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Effect of Sinapic acid on Antiperoxidative and Antioxidant Potential in Normal and Streptozotocin-induced Diabetes in Wistar Rats

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ABSTRACT
The present study was aimed to investigate the effect of Sinapic acid on antioxidant potential in normal and Streptozotocin-induced diabetic rats. Diabetes was induced in female wistar rats by a single intraperitoneal administration of Streptozotocin (45 mg/ kg BW). Rats were divided into six groups: normal (untreated), normal + Sinapic acid (15mg/kg), normal + Sinapic acid (30 mg/kg), diabetic control, diabetic + Sinapic acid (15mg/kg) and diabetic + Sinapic acid (30 mg/kg). Diabetic rats exhibited elevated levels of lipid peroxidation markers such as thiobarbituric acid reactive substances and hydroperoxides in plasma and tissues and decreased levels of antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP_X), reduced glutathione (GSH), ceruloplasmin, vitamin C and vitamin E in serum and tissues. Oral administration of Sinapic acid for a period of 35 days significantly decreased lipid peroxidation markers and increased the antioxidants suggesting the antioxidant potential of Sinapic acid in diabetic rats.

Keywords: Diabetes, Streptozotocin, antioxidant, lipid peroxidation, Sinapic acid.

INTRODUCTION

Diabetes mellitus, a common metabolic disorder, is characterized mainly by chronic hyperglycemia resulting from defects in insulin secretion and /or its action. This eventually leads to improper regulation of carbohydrate, protein and lipid metabolism that ultimately contribute to a key factor in the development and the progression of micro and macrovascular complications. [1] The "top three" countries with largest number of diabetic people are India, China and USA. The prevalence of diabetes in India is expected to increase from current 40.9 to 69.9 million by the year 2025 unless urgent preventive steps are taken. [2] Streptozotocin (STZ) is an alkylating agent antibiotic that experimentally produces diabetes due to β-cell death by the mechanism of DNA damage in rodent islets. [3] β-Cells are very susceptible to oxidative changes since they possess a low antioxidative capacity. [4-5]

Currently, oxidative stress is suggested as mechanism underlying diabetes and diabetic complications [6], which results from an imbalance between radical generating and radical scavenging systems. In diabetes, protein glycation and glucose autoxidation may generate free radicals, which in turn catalyze lipid peroxidation. [7-8] Reactive oxygen species (ROS) are part of the defense mechanism against infection, but excessive generation of free oxygen radicals may damage tissue. [9] Co-operative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes. [10] Enzymatic antioxidants namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and non-enzymatic antioxidants such as vitamins C and E, reduced glutathione (GSH) and ceruloplasmin (CP) play an important role in alleviating tissue damage due to the formation of free radical. [11] The efficiency of this defense mechanism is altered in diabetes [12] and therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue injury. Though several pharmacological agents have been developed for management of diabetes, many traditional plant treatments are still used throughout the world. In India, several indigenous plant products have been used by the practitioners of the Ayurvedic system to treat diabetes. [13] Flavonoids, ubiquitously found in the plant kingdom, are proposed to elicit their beneficial effects *in-vivo* in plant through their ability to scavenge oxygen-free radicals, quench transition metals and / or boost the system. [14-15]

Sinapinic acid or Sinapic acid (Sinapine - Origin: L. Sinapi, sinapis, mustard, Gr., cf. F. Sinapine), is a small naturally occurring carboxylic acid. It is a member of the phenylpropanoid family. Sinapic acid is a cinnamic acid derivative which possesses 4-hydroxy-3 5-dimethoxy cinnamic acid is one of the phenolic acids widely distributed in edible plants such as cereals, nuts, oil seeds and berries. [16]

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Sinapic acid is a major free phenolic acid in rapseed meal, with the majority found in the esterified form of sinapine. [17]. It is well known that phenolic acids exist in the bound form by an ester linkage to other molecule in a plant body. [18] Hence, the present study was aimed at evaluating the effect of Sinapic acid on lipid peroxidation and antioxidants (SOD, CAT, GPx, GSH, ceruloplasmin, vitamin C and vitamin E) in wistar rats subjected to streptozotocin-induced oxidative stress.

