetic rats.

Materials and Methods: 40 male Wistar rats were randomly divided into 4 groups: group I as the healthy control group, group II as the healthy group treated with CA (50 mg/kg i.p. daily), group III as the diabetic control group and group IV as the diabetic group treated with CA (50 mg/kg i.p.daily). Diabetes was induced in the 3rd and 4th groups by the injection of alloxan monohydrate (100 mg/kg s.c). After seven weeks, animals were anaesthetized and blood samples were collected. Then, the serum level of fasting blood glucose (FBS), lipid parameters and the atherogenic index were measured.

Results: The serum level of FBS, cholesterol, low density lipoprotein (LDL-C) and atherogenic index significantly decreased in the diabetic group treated with CA compared with the untreated diabetic group. The serum level of high density lipoprotein (HDL-C) significantly increased in the treated diabetic group compared with the untreated diabetic group.

Conclusion: The results of this study indicated that CA has beneficial effects on serum blood glucose, lipid profile and atherogenic index in type 1 diabetic rats.

Keywords: Diabetes, Caffeic Acid, Serum Lipid Profile, Atherogenic Index, Rat

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Introduction

Diabetes is a major public health problem worldwide (1). The World Health Organization (WHO) estimated that by 2010, more than 200 million people will have diabetes worldwide and by 2025 this number will nearly reach 300 million (2). Oxidative stress plays an important role in the pathogenesis of both types of diabetes (3). The importance of human body protection against hyperglycemia cannot be understated. The direct and indirect effects on human vascular tree are important sources of morbidity and mortality in both type 1 and type 2 diabetes (4). There is a significant progress in the development of drugs

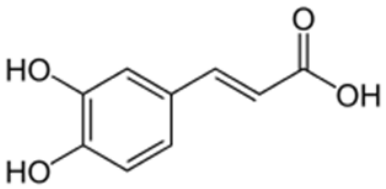

Figure 1. Chemical structure of caffeic acid.

used in the treatment of diabetic complications, but the numerous side effects are still a serious concern (5). Diabetes mellitus management with no side effects is still a challenge for physicians and researchers in the 21st century. In diabetes treatment, herbal drugs have been valued due to their low cost, more efficiency and fewer side effects (6). Many natural polyphenolic antioxidants have been isolated from plant sources, including vitamin E, flavonoids, cinnamic acid derivatives, curcumin, caffeine, catechin, gallic acid derivatives, chlorogenic acid, anthocyanins, tannins, etc. (6). Edible plants contain non-toxic caffeic acid (Figure 1). It is naturally a part of cinnamic acids (5). Naturally, there is caffeic acid (CA) in many vegetables, such as carrot, tomato, strawberry, blueberry, olive oil, coffee (7), artichoke (8), potato, chicory, pear, apple, kiwi, cherry, plum and berry (1). Also, it has been reported to be found in the celery root (9), soybean (10) and Hemelia patens (11). Caffeic acid always acts as a strong antioxidant (12). Apart from the capabilities of CA such as the inhibition of cancer cells proliferation, antimicrobial activity, anti-inflammatory properties, being a powerful antioxidant, immunomodulatory and anti-aging activity; the antidiabetic activity of CA has been the subject of many researches (5).

In this study, we examined the antioxidative activities of CA and its possible protective effect on the serum level of fasting blood glucose (FBS), lipid parameters and atherogenic index in alloxan-induced type 1 diabetic rats.

Materials and Methods

Chemicals

Alloxan monohydrate was purchased from Sigma-Aldrich, an American company, and CA was obtained from Acros Organics, an American company. Phosphotungstic acid and Magnesium chloride and other materials were purchased from Merck, Germany.

Animals

Male Wistar rats (240 - 250 g) were purchased from the animal center of Hamadan University of Medical Sciences. Rats were allowed to adapt themselves to the new situation for two weeks. They were kept under standard conditions (temperature 25 ± 3 °C, relative humidity 55 ± 10%, and 12 hours light and 12 hours dark). Rats were fed a standard chow diet and water was supplied freely for rats throughout the experiment. All experimental guidelines, animal housing conditions and handling were performed in accordance with the guidelines of The National Health and Medical Research Council guidelines.

Diabetes Induction

Diabetes was induced in the third and fourth groups by the subcutaneous injection of alloxan monohydrate (100 mg/kg) (13) after 8 hours of fasting. Experimental studies have indicated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose, which appears only after alloxan treatment (14, 15). Because of acute hypoglycemia, rats received 5% sucrose solution for 24 hours instead of drinking water. 72 hours after alloxan injection, their FBS was measured by glucometer. Rats with fasting blood glucose level of more than 250 mg/dl were used for this experiment.

