Research Article

Effects of cinnamic acid on complications of diabetes

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Background/aim: Diabetes mellitus (DM) is a major health problem worldwide. Cinnamic acid (CA) and its derivatives are synthesized in plants and increasing attention has been given to them in recent years due to the high number of beneficial health properties attributed to their consumption. The aim of this study was to investigate the effects of CA on streptozotocin-induced diabetes in Wistar albino rats.

Materials and methods: DNA damage was evaluated in the blood, liver, and kidney cells of rats by the alkaline comet assay. Oxidative stress parameters such as catalase, superoxide dismutase, glutathione reductase, glutathione-S-transferase, and glutathione peroxidase activities and 8-hydroxy-2'-deoxyguanosine, total glutathione, and malondialdehyde levels; biochemical parameters including insulin, total bilirubin, and BCA protein levels; hepatic enzyme levels such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase; and lipid profile parameters including high-density lipoprotein, low-density lipoprotein, total cholesterol, and triglyceride levels were also evaluated.

Results: DM caused genotoxic damage and alterations in lipid profiles, oxidative stress parameters, and hepatic enzymes levels. CA treatment ameliorated these effects.

Conclusion: It seems that CA might have a role in the prevention of the complications of diabetes.

Key words: Diabetes mellitus, DNA damage, oxidative stress, hepatic enzymes, lipid profile

1. Introduction

Diabetes mellitus (DM) is one of the major health problem worldwide. It is characterized by hyperglycemia resulting from deficiencies in insulin secretion and/or insulin action (1). Chronic hyperglycemia can lead to a variety of complications such as neuropathy, nephropathy, and retinopathy and increased risk of cardiovascular diseases (2). Globally, 2.8% of the world's population suffers from diabetes and this is predicted to be 5.4% by 2025 (3). In Turkey, 7.4% of the population suffers from diabetes and it is estimated that the number of patients will reach 9.6% of the population by 2030 (4).

Studies on diabetes therapy have gained interest due to its unwanted effects on quality of life, e.g., changing lifestyles lead to reduced physical activity and increased obesity (4). In diabetes treatment, the current drugs have higher costs, limited efficacy and tolerability, and/or significant side effects (5). As a result of these factors, patients often have recourse to alternative forms of therapy such as herbal medicines. Herbal medicine usage for diabetes treatment is especially common in West Africa, Central America, and Asia (6,7). According to an estimation published by the World Health Organization (WHO), approximately 80% of diabetic patients presently rely on herbal medicine for their treatment. Unfortunately, pharmacological and toxicological evidence validating the safety and efficacy of these medicinal plants is not readily available (8).

Cinnamic acid (3-phenyl-2-propenoic acid, CA) (Figure 1), a major component of Chinese cinnamon, is a white crystalline phenolic compound with a low-intensity sweet, honey-like aroma (9). It exists in many fruits, vegetables, and beverages including blueberry, kiwi, cherry, plum, apple, pear, chicory, artichoke, potato, cider, and coffee (10). CA and its derivatives possess a variety of pharmacologic properties such as antioxidant, hepatoprotective, antimalarial, and antityrosinase activities (11,12). It was shown that they exert cytoprotective,

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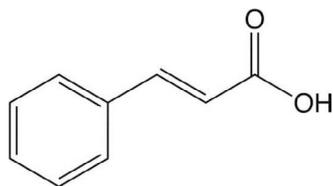* Correspondence: haticegulgoktas@gmail.com

Figure 1. Structure of CA.

antigenotoxic, and antioxidant effects in in vitro and antitumor activities in experimental animal models (13–15). They are important components of flavorings, perfumes, synthetic dyes, and pharmaceuticals (16).

To our best knowledge, there was no study conducted to investigate the effects of CA on DNA damage, oxidative stress parameters, hepatic enzyme levels, and lipid profiles in diabetic rats. Thus, the aim of this study was to evaluate the protective role of CA on diabetes-induced oxidative damage in the blood, liver, and kidney cells of rats. Diabetes was induced by streptozotocin (STZ), a glucosamine–nitrosourea compound derived from *Streptomyces achromogenes* that is used clinically as a chemotherapeutic agent in the treatment of pancreatic β-cell carcinoma. STZ damages pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia (17). In addition, STZ is a source of free radicals that can also contribute to DNA damage and subsequent cell death (18). In this study the comet assay was used for the determination of DNA damage. Oxidative stress parameters such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST), and glutathione peroxidase (GSH-Px) activities and 8-hydroxy-2'-deoxyguanosine (8-OHdG), total glutathione (GSH), and malondialdehyde (MDA) levels in the plasma, liver, and kidney tissues; hepatic enzyme parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), and gamma-glutamyl transferase (GGT) levels in serum; and insulin levels in plasma and blood lipid profiles such as triglyceride, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were also measured.

2. Materials and methods

2.1. Chemicals, kits, and equipment
The chemicals used in this study were purchased from the following suppliers: normal melting agarose and low melting point agarose from Boehringer Manheim (Mannheim, Germany); sodium chloride (NaCl), sodium hydroxide (NaOH), and potassium chloride (KCl) from Merck Chemicals (Darmstadt, Germany); dimethyl sulfoxide (DMSO), ethidium bromide (EtBr), Triton-X-100, phosphate-buffered saline (PBS) tablets, trichloroacetic acid, thiobarbituric acid, ALT assay kit,

AP kit, AST assay kit, GGT assay kit, insulin assay kit, bicinchoninic acid (BCA) protein kit, HDL-LDL assay kit, total cholesterol assay kit, triglyceride assay kit, STZ, and CA from Sigma-Aldrich Chemicals (St Louis, MO, USA); ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-Na$_2$), sodium lauroyl sarcosinate, and Tris from ICN Biomedicals Inc. (Aurora, OH, USA); 8-OHdG assay kit, SOD assay kit, CAT assay kit, GR assay kit, GSH-Px assay kit, GST assay kit, and GSH assay kit from Cayman Chemicals Co. (Ann Arbor, MI, USA); and ketamine hydrochloride from Eczacıbaşı (İstanbul, Turkey). For spectrophotometric and spectrofluorometric measurements SpectraMax M2 (Molecular Devices, Sunnyvale, CA, USA) and for quantification SoftMax Pro Software (Molecular Devices) were used.

