

Original Research Article

Antioxidant and anti-diabetic effects of caffeic acid in a rat model of diabetes

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Abstract

Purpose: To determine the antioxidant and anti-diabetic potential of a natural flavonoid, caffeic acid in a streptozotocin-induced diabetic rat model.

Methods: Experimental diabetes was induced in Wistar rats using streptozotocin injection. Caffeic acid was administered orally on daily basis for 5 weeks. A glucometer was used to monitor fasting blood glucose levels. Insulin levels were estimated using enzyme-linked immunosorbent assay (ELISA). The antioxidant potential of caffeic acid was measured by determining the activities of superoxide dismutase (SOD) and catalase (CAT), and levels of reduced glutathione (GSH) in rat liver. Standard assays were performed to determine the lipid profile of the rats. Histopathological analysis was performed to determine differences in microscopic structures of pancreas among the different treatment groups.

Results: Caffeic acid administration resulted in significant enhancement of serum insulin level, and decrease in blood glucose level of diabetic rat models ($p < 0.05$). Caffeic acid exerted antioxidant effects by significantly increasing GSH levels and activities of CAT and SOD ($p < 0.05$). Histological examination of the pancreas depicted normal islet morphology under caffeic acid administration in diabetic rats.

Conclusion: These results reveal the antioxidant potential and anti-diabetic effect of caffeic acid in a diabetic rat model and point towards the potential applicability of caffeic acid in the management of diabetes mellitus.

Keywords: Diabetes mellitus, Streptozotocin, Caffeic acid, Phenolics, Anti-diabetic, Antioxidant

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INTRODUCTION

Currently, diabetes is one of prevalent human diseases across the globe [1]. Excessive intake of carbohydrate and fat-rich diets has been shown to be responsible for the development of metabolic disturbances leading to the induction of diabetes mellitus in humans [2]. Diabetes

mellitus accounts for a significant level of mortality, mainly in developed countries [3]. The disease develops as a result of abnormal insulin production or impairment of its action, resulting in marked increases in fasting blood glucose levels [4].

Rodent-based diabetic models have been used for the evaluation of different anti-diabetic drugs, and to unveil the molecular events associated with the disease. Diabetes has been induced in rat models using a number of chemical compounds. Streptozotocin is the most commonly used drug for induction of diabetes in rats [4]. It works by destroying the pancreatic β -cells, thereby inhibiting insulin secretion which leads to diabetic state [5]. The currently employed therapeutic agents against diabetes mellitus aim at maintaining normal blood glucose levels. However, owing to the heterogeneous nature of these therapeutic strategies, the successes achieved have been very limited [6].

Against this backdrop, studies are currently directed at achieving better clinical results. Phenolic compounds are one of the dominant classes of natural compounds with promising potential in combating human diseases [7]. These compounds show antioxidant, anti-inflammatory and anticancer effects, and as such have been used in traditional medicines from time immemorial [8]. Previous studies have shown that phenolic compounds exerted anti-diabetic effects in rat models [9].

The present study was designed to evaluate the anti-diabetic effect of caffeic acid (Figure 1) in a rat model of diabetes mellitus.

EXPERIMENTAL

Animals

Healthy male adult male Wistar rats were used in this study. The average body weight of the animals was 165 ± 10 g. The rats were maintained in well-ventilated rooms at a temperature of $25 \pm 3^\circ\text{C}$ and approximately 12h light/12-h dark photo period. Standard pellet-based diet and free access to water were provided to the rats. The study was approved by the Research ethics committee of Huazhong University of Science and Technology (approval no. HUS/6762/2019). All the standard international guidelines were followed during the study as described previously [10].

Induction of diabetes

The rats were fasted for about 14 h prior to intraperitoneal injection of streptozotocin (1mL/rat). After three days of injection, induction of diabetes was confirmed by estimating fasting blood glucose levels. Rats with fasting glucose levels ≥ 240 mg/dL were taken as diabetic and were used in the study. The animals were divided into four experimental groups, with 8 rats

per group. The rat groups were: group I (control, non-diabetic rats), group II (diabetic rats), group III (diabetic rats administered caffeic acid at a dose of 25 mg/kg), and group IV (diabetic rats administered caffeic acid at a dose of 35mg/kg) daily for 5 weeks. At the end of the 5th week, the rats were anaesthetized and subsequently sacrificed. Blood samples were collected and subjected to high speed centrifugation for 15 min to obtain serum samples which were used for biochemical analyses.

Evaluation of blood glucose and plasma insulin levels

A glucometer (Accu-Chek, Mannheim, Germany) was used for determination of fasting glucose levels of rat blood at the end of each week, while ELISA kits (GmbH) were used for determining plasma insulin levels.