Experimental Design

In this study, 40 male rats were randomly divided into four groups (10 per group): The first group as the healthy control group, the second group as the healthy group treated with CA (50 mg/kg i.p. daily), the third group as the diabetic control group and the fourth group as the diabetic group treated with CA (50 mg/kg i.p. daily) (16). Caffeic acid was dissolved in dimethylsolfoxide and then was injected intraperitoneally into the second and the fourth group daily for 7 weeks. After 7 weeks of treatment, animals were anesthetized using diethyl ether and blood samples were collected by heart. Then the samples were kept for clotting in laboratory temperature for 20 minutes and centrifuged at 3200 rpm for 20 minutes for serum separation.

Biochemical Analysis

The serum level of fasting blood glucose (FBS), triglyceride (TG), and total cholesterol (C) of all groups were measured by biochemical analyzer using

commercial kits which were purchased from Pars Azmoon, Iran. The serum level of HDL-C was measured manually by Pars Azmoon kits. The precipitating reagent included phosphotungstic acid (0.55 mM) and magnesium chloride (25 mM). The serum level of LDL-C and VLDL-C was calculated by the formula of Friedewald et al. (17). Moreover, atherogenic index was obtained by the following formulas (18):

Atherogenic index 1 = Total cholesterol/HDL

Atherogenic index 2 = LDL/HDL

Statistical Analysis

All data were expressed as mean ± standard deviation. The means of groups were compared by Kruskal-Wallis H test and Mann-Whitney U test using SPSS software (version 13). P value < 0.05 was considered significant.

Results and Discussion

Table 1 shows the effect of CA on the serum level of FBS, TG, total cholesterol, LDL, VLDL, HDL and atherogenic index in diabetic rats. There was a significant ($p < 0.05$) increase in the serum level of FBS in the diabetic control group compared to the healthy control group. The treatment of diabetic rats with CA ($p < 0.05$) significantly decreased (43.16%) the serum level of FBS among them compared to the diabetic control group. There was not a significant difference concerning the serum level of FBS between the healthy group that were treated with caffeic acid and the healthy control group. The serum level of triglyceride, total cholesterol, LDL-C, and VLDL-C in the diabetic control group significantly increased compared to the healthy control group. The treatment of diabetic rats with CA noticeably decreased the total cholesterol (21.7%) and LDL-C (26.65%) among them compared with the diabetic control group. The treatment of diabetic rats with CA decreased the serum level of triglyceride (21.64%) and VLDL-C (16.43%) among them compared to the diabetic control group, but it was not statistically significant. A significant decrease was observed in the serum level of HDL-C in the diabetic control group compared to the healthy group. The treatment of diabetic rats with CA

Table 1: The effect of caffeic acid on the serum level of fasting blood glucose, triglyceride, total cholesterol, LDL, VLDL, HDL and atherogenic index in alloxan-induced diabetic rats.

parameters	healthy control	healthy treated	diabetic control	diabetic treated
Fasting blood glucose (mg/dl)	152 ± 15.48*	158.25 ± 27.24*	465.75 ± 79.06#	264.75 ± 129.5*#
Triglyceride (mg/dl)	64.17 ± 16.24*	62.86 ± 12.74*	119.8 ± 53.32#	93.87 ± 13.47#
Total cholesterol (mg/dl)	55 ± 3.7*	57.7 ± 2.29	70.83 ± 14#	55.43 ± 8.48*
LDL (mg/d)	22.23 ± 6.22*	26.99 ± 1.38*	35.46 ± 3.49#	26 ± 6.85*
VLDL (mg/dl)	12.83 ± 3.25*	12.57 ± 2.5*	22.47 ± 10.21#	18.77 ± 2.7#
HDL (mg/dl)	20.33 ± 4.58*	17.08 ± 1.3*	8.44 ± 2.28#	13.75 ± 2.07*#
Atherogenic index 1 = Total cholesterol/HDL	2.86 ± 0.58*	3.5 ± 0.36*	5.36 ± 1.2#	4.03 ± 0.61*#
Atherogenic index 2 = LDL/HDL	1.05 ± 0.22*	1.59 ± 0.08*#	2.64 ± 0.46#	1.83 ± 0.48*#

Values were expressed as mean±standard deviation

* p<0.05, when compared with diabetic untreated

# p<0.05, when compared with control group (healthy control)

considerably increased the serum level of HDL-C among them compared to the diabetic control group. There was not a significant difference concerning the serum level of TG, total cholesterol, LDL-C, VLDL-C and HDL-C between the treated healthy group and the healthy control group. There was a significant increase in the serum level of atherogenic index (total cholesterol/HDL-C and LDL-C/HDL-C) in the diabetic control group compared to the healthy control group. The treatment of diabetic rats considerably decreased the serum level of total cholesterol/HDL-C (24.8%) and LDL-C/HDL-C (30.6%) among them compared to the diabetic control group. There was a noticeable difference in the serum level of LDL-C/HDL-C in the healthy treated group compared to the healthy control group.