2.2. Animals
Wistar albino rats (180–250 g, totally 24 rats) were used in all experiments. They were housed in plastic cages with stainless steel grid tops. Rats were maintained on a 12-h light/dark cycle with controlled temperature (23 ± 2 °C) and humidity (50%). Animals were fed with standard laboratory chow and allowed to access feed and drinking water ad libitum. The blood glucose levels of animals were measured before the experiments. The animals were treated humanely and with regard for alleviation of suffering and the study was approved by the Ankara University Animal Ethics Committee (2015-12-138).

2.3. Streptozotocin-induced diabetes mellitus model
Wistar rats were subjected to type 1 diabetes by STZ injection (60 mg/kg freshly prepared in PBS) as previously described (19). Two days later, blood was taken from tails of rats using a lancet to measure the blood glucose levels using a glucometer (Plusmed, İstanbul, Turkey). Rats with a blood glucose level higher than 250 mg/dL were considered to be diabetic.

2.4. Experimental design
The rats were divided into four groups:

Group 1: Sham group (n = 6). This group consisted of animals treated with oral saline alone.

Group 2: Diabetic group (n = 6). This group consisted of animals in which only diabetes was induced and the animals were treated with oral saline.

Group 3: CA-treated group (n = 6). This group consisted of animals treated with an oral dose of 50 mg/kg b.w. CA in saline for 28 days.

Group 4: CA-treated diabetic group (n = 6). This group consisted of animals treated with an oral dose of 50 mg/kg b.w. CA in saline following the induction of diabetes for 28 days.

The CA dose (50 mg/kg b.w. per os) was selected according to our unpublished studies. At the end of the experimental period, all animals were decapitated

under anesthesia (90 mg/kg ketamine hydrochloride, i.p.). Cardiac blood was collected into preservative-free heparin tubes. Whole blood samples were obtained via the intracardiac method. The serum was immediately separated by centrifugation at 2000 rpm for 15 min. The plasma was immediately separated by centrifugation at 4000 rpm for 5 min at 4 °C. The serum and plasma samples were stored at –80 °C until being assayed. Livers and kidneys were removed. The organs were examined for changes in size, color, and texture. The samples were kept in the dark at 4 °C and processed within 4 h for comet assay. The serum, plasma, liver, and kidney homogenates were kept at –80 °C for the determination of oxidative stress parameters, hepatic enzymes, and lipid profiles.

2.5. Comet assay (single-cell gel electrophoresis)

For the comet assay, blood samples and liver and kidney homogenates were used. The liver and kidney tissues were carefully dissected from their attachments and totally excised. Preparation of a single-cell suspension from the organs was performed according to standard procedures (20,21). Briefly, approximately 0.2 g of each organ was placed in 1 mL of chilled mincing solution (HBSS with 20 mM EDTA and 10% DMSO) in a petri dish and chopped into pieces with a pair of scissors. The pieces were allowed to settle and the supernatant containing the single-cell suspension was taken. The concentrations of renal and hepatic tissue cells in the supernatant were adjusted to approximately 2×10^6 cells/mL in HBSS containing 20 mM EDTA/10% DMSO.

The basic alkaline comet assay of Tice et al. (21) as further described by Bacanlı et al. (22) was performed. Cells were embedded on agarose gel and lysed, and fragmented DNA strands were drawn out by electrophoresis to form a comet. After electrophoresis, the slides were neutralized and then incubated in 50%, 75%, and 98% alcohol for 5 min each. The dried microscopic slides were stained with EtBr (20 mg/mL in distilled water, 60 mL/slide) and scored with a Leica fluorescence microscope (Wetzlar, Germany) under green light. The microscope was connected to a charge-coupled device camera and a personal computer-based analysis system (Comet Analysis Software, version 3.0, Kinetic Imaging Ltd., Liverpool, UK) to determine the extent of DNA damage after electrophoretic migration of the DNA fragments in the agarose gel. In order to visualize DNA damage, 100 nuclei per slide were examined at 40× magnification. Results were expressed as the percentage of DNA in the tail (tail intensity).

2.6. Determination of biochemical parameters

The determination of insulin, total bilirubin, and BCA protein levels in the plasma samples was performed spectrophotometrically with assay kits at 450, 530, and 562 nm, respectively. Results were expressed as μIU/mL, mg/dL, and μg/mL, respectively.

2.7. Determination of liver enzyme levels

The determination of ALT, AST, AP, and GGT levels in the serum samples was performed spectrophotometrically with assay kits at 570, 450, and 418 nm, respectively. Results were expressed as mU/mL.

2.8. Determination of lipid levels

The determination of HDL, LDL, total cholesterol, and triglyceride levels in the serum samples was performed spectrophotometrically with assay kits at 570 nm. Results were expressed as μg/mL for HDL, LDL, and total cholesterol and mg/dL for triglyceride levels.

2.9. Determination of oxidative stress parameters

Oxidative stress parameters were assayed in the plasma samples and in liver and kidney homogenates. The liver and kidney tissues were extracted, weighed, and subjected to homogenization and sonication procedures (23). The homogenates of the tissue samples were kept at –80 °C until the time of analysis.

