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EFFECTS OF BARK OF *CINNAMOMUM ZEYLANICUM* ON HYPERGLYCEMIA INDUCED OXIDATIVE STRESS AND DNA DAMAGE IN EXPERIMENTAL DIABETIC RATS

SAPNEH SHARMA[1], ARUN MISHRA[*2]

[1]School of Studies in Biochemistry, Jiwaji University, Gwalior
[2]Department of Biochemistry, MGM Medical College, Indore

ABSTRACT

The aim of the study was to evaluate the antihyperglycemic and antioxidative potential of bark of *Cinnamomum zeylanicum* in alloxan induced diabetic rats and effect on DNA damage. Male rats were divided into four groups comprising of six rats; the third and fourth group were given single intravenous injection of alloxan to develop diabetes while the first and second group received equivalent volume of normal saline and served as control. Two days after alloxan treatment, second and fourth groups were given aqueous suspension of cinnamon bark powder (200mg/kg body weight) dissolved for two weeks. The levels of glutathione peroxidase (GPx), catalase (CAT), superoxide dismutase (SOD), hydrogen peroxide (H_2O_2), malondialdehyde (MDA) and 4-hydroxynonenal (4HNE) were determined in the liver and the brain. Results showed that levels of H_2O_2, MDA and 4HNE were significantly increased in the liver and the brain of diabetic rats when compared with control while the activities of GPx, SOD and CAT were significantly decreased when compared with the control group. The results of present study clearly demonstrated significantly high level of DNA single strand and double strand breaks in the liver and the brain of diabetic rats. Our study revealed that *cinnamomum zeylanicum* bark possesses potent antihyperglycemic and antioxidative effect in experimental diabetic rats. Further studies are required to find out the active compound present in the bark of cinnamon and mechanism of its action for the remedy of hyperglycemia induced disorders.

KEYWORDS: ***Antioxidant enzymes, DNA damage, Cinnamomum zeylanicum; Alloxan induced diabetes***

ARUN MISHRA*

Department of Biochemistry, MGM Medical College, Indore

Corresponding Author

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INTRODUCTION

Diabetes mellitus is a metabolic syndrome which has emerged as one of the most alarming health problems in the present century. The prevalence of diabetes has been increasing globally and number of diabetics is expected to increase to 366 million by 2030.[1] Diabetes mellitus is a metabolic disorder of multiple etiology characterized by chronic hyperglycemia and glycosuria with disturbances in carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both. Chronic hyperglycemia in diabetes starts a cascade of events leading to micro and macrovascular complications which affect many tissues and organs, causing retinopathy, nephropathy, neuropathy, cardiovascular diseases, peripheral vascular diseases.[2,3] Persistent hyperglycemia results in the generation of reactive oxygen species (ROS), ultimately leading to increased oxidative stress in variety of tissues, and playing an important role in diabetic complications.[4] Oxidative stress combined with mitochondrial dysfunction leads to the activation of inflammatory signaling pathways, damaging insulin producing beta cells and further aggravating the complications of diabetes.[5] ROS play an important role in the activation of stress responsive signaling pathways by regulating the expression of genes responsible for cellular damage and other further processes. Free radicals affect the cellular components such as lipids, proteins, DNA and carbohydrates, of which lipids are the most vulnerable molecules. Free radical induced lipid peroxidation causes considerable change to the cell membrane.[6] Peroxidation of lipid membranes has been related to the pathogenesis of many degenerative diseases, namely atherosclerosis, oxidative damage of DNA, carcinogenesis, sickle cell disease and Diabetes Mellitus etc.[7, 8] The level of reactive oxygen species are controlled by the antioxidant enzymes i.e. superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Earlier reports has indicated increased or decreased lipid Peroxidation, SOD, CAT and GPx activities in various tissues, such as liver, brain, kidney, blood vessels, heart, lungs and uterus.[9, 10] Thus, the tissue antioxidant status seems to have an important role in the etiology of diabetic complications. These free radicals induce a variety of lesions in DNA, including oxidized bases, abasic sites, strand breaks, and formation of DNA–protein cross-links. Damages most likely occur when the endogenous antioxidant network and DNA repair systems are disturbed.[8, 11-14] However, it is essential for the cell to repair DNA damage induced by oxidants. Medicinal plants have always been a very good source of drugs and many of the currently available drugs have been derived directly from them. Herbal drugs are safe, with less side effects and low cost. Many plant species have been reported to have hypoglycemic and antidiabetic activity; however, search for new antidiabetic drugs is still continued. Most of the plants contain glycosides, alkaloids, terpenoids, flavanoids, carotenoids etc. which are frequently used for their antidiabetic effect. Plants, such as *Trigonella foenum graecum, Azardica indica*, *Eugenia jambolana*, *Monordica charantia, Ocimum sanctum and Cinnamomum zeylanicum* etc are being commonly studied for the treatment of diabetes.[15-22] *Cinnamomum zeylanicum* commonly known as cinnamon is well known for its unique properties and its bark is used as a spice. Cinnamon, a natural product, is rich in polyphenolic components that have been shown to improve the antioxidant activities. Cinnamon belongs to the Lauraceae family, and its main components are cinnamaldehyde, cinnamic acid, tannin and methylhydroxychalcone polymer (MHCP). Cinnamon bark possesses significant anti-diabetic, anti-allergic, anti-ulcerogenic, antipyretic and antioxidant properties.[21-24] Looking at the potent antidiabetic properties of *Cinnamomum zeylanicum*, the present study was planned to elucidate the antidiabetic and antioxidant potential and to find out whether the effect of oral feeding of aqueous suspension of cinnamon bark has any effect on the DNA damage in the alloxan-induced diabetic rats.