MATERIALS AND METHODS

Experimental Animals

Female albino wistar rats (150-200 g) obtained from Venkateswara Enterprises, Bangalore were used in this study. They were housed in polypropylene cages (47cm × 34cm × 20cm) lined with husk. It was renewed every 24 hours under a 12:12 hour light: dark cycle at around 22ºC and had free access to water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Limited., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% nitrogen free extract (carbohydrates). The diet provided metabolizable energy of 3600 kcal. The experiment was carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Drug and Chemicals

Streptozotocin (STZ) was purchased from Himedia Laboratories Private Limited, Mumbai. Sinapic acid was purchased from Sigma- Aldrich, St. Louis, USA. All other chemicals and biochemicals used in the study were of analytical grade.

Experimental induction of diabetes

STZ was used for the induction of diabetes mellitus in normoglycemic female albino wistar rats. Diabetes was induced in rats by a single intraperitoneal injection of freshly prepared STZ (45 mg/ kg body weight) in citrate buffer (pH 4.5) in a volume of 1 ml/ kg. [19] STZ injected animals were given 10% glucose solution for 5 days to prevent initial drug induced hyperglycemic mortality. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, 48 hours after injection with STZ. Albino rats with a blood glucose level above 240 mg/ dl were considered to be diabetic and were used in the experiment.

Experimental design

In the experiment, a total of 36 rats (18 diabetic surviving rats and 18 control rats) were used. The rats were divided into 6 groups of 6 rats in each group.

Group 1: Normal control rats

Group 2: Control rats administrated orally with Sinapic acid (15mg/kg)

Group 3: Control rats administrated orally with Sinapic acid (30mg/kg)

Group 4: Diabetic control rats

Group 5: Diabetic rats treated orally with Sinapic acid (15mg/kg)

Group 6: Diabetic rats treated orally with Sinapic acid (30mg/kg)

Sinapic acid was dissolved in 0.2% DMSO and administrated to rats orally using an intragastric tube daily for a period of 35 days.

Sample collection

After 35 days of treatment, the animals were fasted for 12 hours and then sacrificed by cervical decapitation. Blood was collected in tubes with EDTA for biochemical analysis. The liver, kidney and pancreas were carefully removed, weighed and washed in ice-cold saline to remove the blood. The liver, kidney and pancreas were sliced into pieces and homogenized in an appropriate buffer pH 7.0. The homogenates were centrifuged at 3000 rpm for 10 min at 0^oC in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Biochemical estimations

Biochemical parameters such as plasma thiobarbituric acid reactive substances were estimated by the method of Yagi. [20] The levels of lipid peroxidation in tissues were estimated by the method of Nichans and Samuelson. [21] The levels of HP were estimated by the method of Jiang *et al.* [22] Superoxide dismutase was assayed by the method of Kakkar *et al.* [23] The activity of catalase was determined by the method of Sinha. [24] Glutathione peroxidase was estimated by the method of Rotruck *et al.* [25] Reduced glutathione (GSH) was estimated by the method of Ellman. [26] Vitamin C in plasma and tissues were estimated by the method of Omaye *et al.* [27] Vitamin E was estimated in plasma and tissues by the method of Baker *et al.* [28] Ceruloplasmin in serum was estimated by the method of Ravin. [29]

Statistical Analysis

Results were expressed as mean ± SD for six rats in each experimental group. Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) 9.05 software. The data were analyzed using one-way analysis of variance (ANOVA) and group means were compared with Duncan's Multiple Range Test (DMRT). P-values < 0.05 were considered as significant.