The determination of CAT, SOD, GR, GST, and GSH-Px enzyme activities and GSH levels in the plasma samples and liver tissues and 8-OHdG levels in the plasma samples was performed spectrophotometrically using kits following the manufacturer's procedures at 540, 440, 340, 340, 420, 420, and 535 nm, respectively. Results were expressed as mmol min^{-1} mg^{-1} for enzyme activities, μM for GSH, pg/mL for 8-OHdG, and nmol/g for MDA levels.

2.10. Statistical analysis

Analysis of data was performed using SPSS 20.0 for Windows (IBM Corp., Armonk, NY, USA). The distribution of the data was checked for normality using the Kolmogorov–Smirnov test. The homogeneity of the variance was verified by the Levene test. The differences among the groups with normal distribution were evaluated by the one-way analysis of variance (ANOVA) test and post hoc analysis of group differences was performed by the LSD test. The differences among the groups without normal distribution were evaluated by Kruskal–Wallis test. The results were given as mean ± standard deviation. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comet assay

The DNA damage, expressed as tail intensity in the blood, liver, and kidney cells of rats, is shown in Figure 2. In all biological samples there were no statistically significant differences in tail intensity between the sham group and the CA-treated groups ($P > 0.05$). The DNA damage was found significantly higher in the diabetic group compared to the sham group ($P < 0.05$). CA treatment in the diabetic group was found to decrease the DNA damage significantly ($P < 0.05$).
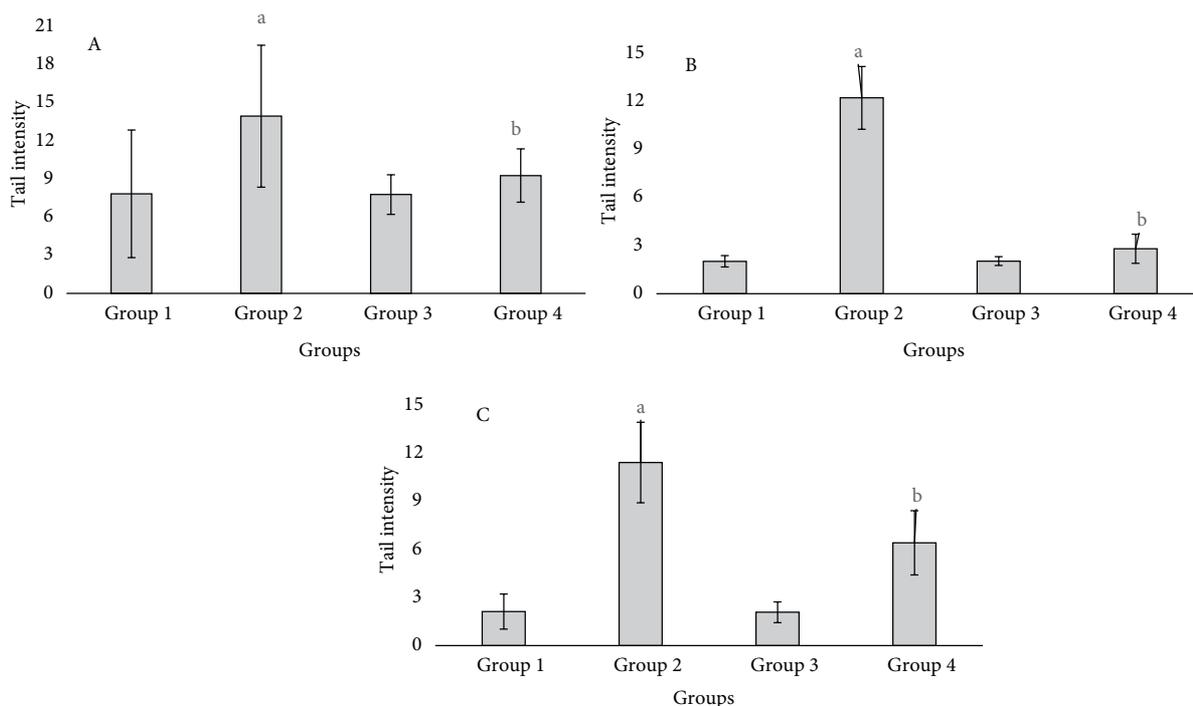
Figure 2. DNA damage of rats: A) blood cells; B) liver cells; C) kidney cells. The values are expressed as mean ± standard deviation. [a] Statistically different from sham group (P < 0.05); [b] statistically different from diabetes group (P < 0.05). Group 1: Sham; Group 2: diabetic rats; Group 3: CA (50 mg/kg)-treated rats; Group 4: CA (50 mg/kg)-treated diabetic rats.

3.2. Biochemical parameters
The insulin, total bilirubin, and BCA protein levels in the plasma samples are shown in Table 1. The insulin levels were significantly decreased in the diabetic group compared to the sham group (P < 0.05) and the levels increased significantly in the CA-treated diabetic group compared to the diabetic group (P < 0.05).

There was no significant increase between the sham and the diabetic group in the total plasma bilirubin and plasma BCA protein levels.

3.3. Liver enzyme levels
The serum ALT, AST, AP, and GGT levels in the groups are shown in Table 2.

The ALT, AST, AP, and GGT levels were significantly increased in the diabetic group compared to the sham group (P < 0.05). The serum AST levels in the CA-treated diabetic group were found to be significantly lower than in the diabetic group (P < 0.05). There were also decreases in the levels of ALT, AP, and GGT in the CA-treated diabetic group compared to the diabetic group, but these differences were not statistically significant.