MATERIAL AND METHODS

Chemicals

Chemicals used in the study were of highest purity/ analytical grade. Sodium chloride, sodium carbonate, potassium chloride, sodium bicarbonate disodium hydrogen phosphate, sodium dihydrogen phosphate, triton X-100, sodium hydroxide, ethylenediamine tetraacetic acid, reduced glutathione, sodium azide, ethanol, beta hydroxytoluene, acetonitrile, methane sulphonic acid, dimethyl sulphoxide and hydrogen peroxide were purchased from HIMEDIA Chemicals, India. Glutathione, Folin-Ciocalteu's reagent, glucose-6-phosphate dehydrogenase, glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate (reduced and oxidized), alloxan, ethidium bromide, agarose and epinephrine were purchased from Sigma Aldrich Chemicals Private Limited, MO, USA.

Experimental animals

Adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing about 110 ± 10 g were used in the present study. Rats were obtained from the animal facilities of Defense Research and Development Establishment, Gwalior, India, and were maintained in light (light-dark cycle of 12 h each) and temperature (25º ± 2ºC) controlled animal room of our department on standard pellet diet (obtained from Amrut Rat & Mice Feed, New Delhi, India) and tap water *ad libitum*. Rats were acclimatized for one week prior to the start of the experiment.

Experimental design

Twenty four rats were randomly divided into four groups of six rats each. Animals were divided into four groups and were given following treatments

Group 1 : Control (healthy rats with normal blood glucose level)

Group 2 : Treated control group (treated with cinnamon bark 200mg/kg body weight for two weeks)

Group 3 : Diabetics (i.v injection of alloxan 55 mg/kg body weight)

Group 4 : Treated diabetic group (treated with cinnamon bark 200 mg/kg body weight for two weeks).

Induction of experimental diabetes and cinnamon treatment

Diabetes was induced in twelve overnight fasted adult rats by single intravenous injection of 55 mg/Kg body weight, of alloxan monohydrate dissolved in normal saline (0.85% NaCl).[25] Diabetes was confirmed by blood glucose estimation after 48 h by an electronic glucometer. The rats with fasting glucose level above 300 mg/dL were considered diabetics[15] and selected for the study. *Cinnamomum zeylanicum* bark was purchased from the local herbal market, cleaned, dried and finely powdered. Aqueous suspension of powdered bark of cinnamon was prepared and 200 mg/kg body weight was given orally to the rats of group II and IV with the help of cannula, daily for two weeks.[26] Rats were humanly killed 24 h after the last treatment by cervical dislocation; different tissues were excised off, washed with 0.9% NaCl and used for different estimations. Animals were ethically handled and humanly killed as per the rules and instructions of Ethical Committee of Animal Care of Jiwaji University, Gwalior, India, in accordance with the Indian National Law on animal care and use. The study was approved by the academic ethical committee of the University [Ethical Code IAEC/ JU/ 2011/ 01].