The toxic impact of alloxan on pancreatic beta cells involves the oxidation of sulfhydryl group (-SH group), inhibition of glucokinase enzyme, generation of free radicals and disturbances of intracellular calcium homeostasis (14).

This study indicated that CA decreases the serum level of FBS in diabetic rats. Caffeic acid acts through various mechanisms against hyperglycemia. Previous studies have showed that CA is able to regulate β-cell and adipocyte GLUT4 performance. Moreover, it inhibits the activity of α-amylase and α-glucosidase in the gastrointestinal tract and increases the activity of glucokinase in hepatocytes (5). A rise in the amount of of hepatic glucokinase increases the consumption of blood glucose for developing the energy or storing the glycogen in liver. Caffeic acid inhibits the activity of glucose-6 phosphatase and phosphoenolpyruvate carboxykinase (11). Another probable mechanism in the antidiabetic function of CA is to increase the transfer of blood glucose to adipocytes (19). Formerly, studies indicated that CA and cinnamic acid have influenced the improvement of glucose consumption in hepatocytes of insulin-resistant rats. These phenolic compounds have probably increased the activity of glycogen-synthase through raising glucokinase expression and at the same time preventing the activity of glycogen-synthase-kinase-3. Therefore, it has improved the consumption of glucose and glycogen syntheses (1). Caffeic acid obtained from Xanthium strumarium in the diabetic rats decreased the plasma glucose (20). Caffeic acid and chlorogenic acid had inhibitory effect on the activities of α-amylase and α-glucokinase pancreas cells, but caffeic acid had a greater inhibitory effect than chlorogenic acid in restraining both enzymes (21). Other caffeic acid derivatives such as chlorogenic acid (22), *p*-hydroxy-cinnamic acid (2) and ferulic acid (23) significantly decreased the level of FBS and hemoglobin A1C in diabetic rats compared to diabetic control group. The present results indicated that CA considerably decreased the serum level of cholesterol, LDL-C and atherogenic index and at the same time remarkably increased the serum HDL-C in type 1 diabetic rats. Hypercholesterolemia and hypertriglyceridemia are independent risk factors which are able, alone or together, to accelerate the progress of coronary artery disease (2). The loss of insulin is associated with the rise of cholesterol level which is due to the attack of lipids from adipose tissue to plasma. The increase of LDL-C concentration in diabetic rats' plasma might be due to the defect of LDL-C receptor through failure in its production function. Besides, the decrease of HDL-C may be due to a reduction in the activity of lecithin cholesterol acyltransferase. The enhancement of insulin secretion inhibits hormone sensitive lipase and also increases the consumption of glucose. Moreover, it reduces the attack of free fatty acids (FFAs) that belong to fat deposits (24). Caffeic acid is a α-tocopherol protectant in LDL (24). The results of this study were in consistent with previous studies. Caffeic acid significantly decreased LDL-C, VLDL-C, triglycerides and total cholesterol level, and at the same time increased HDL-C in diabetic rats (25). Furthermore, the aqueous extract of calamintha officinalis, rosamarinic acid and CA obtained from aerial parts of this plant decreased TBARS, FBS, LDL-C and VLDL-C, and at the same time increased body weight and HDL-C in diabetic rats (26). In another study, caffeic acid decreased the plasma and liver triglycerides as well as cholesterol in mice with a high-fat diet. Moreover, it effectively prevented the cholesterol biosynthesis and suppressed the activity of lipogenesis (27). The oral injection of CA remarkably reduced the serum, liver and kidney level of cholesterol and TG in rats with alcohol-induced toxicity (28). Besides, iron-induced hypercholesterolemia in rats was inhibited by the caffeic acid (29). Other derivatives of hydroxy

cinnamic acid such as sinapic acid significantly reduced the serum total cholesterol, triglycerides, LDL-C and VLDL-C in diabetic rats, and at the same time increased HDL-C among them as compared to diabetic control group (30).

Conclusion

By considering the results of this study and comparing them to previous studies, we can say that CA acts as an effective antioxidant in reducing the serum glucose, LDL-C and atherogenic index in diabetic rats.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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