3.4. Lipid levels
The serum HDL, LDL, total cholesterol, and triglyceride levels are shown in Table 3.

The LDL, total cholesterol, and triglyceride levels were significantly higher in the diabetic group compared to the sham group (P < 0.05). The LDL, total cholesterol, and triglyceride levels in the serum samples of the CA-treated diabetic group were found to be significantly lower than in the diabetic group (P < 0.05). The HDL levels were significantly decreased in the diabetic group compared to the sham group (P < 0.05), but the HDL levels in the serum samples of the CA-treated diabetic group were found to significantly increase in the diabetic group (P < 0.05).

3.5. Oxidative stress parameters
The CAT, SOD, GR, GST, and GSH-Px enzyme activities; the GSH and MDA levels in the plasma, liver, and kidney tissues; and the plasma 8-OHdG levels are shown in Tables 4, 5, and 6, respectively.

The CAT, SOD, GSH-Px, and GST enzyme activities and GSH levels were found to be significantly lower in the diabetic group compared to the sham group (P < 0.05). CAT, SOD, and GSH-Px enzyme activities and GSH levels in the plasma, livers, and kidneys increased significantly in the CA-treated diabetic group compared to the diabetic group (P < 0.05). GST levels of the CA-treated diabetic group were higher than in the diabetic group, but the difference was not statistically significant. There was no significant increase between the sham and CA-treated diabetic groups.

Plasma 8-OHdG and MDA levels and plasma, hepatic, and renal GR enzyme activities were found to significantly

Table 1. Biochemical findings of plasma samples of experimental groups.

	Insulin levels (µIU/mL)	Total bilirubin levels (mg/dL)	BCA protein levels (µg/mL)
Group 1	192.3 ± 50.9	4.3 ± 4.6	3668.3± 68.9
Group 2	68.0 ± 65.7 [a]	7.7 ± 10.8	3726.9 ± 25.0
Group 3	162.5 ± 51.0	2.2 ± 2.4	3731.9 ± 69.6
Group 4	212.9 ± 40.7 [b]	5.1 ± 3.9	3642.8 ± 35.1

The values are expressed as mean ± standard deviation; [a] statistically different from sham group (P < 0.05); [b] statistically different from diabetes group (P < 0.05).
Group 1: Sham; Group 2: diabetic rats; Group 3: CA (50 mg/kg)-treated rats; Group 4: CA (50 mg/kg)-treated diabetic rats.
BCA: Bicinchoninic acid.

Table 2. Liver enzyme levels of plasma samples of experimental groups.

	ALT levels (mU/mL)	AST levels (mU/mL)	AP (mU/mL)	GGT levels (mU/mL)
Group 1	1984.4 ± 2348.2 [b]	79.1 ± 124.9 [b]	39.2 ± 14.0 [b]	1926.4 ± 1696.5 [b]
Group 2	8457.1 ± 8684.3 [a]	1535.8 ± 1924.7 [a]	135.0 ± 24.0 [a]	4213.2 ± 4157.8 [a]
Group 3	1369.7 ± 572.2 [b]	61.0 ± 76.1 [b]	31.0 ± 6.1 [b]	759.4 ± 1324.1 [b]
Group 4	7480.9 ± 6711.0	659.4 ± 647.5 [b]	89.4 ± 7.5	3191.0 ± 3196.3

The values are expressed as mean ± standard deviation; [a] statistically different from sham group (P < 0.05); [b] statistically different from diabetes group (P < 0.05).
Group 1: Sham; Group 2: diabetic rats; Group 3: CA (50 mg/kg)-treated rats; Group 4: CA (50 mg/kg)-treated diabetic rats.
ALT: Alanine aminotransferase; AST: aspartate aminotransferase; AP; alkaline phosphatase, GGT: gamma-glutamyl transferase.

increase in the diabetes group compared to the sham group (P < 0.05). The levels were found to significantly decrease in the CA-treated diabetic group compared to the diabetic group (P < 0.05). CA alone did not cause significant changes in any of the studied oxidative stress parameters compared to the sham group (P > 0.05).

4. Discussion
The antihyperglycemic activity of medicinal plants or plant-derived products needs extensive research as the number of diabetic patients is continuously on the rise according to WHO projections (24). In animal studies, phenolic acids have been reported to exert antiinflammatory, antioxidant, antidiabetic, antimutagenic, anticarcinogenic, and body mass-reducing activities. Cinnamic acid is the parent compound of its esters, which are more volatile to be transported to other parts easily (25,26).

It is known that oxidative stress results from an imbalance between the generation of oxygen-derived radicals and the antioxidant system. Numerous studies have shown that diabetes is associated with increased formation of free radicals and decrease in antioxidant potential. In both types of diabetes, oxidative stress is increased. Multiple factors can cause oxidative stress in diabetes. The most important factor is glucose autoxidation, leading to the production of free radicals. Other factors include cellular oxidation/reduction imbalances and reduction in antioxidant defenses (including decreased cellular antioxidant levels and a reduction in the activity of enzymes that dispose of free radicals). Another important factor is the interaction of advanced glycation end products (AGEs) with specific cellular receptors called AGE receptors. Elevated levels of AGEs are formed under hyperglycemic conditions. Their formation is initiated when glucose interacts with specific amino acids on proteins, forming a compound that then undergoes further chemical reactions. Glycation of protein alters protein and cellular function and the binding of AGEs to their receptors, which can lead to modification in cell signaling and further production of free radicals (27). The other nonenzymatic factors are activation of NAD(P)H oxidases, nitric oxide synthase, and a specific enzyme activity, xanthine oxidase, which produces oxidant species and subsequent oxidative stress (28–30).

Table 3. Lipid levels of serum samples of experimental groups.