Estimation of lipid peroxidation and hydrogen peroxide

Malondialdehyde *(*MDA) and 4-hydroxynonanal (4HNE), the two major end products of lipid peroxidation, were estimated by the method of Jacobson et al with minor modifications.[27] A 10% of tissue homogenate was prepared in Tris HCl buffer (20 mM, pH 7.4). Prior to homogenization, 10 µl of 0.5 M butylated hydroxytoluene (BHT) in acetonitrile was added per 1 ml of tissue homogenate. After homogenization, the homogenates were centrifuged at 3000 g at 4°C for 10 min and clear supernatant was used for the assay. Briefly 200 µl of supernatant was transferred to 650 µl of 10.3 mM 1-methyl-2-phenylindole in acetonitrile and vortex mixed. To assay MDA + 4HNE, 150 µl of 15.4 M methanesulfonic acid (MSA) was added, vortexed and incubated at 45°C for 40 min. To assay MDA alone, 150 µl of 37% HCl was added instead of MSA, vortexed, incubated at 45°C for 60 min. After incubation samples were kept on ice, centrifuged at 9500 g for 5 min and absorbance was measured at 586 nm. The levels of MDA and 4HNE are expressed as nmol g^{-1} tissue using extinction coefficient 1.1×10^5 M^{-1} cm^{-1}. Hydrogen peroxide (H_2O_2) levels were measured by the method of Pick, 1996.[28] For assay of H_2O_2 100 µl of tissue homogenate prepared in Tris HCl buffer (20 mM, pH 7.4), 100 µl of assay solution (containing 0.2 ml phenol red, 0.2 g/l and 0.2 ml of horse radish peroxidase, 20 U/ml in potassium phosphate buffer, 0.05M, pH 7.0 and 9.6 ml of 0.9% NaCl), was taken and reaction was started by the addition of 10 µl of 1.0 N NaOH. Absorbance was recorded at 600 nm in a microplate using ELISA reader. Hydrogen peroxide standard curve was plotted by taking different concentrations of H_2O_2, ranging from 20 to 100 µmol in a total volume of 100 µl and processed in the same way. Results are expressed as µmol H_2O_2 formed/ml homogenate.

Estimation of enzyme activities

Superoxide dismutase (SOD) activity was assayed by estimation of inhibition of auto oxidation of epinephrine.[29] A 10% of tissue homogenate was prepared in 0.9% NaCl, centrifuged at 15,000 g for 15 min and the corresponding supernatant was used for enzyme assay. Reaction mixture containing 0.5 ml sodium carbonate buffer (0.3 M, pH 10.2), 0.5 ml EDTA (0.6 mM), 0.5 ml homogenate and 1.0 ml distilled water was incubated at room temperature for 10 min. Reaction was initiated by addition of 0.5 ml epinephrine (1.8 mM) and absorbance change per min was recorded for 5 min at 480 nm. Specific activity is expressed as % inhibition of auto oxidation of epinephrine by the enzyme min^{-1} mg^{-1} protein. Glutathione peroxidase (GPx) activity was estimated as described by Paglia and Valtntine.[30] A 10% of tissue homogenate was prepared in 1.15% KCl, centrifuged at 5000 g for 10 min and the supernatant was used for enzyme assay. Reaction mixture containing 0.3 ml sodium phosphate buffer (0.1 M, pH 7.4), 0.1 ml GSH (0.15 M), 0.05 ml sodium azide (2.25 M), 0.05 ml homogenate, 0.1 ml NADPH (0.84 mM) and 0.05 ml glutathione reductase (2U/ml) was incubated at room temperature for 10 min. Reaction was initiated by addition of 0.05 ml H_2O_2 (0.0011 M) and absorbance change per min was recorded for 5 min at 340 nm. Specific activity is expressed as nmole NADPH oxidized min^{-1} mg^{-1} protein. Catalase (CAT) activity was estimated by the method of Aebi *et al*.[31] A 10% of tissue homogenate was prepared in 1.15% KCl, centrifuged at 5,000 g for 10 min and the supernatant thus obtained was used for enzyme assay. Reaction mixture containing 0.8 ml phosphate buffer (K_2HPO_4/ NaH_2PO_4, 50 mM, pH 7.0), 0.1 ml homogenate and 0.1 ml triton X-100 (0.02%) was incubated at room temperature for 10 min. Reaction was initiated by addition of 2.0 ml H_2O_2 (0.03 M prepared in potassium phosphate buffer, pH 7.0) and absorbance change per min was recorded for 5 min at 240 nm. Specific activity is expressed as micromole H_2O_2 decomposed min^{-1} mg^{-1} protein.