RESULTS

Effect of Sinapic acid on TBARS and hydroperoxides

The effect of Sinapic acid on the concentration of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in normal and STZ-induced diabetic rats are presented in Table 1 and 2 respectively. The diabetic rats showed significant increase in the concentration of TBARS and HP in plasma and tissues (liver, kidney and pancreas). Oral administration of Sinapic acid in STZ-induced diabetic rats significantly decreased the levels of TBARS and HP in plasma and tissues.

Effect of Sinapic acid on antioxidants

The activity of enzymatic antioxidants such as SOD, CAT and GPx in the liver, kidney and pancreas of normal and STZ-induced diabetic animals are shown in Table 3 and 4. A significant reduction in the activity of SOD and CAT in the liver, kidney and pancreas of diabetic rats were observed while the activity of GPx decreased in the liver and pancreas and increased in kidney of diabetic rats. Oral administration of Sinapic acid exerted a significant effect on the antioxidants in STZ- induced diabetic rats.

The effect of Sinapic acid on serum ceruloplasmin and GSH in liver, kidney and pancreas of normal and STZ-induced diabetic rats are presented in Table 5. The levels of vitamin C and E in serum, liver and kidney of normal and STZ-induced diabetic rats are shown in Table 6. STZ-induced diabetic rats showed significant decrease in the levels of all the non-enzymatic antioxidants in serum and tissues. Rats treated with Sinapic acid significantly increased the levels of these

Table 1: Effect of Sinapic acid on the levels of TBARS in plasma and tissues of normal and STZ-induced diabetic rats

Groups	Plasma TBARS (nmol/ml)	TBARS (mM/100g tissue)		
		Liver	Kidney	Pancreas
Normal control	0.141 ± 0.02[a]	0.67 ± 0.07[a]	0.75 ± 0.05[a]	0.33 ± 0.03[a]
Normal + Sinapic acid (15mg/kg)	0.145 ± 0.03[a]	0.64 ± 0.03[a]	0.72 ± 0.03[a]	0.36 ± 0.04[a]
Normal + Sinapic acid (30mg/kg)	0.141 ± 0.03[a]	0.66 ± 0.05[a]	0.77 ± 0.05[a]	0.28 ± 0.15[a]
Diabetic control	0.455 ± 0.03[b]	3.64 ± 0.21[b]	3.64 ± 0.25[b]	2.40 ± 0.12[b]
Diabetic + Sinapic acid (15mg/kg)	0.340 ± 0.01[c]	2.55 ± 0.14[c]	2.54 ± 0.19[c]	1.48 ± 0.05[c]
Diabetic + Sinapic acid (30mg/kg)	0.25 ± 0.03[d]	1.85 ± 0.07[d]	1.85 ± 0.15[d]	0.80 ± 0.06[d]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 2: Effect of sinapic acid on the levels of lipid hydroperoxide (HP) in plasma and tissues of normal and STZ- induced diabetic rats

Groups	Plasma Hydroperoxides (mM/dl)	Lipid hydroperoxide (mM/g tissue)		
		Liver	Kidney	Pancreas
Normal control	10.24 ± 0.98[a]	68.92 ± 2.79[a]	43.63 ± 2.28[a]	25.34 ± 2.90[a]
Normal+ Sinapic acid (15mg/kg)	10.65 ± 0.99[a]	66.73 ± 2.34[a]	41.85 ± 1.35[a]	25.23 ± 2.85[a]
Normal + Sinapic acid (30mg/kg)	10.52 ± 0.85[a]	66.93 ± 2.21[a]	42.15 ± 2.23[a]	25.19 ± 2.1[a]
Diabetic control	26.54 ± 1.59[b]	96.48 ± 4.91[b]	72.49 ± 2.38[b]	37.58 ± 2.67[b]
Diabetic + Sinapic acid (15mg/kg)	13.05 ± 0.65[c]	78.89 ± 1.54[c]	64.88 ± 3.24[c]	26.61 ± 1.23[c]
Diabetic + Sinapic acid (30mg/kg)	11.37 ± 0.59[d]	74.71 ± 1.50[d]	58.57 ± 3.07[d]	24.05 ± 1.12[d]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 3: Effect of sinapic acid on the activities of SOD and CAT in tissues of normal and STZ-induced diabetic rats