	HDL levels (µg/mL)	LDL levels (µg/mL)	Cholesterol levels (µg/mL)	Triglyceride levels (mg/dL)
Group 1	0.7 ± 0.1 [b]	0.3 ± 0.0 [b]	0.2 ± 0.0 [b]	159.0 ± 26.0 [b]
Group 2	0.3 ± 0.2 [a]	0.5 ± 0.0 [a]	0.7 ± 0.0 [a]	514.4 ± 289.0 [a]
Group 3	0.6 ± 0.2 [b]	0.3 ± 0.1 [b]	0.2 ± 0.0 [b]	167.7 ± 24.7 [b]
Group 4	0.5 ± 0.1 [b]	0.4 ± 0.0 [b]	0.2 ± 0.0 [b]	433.4 ± 54.0 [a, b]

The values are expressed as mean ± standard deviation; [a] statistically different from sham group ($P < 0.05$); [b] statistically different from diabetes group ($P < 0.05$).
Group 1: Sham; Group 2: diabetic rats; Group 3: CA (50 mg/kg)-treated rats; Group 4: CA (50 mg/kg)-treated diabetic rats.
HDL: High-density lipoprotein; LDL: low-density lipoprotein.

Table 4. Oxidative stress parameters in the plasma samples of experimental groups.

	Group 1	Group 2	Group 3	Group 4
CAT activity (nmol min^{-1} mL^{-1})	153.6 ± 13.2 [b]	89.2 ± 34.1 [a]	151.6 ± 4.7 [b]	151.9 ± 26.5 [b]
SOD activity (U/mL)	0.9± 0.6 [b]	0.4 ± 0.3 [a]	1.4 ± 0.2 [b]	0.8 ± 0.2 [b]
GSH-Px activity (nmol min^{-1} mL^{-1})	114.4± 2.4 [b]	42.4 ± 9.4 [a]	115.4 ± 1.1 [b]	95.1 ± 7.4 [b]
GR activity (nmol min^{-1} mL^{-1})	2.4 ± 0.4 [b]	9.1 ± 3.4 [a]	2.9 ± 0.8 [b]	4.1 ± 1.8 [b]
GSH levels (µM)	4.1 ± 1.2 [b]	2.1 ± 0.1 [a]	4.2 ± 0.3 [b]	3.0 ± 0.9 [b]
GST (nmol min^{-1} mL^{-1})	7.6 ± 0.7 [b]	3.7 ± 2.0 [a]	8.9 ± 0.9	6.0 ± 2.0
8-OHdG levels (ng/mL)	11.1 ± 5.9 [b]	20.1 ± 7.0 [a]	10.4 ± 7.2 [b]	7.8 ± 2.0 [a, b]
MDA levels (nmol/g)	11.4 ± 4.1 [b]	26.4 ± 4.4 [a]	11.7 ± 3.5 [b]	14.1 ± 2.6 [b]

The values are expressed as mean ± standard deviation; [a] statistically different from sham group ($P < 0.05$); [b] statistically different from diabetes group ($P < 0.05$).
Group 1: Sham; Group 2: diabetic rats; Group 3: CA (50 mg/kg)-treated rats; Group 4: CA (50 mg/kg)-treated diabetic rats
CAT: Catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GST; glutathione S-transferase, 8-OHdG: 8-hydroxy-2'-deoxyguanosine; MDA: malondialdehyde.

Table 5. Oxidative stress parameters in the livers of experimental groups.

	Group 1	Group 2	Group 3	Group 4
CAT activity (nmol min^{-1} mL^{-1})	15.1 ± 2.1 [b]	10.4 ± 4.7 [a]	16.0 ± 2.1 [b]	15.0 ± 3.9 [b]
SOD activity (U/mL)	0.4 ± 0.3 [b]	0.2 ± 0.1 [a]	0.7 ± 0.2 [b]	0.4 ± 0.4 [b]
GSH-Px activity (nmol min^{-1} mL^{-1})	124.1 ± 2.5 [b]	56.5± 5.4 [a]	119.5 ± 3.4 [b]	92.4 ± 8.8 [b]
GR activity (nmol min^{-1} mL^{-1})	3.7 ± 1.2 [b]	11.3 ± 6.2 [a]	4.7 ± 3.8 [b]	6.3 ± 2.0 [b]
GSH levels (µM)	9.9 ± 3.6 [b]	5.4 ± 0.5 [a]	8.1 ± 4.4 [b]	7.8 ± 1.2 [b]
GST (nmol min^{-1} mL^{-1})	7.6 ± 0.7 [b]	3.7 ± 2.0 [a]	73.4 ± 7.8	75.1 ± 8.4
MDA levels (nmol/g)	13.4 ± 5.1 [b]	24.3 ± 4.7 [a]	15.2 ± 1.7 [b]	16.8 ± 3.1 [b]

The values are expressed as mean ± standard deviation; [a] statistically different from sham group ($P < 0.05$); [b] statistically different from diabetes group ($P < 0.05$).
Group 1: Sham; Group 2: diabetic rats; Group 3: CA (50 mg/kg)-treated rats; Group 4: CA (50 mg/kg)-treated diabetic rats.
CAT: Catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GST; glutathione S-transferase, MDA: malondialdehyde.

Table 6. Oxidative stress parameters in the kidneys of experimental groups.