Protein in the tissues samples was estimated by the method of Lowry *et al.* using bovine serum albumin as standard.[32]

Studies on expression

Total RNA from the tissues was separated by the method described by Sambrook and Russell.[33] Briefly, 100 mg of fresh tissue was homogenized in 1.0 ml ice cold monophasic lysis reagent (TRI reagent). The homogenate was incubated for 5 min at room temperature followed by addition of 0.2 ml chloroform with vigorous shaking. The mixture was centrifuged at 3,360 g for 15 min in cold and the upper aqueous layer was separated. The RNA was precipitated by the addition of 0.25 ml isopropanol and 0.25 ml RNA precipitation solution (1.2 M NaCl and 0.8 M trisodium citrate), mixed thoroughly and incubated at room temperature for 10 min. The solution was centrifuged at 3,360 g for 10 min and the pellet containing RNA was separated. The pellet was washed twice with 75% ethanol and dissolved in 50 µl DEPC treated water. The reverse transcriptase (RT) PCR was performed using Qiagen one step RT PCR kit. A master mix was prepared containing RT PCR buffer, dNTP mix, Q solution, primers, reverse transcriptase enzyme and template RNA as per manufacturer's protocol. The primers used for superoxide dismutase, glutathione

peroxidase, catalase and 18S were designed using the primer3 software.

Superoxide Dismutase
Left primer CCACTGCAGGACCTCATTTT
Right primer CACCTTTGCCCAAGTCATCT

Glutathione peroxidase
Left primer ATAGAAGCCCTGCTGTCCAA
Right primer GAAACCGCCTTTCTTTAGGC

Catalase
Left primer ACATGGTCTGGGACTTCTGG
Right primer CAAGTTTTTGATGCCCTGGT

18S RNA
Left primer CGCGGTTCTATTTTGTTGGT
Right primer AGTCGGCATCGTTTATGGTC

18S RNA was used as housekeeping gene. First strand of cDNA was synthesized using Omniscript reverse transcriptase at 50ºC for 30 min. 35 cycles of PCR were done with a profile of 94º C for 1 min, 59º C for 1 min and 72ºC for 1 min. The amplicons were analyzed on 1.5 % TAE-Agarose gel. The bands were identified based on the product size using a 100 bp DNA ladder; documented using a gel documentation system. The bands were quantified densitometrically with the quantity one software. The results were normalized to the levels obtained for the 18S gene by taking a ratio of the value obtained for the gene of interest to that of 18S. The values of the diabetic samples were expressed as percentages with respect to the control.