Groups	SOD (Units[a] / mg protein)			CAT (Units[b] / mg protein)		
	Liver	Kidney	Pancreas	Liver	Kidney	Pancreas
Normal control	10.33 ± 0.48[a]	12.54 ± 0.92[a]	6.19 ± 0.36[a]	67.54 ± 3.02[a]	43.57 ± 3.31[a]	4.39 ± 0.08[a]
Normal+ Sinapic acid (15mg/kg)	10.75 ± 0.56[a]	12.36 ± 0.91[a]	6.15 ± 0.34[a]	67.75 ± 3.35[a]	43.76 ± 3.35[a]	4.36 ± 0.07[a]
Normal + Sinapic acid (30mg/kg)	10.59 ± 0.51[a]	12.78 ± 0.99[a]	6.13 ± 0.35[a]	67.28 ± 3.01[a]	44.05 ± 3.30[a]	4.23 ± 0.05[a]
Diabetic control	5.56 ± 1.20[b]	6.35 ± 0.46[b]	3.55 ± 0.15[b]	35.52 ± 2.10[b]	21.29 ± 1.56[b]	2.04 ± 0.1[b]
Diabetic + Sinapic acid (15mg/kg)	7.34 ± 1.31[c]	8.60 ± 1.51[c]	4.21 ± 0.54[c]	51.60 ± 3.09[c]	27.44 ± 1.89[c]	3.34 ± 0.19[c]
Diabetic + Sinapic acid (30mg/kg)	8.47 ± 1.45[d]	14.20 ± 0.52[d]	5.14 ± 0.59[d]	58.48 ± 3.13[d]	33.52 ± 2.93[d]	3.74 ± 0.25[d]

U[a] - Enzyme concentration required to inhibit the chromogen produced by 50% in one minute.
U[b] - µmol of hydrogen peroxide consumed per minute.
Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 4: Effect of sinapic acid on GPx activity in tissues of normal and STZ-induced diabetic rats

Groups	GPx (Units[a] /min/mg protein)		
	Liver	Kidney	Pancreas
Normal control	8.40 ± 0.52[a]	3.25 ± 0.87[a]	32.51 ± 2.89[a]
Normal+ Sinapic acid (15mg/kg)	8.41 ± 0.50[a]	3.21 ± 0.85[a]	33.74 ± 2.85[a]
Normal + Sinapic acid (30mg/kg)	8.41 ± 0.50[a]	3.20 ± 0.35[a]	32.90 ± 1.25[a]
Diabetic control	4.30 ± 0.31[b]	9.60 ± 2.35[b]	15.40 ± 1.25[b]
Diabetic + Sinapic acid (15mg/kg)	6.17 ± 1.20[c]	6.26 ± 1.21[c]	26.30 ± 2.57[c]
Diabetic + Sinapic acid (30mg/kg)	6.75 ± 0.25[d]	5.36 ± 0.05[d]	28.56 ± 2.55[d]

U[a] - µg of glutathione consumed.
Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 5: Effect of sinapic acid on serum ceruloplasmin and GSH in tissues of normal and STZ-induced diabetic rats

Groups	Ceruloplasmin (mg/dl)	GSH(mg/dl)		
		Liver	Kidney	Pancreas
Normal control	15.57 ± 2.8[a]	8.39 ± 1.52[a]	8.57 ± 1.05[a]	13.18 ± 1.8[a]
Normal+ Sinapic acid (15mg/kg)	15.33 ± 2.1[a]	8.35 ± 1.32[a]	8.37 ± 0.20[a]	13.36 ± 1.61[a]
Normal + Sinapic acid (30mg/kg)	14.64 ± 2.4[a]	8.50 ± 0.57[a]	8.44 ± 0.36[a]	13.11 ± 1.43[a]
Diabetic control	11.90 ± 2.5[b]	4.25 ± 0.47[b]	4.60 ± 0.77[b]	5.56 ± 0.12[b]
Diabetic + Sinapic acid (15mg/kg)	13.49 ± 1.9[c]	5.48 ± 0.35[c]	5.56 ± 0.34[c]	7.63 ± 0.18[c]
Diabetic + Sinapic acid (30mg/kg)	27.36 ± 1.5[d]	6.64 ± 0.38[d]	6.36 ± 1.43[d]	9.22 ± 1.46[d]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Table 6: Effect of Sinapic acid on the levels of vitamin C and vitamin E in serum and tissues of normal and STZ-induced diabetic rats