	Group 1	Group 2	Group 3	Group 4
CAT activity (nmol min^{-1} mL^{-1})	166.4 ± 8.2 [b]	65.2 ± 3.7 [a]	167.0 ± 4.3 [b]	127.0 ± 23.5 [a, b]
SOD activity (U/mL)	0.8 ± 0.5 [b]	0.3 ± 0.0 [a]	0.8 ± 0.2 [b]	0.6 ± 0.3 [b]
GSH-Px activity (nmol min^{-1} mL^{-1})	149.4 ± 9.4 [b]	64.4 ± 1.4 [a]	132.4 ± 1.1 [b]	130.4 ± 2.4 [b]
GR activity (nmol min^{-1} mL^{-1})	4.4 ± 1.4 [b]	12.0 ± 3.4 [a]	5.0 ± 2.1 [b]	6.8 ± 1.1 [b]
GSH levels (µM)	8.3 ± 0.6 [b]	5.3 ± 0.1 [a]	8.0 ± 0.3 [b]	7.9 ± 0.8 [b]
GST (nmol min^{-1} mL^{-1})	7.6 ± 0.7 [b]	3.7 ± 2.0 [a]	42.1 ± 10.4	48.2 ± 11.4
MDA levels (nmol/g)	12.4 ± 4.0 [b]	23.8 ± 6.1 [a]	14.4± 5.7 [b]	14.8 ± 8.4 [b]

The values are expressed as mean ± standard deviation; [a] statistically different from sham group (P < 0.05); [b] statistically different from diabetes group (P < 0.05).
Group 1: Sham; Group 2: diabetic rats; Group 3: CA (50 mg/kg)-treated rats; Group 4: CA (50 mg/kg)-treated diabetic rats.
CAT: Catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GST; glutathione S-transferase, MDA: malondialdehyde.

Numerous reports have documented elevations in peroxide levels in plasma, red blood cells, and tissues of animals with chemically induced diabetes (31,32). In diabetic patients, both increases and decreases in the activities of key antioxidant enzymes including CAT, SOD, GSH-Px, and GR have been reported (33,34). In a pediatric study, antioxidant activity was found to be decreased in relation to poor glycemic control (35). It is also shown that oxidative stress exists in diabetic patients, as evidenced by increased total antioxidant capacity in the saliva and blood of patients (36).

In the present study, we evaluated the ameliorative effects of CA against STZ-induced diabetes and its complications in Wistar albino rats. We found that STZ-induced diabetes caused an increase in hepatic enzyme (ALT, AST, AP, and GGT) levels and a decrease in insulin levels compared to the sham group. There was no significant increase between the sham and diabetic groups in the total bilirubin levels of plasma samples. As far as we know, this is the first study about the correlation between diabetes and BCA protein levels. We found no significant differences between the sham and diabetic groups in BCA protein levels in the plasma samples. Diabetic rats have significantly lower HDL but significantly higher LDL, total cholesterol, and triglyceride levels when compared to the sham group. When evaluating the oxidative stress parameters, GSH levels and activities of CAT, SOD, GSH-Px, and GST were significantly lower while 8-OHdG and MDA levels and activities of GR were significantly higher in the diabetic group compared to the sham group. CA itself did not induce abnormalities in the oxidative stress parameters. It also ameliorated the oxidative damage induced by diabetes.

Inhibition of α-glucosidase may be effective in diabetes therapy. Due to this effect, mammalian α-glucosidase inhibitors from natural sources can be beneficial in the prevention and treatment of DM. Adisakwattana et al. (37) demonstrated the α-glucosidase inhibitory activity of cinnamic acid derivates against intestinal sucrase inhibitors. It was shown that cinnamon extracts (50, 100, 150, and 200 mg/kg), which include CA significantly, decreased the blood glucose and lipid levels in mice (38). Ping et al. (39) studied the hypoglycemic effect of cinnamon oil, which contains water-soluble polyphenol type A polymer, cinnamaldehyde, and cinnamic acid as active compounds, in a type 2 diabetic animal model. They found that fasting blood glucose concentration was significantly decreased in the 100 mg/kg group compared to other groups. In addition, they found significant decreases in plasma C-peptide, serum triglyceride, total cholesterol, and blood urea nitrogen levels while serum HDL levels were significantly increased after 35 days. Huang et al. (40) reported that caffeic and cinnamic acids improved glucose uptake in TNF-α-treated insulin-resistant FL83B cells. The same group treated the mouse FL83B cells with TNF-α to induce insulin resistance to evaluate the effects of caffeic and cinnamic acids on glucose metabolism. They found that caffeic and cinnamic acids increased the expression of glycogen synthase, whereas the expression of glycogen synthase kinase and phosphorylation of glycogen synthase in Ser641 in insulin-resistant mouse hepatocytes was decreased. The compounds suppressed the expression of hepatic nuclear factor-4 in TNF-α-treated mouse FL83B hepatocytes. They concluded that caffeic and cinnamic acids ameliorated glucose metabolism by promoting glycogenesis and inhibiting gluconeogenesis in TNF-α-

treated insulin-resistant mouse hepatocytes (41). Rao and Rao (42) reported the antihyperglycemic effect of *Syzygium alternifolium* seeds, which contain CA. Treatment with 50 mg/kg b.w. daily of fraction C (which includes CA) for 30 days resulted in a significant decrease in the fasting blood glucose levels of diabetic rats. The altered enzyme activities of carbohydrate metabolism in the livers and kidneys of diabetic rats were significantly reverted to near normal levels by the administration of fraction C (43). Adisakwattana et al. (44) reported that CA and its derivatives (p-methoxycinnamic acid and ferulic acid) have insulin-secreting activity in pancreatic β cells. Cinnamaldehyde isolated from *Cinnamomum cassia* has been shown to have hypoglycemic and hypolipidemic effects in STZ-induced diabetic rats (45). Lakshmi et al. (46) reported that CA activates insulin-mediated glucose transport. Yibchok-anun et al. (47) showed that oral administration of p-methoxycinnamic acid (40–100 mg/kg) significantly decreased plasma glucose and also increased plasma insulin concentrations in both healthy and STZ-induced diabetic rats.