Estimation of DNA damage by Single cell gel electrophoresis (Comet Assay)
Single strand breaks were measured by alkaline comet assay as described by Sasaki *et al*.[34] Fresh tissues were collected and homogenates (25% w/v) were prepared in homogenizing buffer (0.075 M NaCl containing 0.024 M EDTA, pH 7.5) with single stroke. To obtain nuclei, homogenates were centrifuged at 700 g for 10 min and the resulting pellets were gently re-suspended in 4.0 ml of chilled homogenizing buffer. 75 µl of normal melting agarose (1% prepared in 0.1 M sodium phosphate buffer, pH 7.2 containing 0.9% NaCl) was quickly layered on an end frosted slide, covered gently with another slide, and allowed to solidify. The upper slide was gently removed and the pre-coated slides were coated with 100 µl of mixture containing equal volume of sample and low melting agarose (2% in phosphate buffer saline). Slides then immerged in lysis buffer (containing 0.25 M NaCl, 100 mM EDTA, 10 mM Trizma base, 1% sarcosine, pH 10.0 adjusted with 10.0 N NaOH; 5% DMSO and 1% Triton X-100 was added just before use) for 1 h at 4° C in dark. After lysis, the slide were rinsed with chilled distilled water, transferred on a horizontal electrophoresis platform and immersed in electrophoresis buffer (300 mM NaOH and 1 mM EDTA, pH > 13.0) for 20 min for unwinding of DNA. Electrophoresis was performed for 15 min at constant voltage (1 V/cm and 300 mA). After electrophoresis, the slides were washed thrice with neutralizing buffer (0.4 M Tris-HCl, pH 7.4) for 5 min each, dehydrated in absolute methanol for 10 min and left at room temp to dry. The whole procedure was performed in dim light to minimize artefactual DNA damage. Just before visualization, each slide was stained with 50 µl EtBr (20 µg/ml), rinsed with water, and covered with a cover slide. A total of 50 cells were scored per tissue per animal under fluorescence microscope (Leica 4000B Digital microscope). Analyses were performed on the basis of the type of comet visualized on the slide. The nuclei were divided into five types with the help of software comet Score, as stage 0, I, II, III and IV on the basis of % DNA in the comet tail: 0-10% as stage 0, 10-25% as stage I, 25-50% as stage II, 50-75% as stage III and > 75% DNA in as sage IV as shown in fig. 1. The nuclei of each stage were counted in a total of 50 nuclei per slide and the DNA damage index was calculated as # 0 + # 1 + # 2 + # 3 + # 4/ # of cell scored.

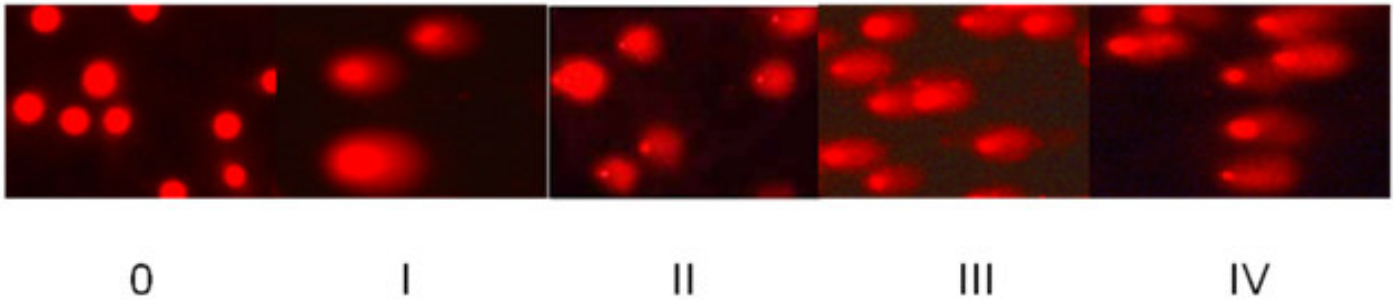

Figure 1
Stages of DNA damage

STATISTICAL ANALYSES

Results are expressed as mean ± S.E. of six sets of observations taken on different days. Statistical analyses were performed using Sigma Stat Statistical software version 2.0. All the statistical analyses were performed using one-way analysis of variance post hoc Bonferroni's multiple comparison test applied across the treatment groups. Significance was based on *P* value < 0.05.

RESULTS

Effect of cinnamon bark powder on the blood glucose level in control and diabetic rats
The blood glucose level of all the rats was tested by taking the blood from the tail vein and using electronic glucometer. It was observed that oral administration of aquous extract of cinnamon bark powder significantly decreased the the blood glucose levels in diabetic as well as control rats. Results of the present study showed

that oral administration of 200 mg/kg body weight cinnamon powder daily for 14 days, to the diabetic rats caused 9.1% decrease on 7th day and 34.7% decrease in the blood glucose level on day 14th of the start of treatmet. The same cinnamon treatmemt to the control rats showed 16.4% decrease in the blood glucose level on day 14th when compared with day 0 (Table 1). The results clearly showed the hypoglycemic potential of cinnamon bark.