Groups	Vitamin C (mg/dl)			Vitamin E (mg/dl)		
	Serum	Liver	Kidney	Serum	Liver	Kidney
Normal control	2.42 ± 0.08[a]	0.54 ± 0.12[a]	0.53 ± 0.12[a]	7.29 ± 0.16[a]	5.30 ± 0.4[a]	5.27 ± 0.35[a]
Normal+ Sinapic acid (15mg/kg)	2.34 ± 0.22[a]	0.55± 0.13[a]	0.52 ± 0.11[a]	7.01 ± 0.14[a]	5.22 ± 0.41[a]	5.15 ± 0.31[a]
Normal + Sinapic acid (30mg/kg)	2.23 ± 0.06[a]	0.53 ± 0.12[a]	0.52 ± 0.11[a]	7.01 ± 0.40[a]	5.31 ± 0.40[a]	5.13 ± 0.34[a]
Diabetic control	0.33 ± 0.12[b]	0.73 ± 0.22[b]	0.35 ± 0.06[b]	15.35 ± 0.06[b]	1.66 ± 0.06[b]	3.31 ± 0.14[b]
Diabetic + Sinapic acid (15mg/kg)	1.88 ± 0.24[c]	0.85 ± 0.25[c]	0.41 ± 0.08[c]	17.33 ± 0.08[c]	2.31 ± 0.08[c]	7.49 ± 0.75[c]
Diabetic + Sinapic acid (30mg/kg)	1.94 ± 0.21[d]	0.92 ± 0.21[d]	0.46 ± 0.08[d]	18.25 ± 0.13[d]	3.74 ± 0.13[d]	8.31 ± 0.54[d]

Each value is mean ± S.D. for six rats in each group. Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

antioxidants in serum and tissues when compared with diabetic control rats.

DISCUSSION

Formation of lipid peroxides by the action of free radicals on unsaturated fatty acids has been implicated in the pathogenesis of atherosclerosis and vascular diseases. [30] Oxidative stress may cause oxidative damage of cellular