Determination of antioxidant parameters

Activities of SOD and CAT, and levels of GSH were determined using the following standard protocols: A reaction mixture consisting of phosphate buffer (25 mM, pH 7.8); EDTA (0.25 mM), quercetin (0.05 μM) and TEMED (0.8mM) was used to assay SOD activity by estimating the inhibition of quercetin oxidation at 406nm. One unit of SOD activity was taken as amount of enzyme that produced 50% inhibition of oxidation. Reduction of H_2O_2 was used to measure CAT activity at 240 nm, with a reaction mixture containing phosphate buffer (50 mM, pH 7.0); EDTA (0.5 mM), H_2O_2 (10 mM) and TritonX100 (0.012 %). The activity of CAT activity was expressed as $\mu\text{mol H}_2\text{O}_2$ decayed/min/mg protein. In the determination of GSH levels, the samples were added to a 96-well plate. The final volume was made up to 100 μL using phosphate buffer (100 mM, pH 8.0); EDTA (5 mM) and O-phthalaldehyde (25 μL). The plate was then subjected to incubation at 37°C for 45 min, after which GSH levels were determined by recording the fluorescent emission at 425 nm after the sample excitation at 340 nm.

Measurement of serum lipid profile

The levels of serum triglycerides (TGIs), total cholesterol (TChl) level and high-density lipoprotein cholesterol (HDL-C) were determined using Randox assay kits (Randox Laboratories Ltd., United Kingdom). Friedewald's formula was used to calculate the concentration of low-density lipoprotein (LDL-C) cholesterol as in Eq 1.

$$\text{LDL-C} = [(\text{TChl}-\text{HDL-C})-\text{TGIs}]/5 \dots\dots\dots (1)$$

Histopathological examination of pancreatic tissue

Three rats were randomly taken from each animal group and were sacrificed under anaesthesia. The pancreas was removed from the sacrificed animals and fixed using 10 % formalin (neutral-buffered). The tissues were fixed by dehydration with alcohol, followed by paraffin embedding. Thereafter, 4.5- μ m sections were sliced from the rat pancreatic tissues and stained with haematoxylin and eosin (H&E). The sections were mounted using disterenephthalate xylene, and histological examination was done by a trained pathologist who was blind to the treatments used. The islet cell injuries were analysed through microscopic examination of at least a dozen fields from each pancreatic section.

Statistical analysis

The experiments were performed in three replicates, and the data are presented as mean \pm standard deviation (SD). The *p*-values were calculated using Student's *t*-test. Statistical analysis was performed using GraphPad Prism 7.0 offline software. Values of *p* < 0.05 were taken as indicative of statistical significance.

RESULTS

Caffeic acid restored blood glucose and insulin levels in diabetic rats

The diabetic rats showed significantly higher levels of blood glucose, low plasma insulin and low body weight, relative to the normal control group (Table 1). However, daily oral administration of caffeic acid at doses of 25 mg/kg body weight (group III) and 35 mg/kg body weight (group IV) led to significant reductions in the blood glucose levels, when compared to the diabetic group (group II; Figure 2). Caffeic acid treatment also significantly enhanced plasma insulin levels (Figure 3).

Caffeic acid exerted antioxidant effects in diabetic rats

The streptozotocin-induced diabetic rats (group II) exhibited significantly reduced liver activities of SOD (Figure 4 A) and CAT (Figure 4 B), and reduced GSH levels (Figure 4 C), when compared with the normal control group (Group I). However, the caffeic acid-administered animal groups (groups III and IV exhibited SOD, CAT and GSH levels comparable to those of normal rats.

Effect of caffeic acid on serum lipid profile

Results of serum lipid profile determination in different animal groups showed that the concentrations of TGIs, TChI and low-density lipoprotein cholesterol (LDL-C) were increased, whereas the concentration of HDL-C was decreased markedly in the diabetic group (group II), relative to the normal control group (Table 2). Interestingly, oral treatment of caffeic acid at doses of 25 mg/kg body weight (group III) and 35mg/kg body weight (group IV) resulted in marked decrease in the concentrations of TGIs, TChI and LDL-C, and increases in HDL-C concentration.

Histopathological features of pancreas

Pancreatic sections from the normal animal group depicted normal-sized islets (Figure 5 A). However, microscopic examination of pancreatic sections from the diabetic control group (group II) revealed that the diabetic rats had decreased number of β -cells, indicating evidence of β -cells destruction (Figure 5 B). The islet structure was almost fully preserved in sections from groups III and IV (caffeic acid-treated groups), and there was hardly any pathological changes in their architecture (Figure 5 C and D).