There are limited studies about genotoxic damage caused by DM. In these studies, it is claimed that reactive oxygen species production could cause DNA damage in diabetes. Kushawa et al. (48) determined the DNA damage in lung, liver, aorta, heart, kidney, pancreas, and blood samples of experimentally induced diabetic rats by standard comet, endonuclease III, and formamidopyrimidine modified comet assays. They showed that DNA damage was significantly higher in the diabetic group compared to the nondiabetic group.

In this study, the DNA damage in the blood, liver, and kidney cells in the diabetes-induced rats were found to be higher compared to the sham group. CA treatment (50 mg/kg daily, per os) was found to significantly decrease the DNA damage in the blood, liver, and kidney cells of the diabetic rats. Our results are in accordance with previous reports about diabetes, its complications, and the effects of natural antioxidants against diabetes.

In conclusion, diabetes is affecting a significant proportion of the population worldwide. It affects many organs, including the pancreas, kidneys, and liver. The disease is associated with a reduced quality of life and increased risk factors for mortality and morbidity. In diabetes treatment, traditional herbal folk medicines are getting popular. Due to their antioxidant properties, herbal products give positive and promising results. With the overall results of the present study, we have demonstrated that STZ-induced diabetic rats have DNA damage and alterations in hepatic enzymes, lipid profiles, and biochemical and oxidative stress parameters. CA treatment was found to be protective against diabetes-induced damage in the blood, liver, and kidneys of rats. Further in vitro, in vivo, and epidemiological studies are required to clear up the role of CA in prevention and management of diabetes and its complications.

References

1. Eldar-Finkelman H, Kaidanovich O. The role of glycogen synthase kinase-3 in insulin resistance and type 2 diabetes. Expert Opin Ther Targets 2002; 6: 555-561.

2. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, Lin JK, Farzadfar F, Khang YH, Stevens GA et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. Lancet 2011; 378: 31-40.

3. Fatima A, Agrawal P, Singh PP. Herbal option for diabetes: an overview. Asian Pac J Trop Dis 2012; 2: S536-S44.

4. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010; 87: 4-14.

5. Rotenstein LS, Kozak BM, Shivers JP, Yarchoan M, Close J, Close KL. The ideal diabetes therapy: what will it look like? How close are we? Clin Diabetes 2012; 30: 44-53.

6. Yusuff KB, Obe O, Joseph BY. Adherence to anti-diabetic drug therapy and self management practices among type-2 diabetics in Nigeria. Pharm World Sci 2008; 30: 876-883.

7. Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 2005; 99: 325-348.

8. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: pharmacological and toxicological considerations. J Ethnopharmacol 2014; 155: 857-924.

9. Eilerman RG. Cinnamic acid, cinnamaldehyde, and cinnamyl alcohol. In: Seidel A, editor. Kirk-Othmer Encyclopedia of Chemical Technology. 5th ed. New York, NY, USA: John Wiley & Sons, Inc.; 2001. pp. 595-603.

10. Manach C, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr 2004; 79: 727-747.

11. Lee EJ, Kim SR, Kim J, Kim YC. Hepatoprotective phenylpropanoids from *Scrophularia buergeriana* roots against CCl$_4$-induced toxicity: action mechanism and structure-activity relationship. Planta Med 2002; 68: 407-411.

12. Wiesner J, Mitsch A, Wissner P, Jomaa H, Schlitzer M. Structure-activity relationships of novel anti-malarial agents. Part 2: cinnamic acid derivatives. Bioorg Med Chem Lett 2001; 12: 423-424.

13. Banskota AH, Nagaoka T, Sumioka LY, Tezuka Y, Awale S, Midorikawa K, Matsushige K, Kadota S. Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines. J Ethnopharmacol 2002; 80: 67-73.

14. Cartron E, Carbonneau MA, Fouret G, Descomps B, Léger CL. Specific antioxidant activity of caffeoyl derivatives and other natural phenolic compounds: LDL protection against oxidation and decrease in the proinflammatory lysophosphatidylcholine production. J Nat Prod 2001; 64: 480-486.

15. Taner G, Özkan Vardar D, Aydin S, Aytaç Z, Başaran A, Başaran N. Use of in vitro assays to assess the potential cytotoxic, genotoxic and antigenotoxic effects of vanillic and cinnamic acid. Drug Chem Toxicol 2017; 40: 183-190.

16. Bickers D, Calow P, Greim H, Hanifin J, Rogers A, Saurat J, Sipes IG, Smith RL, Tagami H. A toxicologic and dermatologic assessment of cinnamyl alcohol, cinnamaldehyde and cinnamic acid when used as fragrance ingredients: the RIFM expert panel. Food Chem Toxicol 2005; 43: 799-836.

17. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia 2008; 51: 216-226.

18. King AJ. The use of animal models in diabetes research. Br J Pharmacol 2012; 166: 877-894.

19. Cumaoğlu A, Ozansoy G, Irat AM, Arıcıoğlu A, Karasu Ç, Arı N. Effect of long term, non cholesterol lowering dose of fluvastatin treatment on oxidative stress in brain and peripheral tissues of streptozotocin-diabetic rats. Eur J Pharmacol 2011; 654: 80-85.

20. Patel R, Shervington A, Pariente JA, Martinez-Burgos MA, Salido GM, Adeghate E, Salido GM, Adeghate E, Singh J. Mechanism of exocrine pancreatic insufficiency in streptozotocin-induced type 1 diabetes mellitus. Ann N Y Acad Sci 2006; 1084: 71-88.

21. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 2000; 35: 206-221.

22. Bacanlı M, Aydın S, Taner G, Göktaş H, Şahin T, Başaran A, Başaran N. Does rosmarinic acid treatment have protective role against sepsis-induced oxidative damage in Wistar albino rats? Hum Exp Toxicol 2016; 35: 877-886.