membranes and changes in the structural and functional integrity of sub-cellular organelles and may produce effects that result in various complications in diabetic disease. [31-34]
In the present study, an increase in the levels of plasma and tissue TBARS, an index of lipid peroxidaton and hydroperoxides were observed in STZ-induced diabetic rats. An observed increase in the levels of TBARS in liver, kidney and pancreas may be due to increased susceptibility of the tissues of diabetic rats to lipid peroxidaton. An increased level of hydroperoxide in the liver, kidney and pancreas observed in the study may be due to decrease in the activities of antioxidant enzymes, which is favourable factor for uncontrolled generation of lipid hydroperoxides. [35] It has been reported that an increase in the levels of lipid peroxides could be generally thought to be the consequence of increased production and liberation into the circulation. [36] Diabetic rats treated with Sinapic acid brought lipid peroxidation markers back to near normal which could be the result of improved antioxidant status. Our result confirmed the possibility of the major function of compound Sinapic acid may be the protection of vital tissues including liver, kidney and pancreas, thereby reducing the causation of diabetes.
A significant decrease in the activity of enzymatic and the levels of non- enzymatic antioxidants were observed in STZ-induced diabetic rats. Reduced activities of SOD and CAT in liver, kidney and pancreas tissues have been observed in diabetic rats and this activity may result in a number of deleterious effects due to accumulation of superoxide radicals and hydrogen peroxide radical. [37] Oral administration of Sinapic acid significantly increased the activities of SOD and CAT in liver, kidney and pancreas of diabetic rats. The higher SOD activity is believed to be due to increase dismutation of superoxide anions due to their increased production. [38] The result of SOD and CAT activity clearly shows that Sinapic acid contains a free radical scavenging activity, which could exert a beneficial action against pathological alteration caused by the presence of superoxide radicals and hydrogen peroxide radical.
Depression of glutathione peroxidase activity observed in diabetic liver and pancreas in the investigation has been shown to be an important response to increased peroxidase stress. [39] Kashiwagi *et al.* [40] reported that the elevation of glucose concentration reduces the activity of glutathione peroxidase, leading to an accumulation of H_2O_2. Hydrogen peroxide catabolism leads to the formation of superoxide anion. [41] Gpx in the kidney of diabetic rat increased as compared to the control. The decreased catalase activity and increased Gpx activity in the kidney suggests that there may be compensatory mechanism among the antioxidant enzymes in response to increased stress so that tissues lacking significant catalase activity may be critically dependent on activity of Gpx. Oral administration of Sinapic acid significantly decreased the activity of glutathione peroxidase in kidney and increased in liver and pancreas of diabetic rats.
GSH is known to protect the cellular system against the toxic effect of lipid peroxidation. [42] Diabetic rats showed a significant decrease in the level of GSH in liver, kidney and pancreas which have been considered to be an index of increased oxidative stress. [43] Depletion of reduced GSH either by conjugation and removal from the cell or oxidation to GSSG could significantly affect the overall redox potential of the cell. [44] Normal GSH levels are maintained via its synthesis by γ-glutamyl cysteine synthase, regeneration of GSH from GSSH by glutathione reductase and glucose - 6 - phosphate dehydrogenase. It has been reported that the activities of these enzymes are decreased in diabetic patients and animals [45] possibly due to their glycation by uncontrolled hyperglycemia and hence leading to decrease in the level of GSH. [46] Sinapic acid treated rats showed significant elevation in the level of GSH.
Ceruloplasmin forms a major part of the extracellular antioxidant defense. It also inhibits iron and copper dependent lipid peroxidation and also has a superoxide radical scavenging activity. [47] The diabetic rats in the present study showed a significant decrease in the level of serum ceruloplasmin which may be due to the generation of increased free radicals by STZ. Administration of Sinapic acid showed significant reversal of ceruloplasmin level in serum of diabetic rats.
Vitamin C is an excellent hydrophilic antioxidant. Vitamin C has been reported to contribute up to 24 percent of total peroxyl radical-trapping antioxidant activity. [48] Vitamin C also acts as a co-antioxidant by regenerating the vitamin A, E and GSH from radicals. [49] A decreased level of vitamin C in serum, liver and kidney of diabetic rats reported in the present study could be due to the increased utilization of vitamin C in deactivation of the increased level of reactive oxygen species. GSH is required for the recycling of vitamin C. [50-51] Administration of Sinapic acid improved the level of vitamin C in serum and tissues of diabetic rats.
The decreased level of vitamin E found in serum, liver and kidney of diabetic rats as compared with the control rats could be due to the increased oxidative stress, which accompanies the decrease in the level of antioxidant and may be related to the causation of diabetes mellitus. [52] Low levels of vitamin E observed in STZ induced diabetic rats suggest decreased regeneration of vitamin E from its radical. Regeneration of vitamin E requires ascorbic acid, an aqueous phase antioxidant, which requires GSH. [53] Oral administration of Sinapic acid improved the level of vitamin E level in serum and tissues of diabetic rats.
In conclusion, the present study revealed that Sinapic acid possesses a potential antiperoxidative and antioxidant activities in STZ-induced diabetic rats by decreasing the levels of lipid peroxidation products and increasing the levels and activities of antioxidants. The possible mechanism of action of Sinapic acid responsible for antioxidant effect needs further investigation.

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