Table 1
Effect of oral administration of Cinnamon on the blood glucose levels in normal and diabetic rats

Groups	Blood glucose level[a]		
	Day 0	Day 7	Day 14
Control	78.8 ± 1.77	98.8 ± 6.94	74.8 ± 2.27
Control + *Cz*	98.6 ± 3.40	84.8 ± 3.35*	82.4 ± 1.96**
Diabetic	357.4 ± 8.30***	390.6 ± 4.95#	457.4 ± 8.30***
Diabetic + *Cz*	337.0 ± 2.86***	306.2 ± 3.70***, cd***	229.0 ± 1.90***, cd***

[a]A drop of blood was taken from the tail vein and glucose level was measured using electronic glucometer. Blood glucose levels are expressed as mg/ dL
Results are mean ± S.E. of six set of observation. * P < 0.05, ** P < 0.01, *** P < 0.001 and # P > 0.05 when compared with respective control, cdcomparison between diabetic and diabetic + treatment group. Control and diabetic rats were given aqueous suspension of powdered cinnamomum zeylanicum (Cz) bark powder orally, 200 mg/kg body weight, with the help of cannula, daily for two week.

Effect of cinnamon bark powder on the levels of MDA, 4HNE and hydrogen peroxide in the liver and brain of control and diabetic rats

The results of the presnt study clearly showed that alloxan adminstration in rats caused hyperglyvemia which in tur resulted in the accumulation of malondialdehyde (MDA) and 4-hydroxynonanal (4HNE), the two major end products of lipid peroxidation, in the liver and the brain of rats when comapared with control. MDA and 4HNE levels were increased by 26.1% and 79.3% in the liver and 35.3% and 52.8% in the brain, respectively, when compared with control (table 2). When the diabetic rats were given cinnamon treatment for two weeks, 18.5% and 13.8% recovery in MDA levels while 47.1% and 28.5% recovery in the 4HNE levels of the liver and the brain, respectively, were observed when compared with control group (Table 2). The results clearly showed antioxidative potential of cinnamon bark. The presence of diabetes induced oxidative stress was also confirmed by monitoring the levels of hydrogen peroxide (H_2O_2), the most stable ROS in rat tissues. The results showed that there was significantly high accumilation of H_2O_2 in the liver and the brain of diabetic rats. It was observed that hepatic H_2O_2 level was increased by 235% while 29.5% increase was observed in the brain of diabetic rats when compared with control. cinnamon treatment for 15 days, caused decrese in the levels of H_2O_2 in the dibetic rat tissues while the level of H_2O_2 in the tissues of control rats remained almost unaltered when compered with untreated group. Cinnamon treatment to the diabetic rats showed 155.2% recovery in the liver and 19% recovery in the brain H_2O_2 levels when compared with control group (Table 3).

Table 2
Effect of oral administration of cinnamon for two weeks on the levels of MDA and 4HNE in the liver and the brain of control and diabetic rats

Groups	Liver		Brain	
	MDA[a]	4HNE[a]	MDA[a]	4HNE[a]
Control	46.2 ± 0.8	4.5 ± 0.1	25.5 ± 1.2	2.6 ± 0.2
Control + *Cz*[b]	44.6 ± 1.2#	5.9 ± 0.8≠	25.3 ± 2.1#	3.1 ± 0.3#
Diabetic	58.3 ± 2.2***	8.0 ± 0.3***	34.5 ± 0.6***	3.9 ± 0.4***
Diabetic + *Cz*[b]	49.8 ± 3.9**, cd*	5.9 ± 0.3**, cd**	30.9 ± 0.6**, cd**	3.2 ± 0.1**, cd*

[a]MDA and 4HNE levels are expressed as nmol g^{-1} tissue.
Results are mean ± S.E. of six set of observation. * P < 0.05, ** P < 0.001, *** P < 0.0001 and # P > 0.05 when compared with respective control, cd comparison between diabetic and diabetic + treatment group.
[b]Control and diabetic rats were given aqueous suspension of powdered cinnamon bark powder orally, 200 mg/kg body weight, with the help of cannula, daily for two week.

Table 3
Effect of oral administration of cinnamon for two weeks on the levels of hydrogen peroxide in the liver and the brain of control and diabetic rats

Groups	Hydrogen peroxide[a]	
	Liver	Brain
Control	1191.3 ± 57.40	359.1 ± 4.07
Control + *Cz*[b]	1190.0 ± 193.4[#]	341.1 ± 23.3[#]
Diabetic	3999.6 ± 108.6***	465.3 ± 8.90***
Diabetic + *Cz*[b]	2151.3 ± 55.20**. [cd]***	397.1 ± 4.07***. [cd]***

[a]H_2O_2 levels are expressed as µmole ml^{-1} homogenate.
Results are mean ± S.E. of six set of observation. * P < 0.05, ** P < 0.001, *** P < 0.0001 and [#] P > 0.05 when compared with respective control, [cd] comparison between diabetic and diabetic + treatment group.
[b]Control and diabetic rats were given aqueous suspension of powdered cinnamon bark powder orally, 200 mg/kg body weight, with the help of cannula, daily for two week.