23. Sier C, Kubben F, Ganesh S, Heerding M, Griffioen G, Hanemaaijer R, van Krieken JH, Lamers CB, Verspaget HW. Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. Br J Cancer 1996; 74: 413-417.

24. Rathmann W, Giani G. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 2568-2569.

25. Hsu CL, Yen GC. Phenolic compounds: evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. Mol Nutr Food Res 2008; 52: 53-61.

26. Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci 2007; 30: 3268-3295.

27. Penckofer S, Schwertz D, Florczak K. Oxidative stress and cardiovascular disease in type 2 diabetes: the role of antioxidants and pro-oxidants. J Cardiovasc Nurs 2002; 16: 68-85.

28. Desco MC, Asensi M, Márquez R, Martínez-Valls J, Vento M, Pallardó FV, Sastre J, Viña J. Xanthine oxidase is involved in free radical production in type 1 diabetes protection by allopurinol. Diabetes 2002; 51: 1118-1124.

29. Inoguchi T, Li P, Umeda F, Yu HY, Kakimoto M, Imamura M, Aoki T, Etoh T, Hashimoto T, Naruse M et al. High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. Diabetes 2000; 49: 1939-1945.

30. Cosentino F, Hishikawa K, Katusic ZS, Lüscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997; 96: 25-28.

31. Armstrong D, Al-Awadi F. Lipid peroxidation and retinopathy in streptozotocin-induced diabetes. Free Radic Biol Med 1991; 11: 433-436.

32. Rungby J, Flyvbjerg A, Andersen HB, Nyborg K. Lipid peroxidation in early experimental diabetes in rats: effects of diabetes and insulin. Acta Endocrinol 1992; 126: 378-380.

33. Godin DV, Wohaieb SA. Reactive oxygen radical processes in diabetes. In: Singal PK, editor. Oxygen Radicals in the Pathophysiology of Heart Disease. Developments in Cardiovascular Medicine. 1st ed. Boston, MA, USA: Springer; 1988. pp. 303-322.

34. Godin DV, Wohaieb SA, Garnett ME, Goumeniouk A. Antioxidant enzyme alterations in experimental and clinical diabetes. Mol. Cell. Biochem 1988; 84: 223-231.

35. Asayama K, Uchida N, Nakane T, Hayashibe H, Dobashi K, Amemiya S, Kato K, Nakazawa S. Antioxidants in the serum of children with insulin-dependent diabetes mellitus. Free Radic Biol Med 1993; 15: 597-602.

36. Astaneie F, Afshari M, Mojtahedi A, Mostafalou S, Zamani MJ, Larijani B, Abdollahi M. Total antioxidant capacity and levels of epidermal growth factor and nitric oxide in blood and saliva of insulin-dependent diabetic patients. Arch Med Res 2005; 36: 376-381.

37. Adisakwattana S, Chantarasinlapin P, Thammarat H, Yibchok-Anun S. A series of cinnamic acid derivatives and their inhibitory activity on intestinal α-glucosidase. J Enzyme Inhib Med Chem 2009; 24: 1194-1200.

38. Kim SH, Hyun SH, Choung SY. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J Ethnopharmacol 2006; 104: 119-123.

39. Ping H, Zhang G, Ren G. Antidiabetic effects of cinnamon oil in diabetic KK-Ay mice. Food Chem Toxicol 2010; 48: 2344-2349.

40. Huang DW, Shen SC, Wu JSB. Effects of caffeic acid and cinnamic acid on glucose uptake in insulin-resistant mouse hepatocytes. J Agric Food Chem 2009; 57: 7687-7692.

41. Huang DW, Shen SC. Caffeic acid and cinnamic acid ameliorate glucose metabolism via modulating glycogenesis and gluconeogenesis in insulin-resistant mouse hepatocytes. J Funct Foods 2012; 4: 358-366.

42. Rao BK, Rao CA. Hypoglycemic and antihyperglycemic activity of *Syzygium alternifolium* (Wt.) Walp. seed extracts in normal and diabetic rats. Phytomedicine 2001; 8: 88-93.

43. Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EGT, Swapna S, Ramesh B, Rao CA. Antihyperglycemic and antihyperlipidemic activities of methanol:water (4:1) fraction isolated from aqueous extract of Syzygium alternifolium seeds in streptozotocin induced diabetic rats. Food Chem Toxicol 2010; 48: 1078-1084.

44. Adisakwattana S, Moonsan P, Yibchok-Anun S. Insulin-releasing properties of a series of cinnamic acid derivatives in vitro and in vivo. J Agric Food Chem 2008; 56: 7838-7844.

45. Babu PS, Prabuseenivasan S, Ignacimuthu S. Cinnamaldehyde—a potential antidiabetic agent. Phytomedicine 2007; 14: 15-22.

46. Lakshmi BS, Sujatha S, Anand S, Sangeetha KN, Narayanan RB, Katiyar C, Kanaujia A, Duggar R, Singh Y, Srinivas K. Cinnamic acid, from the bark of *Cinnamomum cassia*, regulates glucose transport via activation of GLUT4 on L6 myotubes in a phosphatidylinositol 3-kinase-independent manner. J Diabetes 2009; 1: 99-106.

47. Yibchok-anun S, Adisakwattana S, Moonsan P, Hsu WH. Insulin-secretagogue activity of p-methoxycinnamic acid in rats, perfused rat pancreas and pancreatic β-cell line. Basic Clin Pharmacol Toxicol 2008; 102: 476-482.

48. Kushwaha S, Vikram A, Trivedi P, Jena G. Alkaline, Endo III and FPG modified comet assay as biomarkers for the detection of oxidative DNA damage in rats with experimentally induced diabetes. Mutat Res Genet Toxicol Environ Mutagen 2011; 726: 242-250.