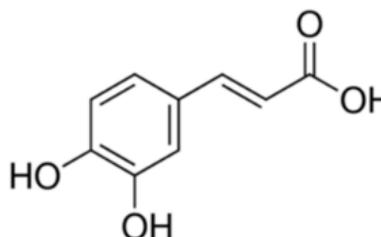


Figure 1: Molecular structure of caffeic acid.

Table 1: Effect of caffeic acid treatment on the body weight of diabetic rats

Animal group	Initial weight (g)	Final weight (g)
Group I (Normal)	172 \pm 0.87	219 \pm 0.76 ^a
Group II (Diabetic)	174 \pm 0.85	143 \pm 0.54 ^b
Group III (Diabetic + 25mg/kg caffeic acid)	169 \pm 0.92	210 \pm 0.95 ^a
Group IV (Diabetic + 35mg/kg caffeic acid)	171 \pm 0.86	220 \pm 1.3 ^a

Values are mean \pm SD. Values with different superscripts differ significantly with each other (*p* < 0.05) as assessed using Duncan's multiple range test

Table 2: Effect of caffeic acid treatment on the lipid profile of diabetic rats

Group	Lipid profile			
	Triglycerides	Total cholesterol	HDL Cholesterol	LDL Cholesterol
Group I (Normal)	71.87 ± 1.21 ^a	91.42 ± 1.56 ^a	52.83 ± 0.96 ^a	22.66 ± 0.69 ^a
Group II (Diabetic)	157.12 ± 2.17 ^b	158.43 ± 1.86 ^b	28.71 ± 0.86 ^b	97.22 ± 1.33 ^b
Group III (Diabetic + 25 mg/kg caffeic acid)	74.08 ± 1.16 ^a	93.52 ± 1.44 ^a	49.93 ± 1.09 ^a	26.17 ± 0.78 ^a
Group IV (Diabetic + 35 mg/kg caffeic acid)	72.64 ± 1.43 ^a	92.03 ± 1.51 ^a	51.63 ± 1.17 ^a	23.94 ± 0.79 ^a

Values are mean ± SD values. Values with different superscripts in a particular column differ significantly with each other ($p < 0.05$), as assessed via Duncan's multiple range test.

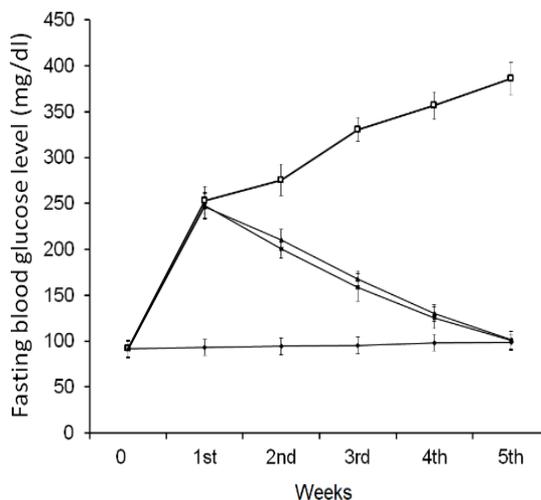


Figure 2: Effect of caffeic acid treatment on fasting blood glucose levels in different rat groups. The experiments were performed in triplicate. **Key:** ♦ = normal rat group, □ = diabetic rat group, ▲ = diabetic + 25 mg caffeic acid group, and ■ = diabetic + 35 mg caffeic acid group. Values are expressed as mean ± SD ($p < 0.05$)

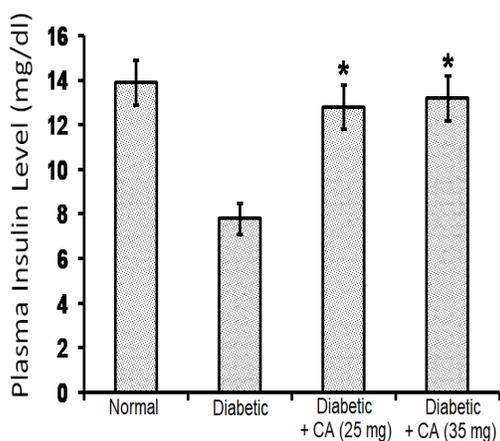


Figure 3: Effect of caffeic acid treatment on plasma insulin levels in different rat groups. The experiments were performed in triplicate. Values are expressed as mean ± SD ($p < 0.05$)

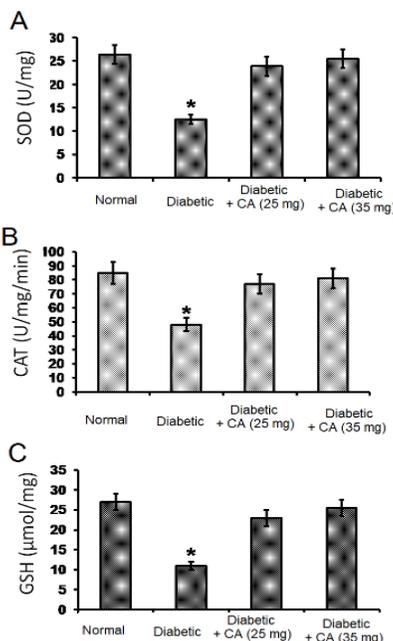


Figure 4: Antioxidant potential of caffeic acid. (A) SOD activity (B) CAT activity (C) GSH levels in different rat groups. The experiments were performed in triplicate, and values are expressed as mean ± SD ($*p < 0.05$)

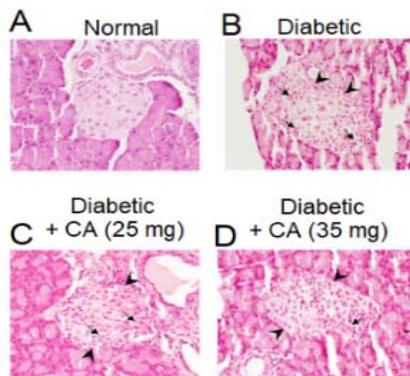


Figure 5: Effect of caffeic acid on the histopathological features of different rat groups (H & E staining). (A) Normal, (B) Diabetic, (C) Diabetic + caffeic acid (25 mg/kg) (D) Diabetic + caffeic acid (35 mg/kg). The experiments were performed in triplicate

DISCUSSION

Recent studies have shown that high incidence of diabetes mellitus has imposed tremendous concerns on human health across the globe. The disease results in high level of lethality in the developed countries. Against this backdrop, researchers are actively involved in exploring possibilities of evolving various treatment strategies which can prove handy in the fight against diabetes. Studies conducted in recent years have shown the importance of animal models in understanding the molecular mechanism of diabetes mellitus. Diabetes is chemically induced in mice using chemical compounds, for example streptozotocin. In rats, streptozotocin induces high blood glucose levels, low plasma insulin and very low body weight, the weight loss being due to excessive protein breakdown as energy source [11]. In the current study, the diabetic rats consistently showed very high glucose levels, low body weight and low plasma insulin.

However, when the diabetic rats were treated with caffeic acid, there were significant decreases in fasting blood glucose levels, most likely due to the enhancement of insulin levels. The increase in insulin might have resulted from increased insulin secretion from pancreas and/or regeneration of pancreatic β -cells, as has been proposed in previous studies [12,13]. Similar anti-diabetic effects have been observed for caffeic acid esters in previous reports [14]. Due to decrease in insulin levels in the diabetic state, blood glucose is not used for energy production, and as such, excessive fatty acid levels accumulate in the liver due to their mobilization from the adipose tissue. These are converted to triglycerides in the liver, thereby manifesting as abnormal lipid profile [15].

The potency of anti-diabetic agents is also expressed in terms of their effect on the lipid profile of diabetic patients. In the present study, caffeic acid was found to decrease the blood glucose levels of the diabetic rats. It has been shown that there is excessive production of reactive oxygen species (ROS) in diabetes due to reduction in anti-oxidant potential, manifested in decreases in levels of enzymatic (SOD and CAT) and non-enzymatic (GSH) antioxidants [16]. Hence, in this study, caffeic acid has emerged as a potential anti-diabetic agent since its treatment led to significant enhancement in antioxidant potential of diabetic rats. Moreover, the anti-diabetic potential of caffeic acid was evident in its potential to exert protective effects on pancreatic islets of streptozotocin-induced diabetes rats.

CONCLUSION

The findings of this study reveal that caffeic acid enhances serum insulin levels, and decreases fasting blood glucose level of diabetic rats. Caffeic acid also exerts antioxidant effects by increasing the levels of GSH, CAT and SOD. These results suggest the potent antioxidant and anti-diabetic potential of caffeic acid in diabetic rat model may be the potentials of the compound for the clinical management of diabetes in humans.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Wenguang Xu and Xiuying Wen performed the study and contributes in the writing of manuscript. Ming Xiao, Qijian Mei contributes in the statistical analysis and collected the data. Qiong Luo was designed the protocol, supervise the study and writes the manuscript.

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