Effect of cinnamon on the levels of antioxidant enzymes in the liver and brain of control and diabetic rats

Studies were carried out to monitor the effect of diabetes on the activities of antioxidant enzymes namely, glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in the tissues of rats. Experiments were also carried out to evaluate the protective effects of cinnamon treatment on the activities of these enzymes in tissues of control and diabetic rats. The results showed that the hyperglycemia induced by alloxan treatment, decreased the activities of these antioxidant enzymes in the liver and the brain, when compared with control. The hepatic activities of GPx, SOD and CAT were decreased by 26.1%, 36.6% and 67.6% while 29.4%, 17.4% and 66.6% decrease, respectively, was observed in the brain of diabetic rats when compared with control (Table 4). Administration of cinnamon for 14 days showed some protection against diabetes induced alterations in the activities of GPx, SOD and CAT. When the cinnamon treatment was given to the diabetic rats, 17.35%, 24.4% and 22.46% recovery in the activities of hepatic GPx, SOD and CAT, respectively, while the recovery in the brain was 24.3%, 9.3% and 17.9%, respectively, when compared with control group (Table 4).

Table 4
Effect of oral administration of cinnamon for two weeks on antioxidant enzyme activities in the liver and the brain of control and diabetic rats

Groups		GPx[e]	SOD[f]	CAT[g]
Liver				
	Control	3200.0 ± 182.7	558.2 ± 11.8	18.74 ± 0.30
	Control + *Cz*	3106.5 ± 166.5[#]	565.4 ± 13.7[#]	19.14 ± 0.59[#]
	Diabetic	2362.8 ± 59.13***	353.6 ± 6.42***	6.06 ± 0.17***
	Diabetic + *Cz*	2920.6 ± 49.11**, [cd]**	489.6 ± 7.88**. [cd] **	10.28 ± 0.24***. [cd]***
Brain				
	Control	38.25 ± 1.97	453.8 ± 12.14	2.34 ± 0.09
	Control + *Cz*	44.68 ± 1.94[#]	454.8 ± 10.16[#]	2.54 ± 0.04[#]
	Diabetic	27.00 ± 1.32***	374.8 ± 20.17***	0.78 ± 0.03***
	Diabetic + *Cz*	36.28 ± 0.74**, [cd]**	417.0 ± 2.78**, [cd]**	1.20±0.06**, [cd]**

[e]GPx activity is expressed as nmole NADPH oxidized $min^{-1}mg^{-1}$ protein
[f]SOD activity as % inhibition of auto oxidation of epinephrine by the enzyme $min^{-1}mg^{-1}$ protein and
[g]CAT activity is expressed as micromole H_2O_2 decomposed $min^{-1}mg^{-1}$ protein
Results are mean ± S.E. of six set of observation. * P < 0.05, ** P < 0.001, *** P < 0.0001 and [#] P > 0.05 when compared with respective control, [cd] comparison between diabetic and diabetic + treatment group.
[b]Control and diabetic rats were given aqueous suspension of powdered cinnamon bark powder orally, 200 mg/kg body weight, with the help of cannula, daily for two week.

Effect of cinnamon on the mRNA level of antioxidant enzymes in the tissues of control and diabetic rats

It was observed that experimental diabetes induced by alloxan, altered the expression of GPx, SOD and CAT genes in the liver and the brain of rats when compared with control. RT PCR results revealed that, experimental diabetes caused decrease in the gene expression of GPx, SOD and CAT by 29%, 26%, 23% in the liver and 23%, 39%, 35% in the brain respectively, when compared with the control. *Cz* treated diabetic rats showed increased gene expression of GPx, SOD and CAT by 21%, 24%, 12% in the liver and 19%, 41%, 33% in the brain, respectively, when compared with untreated diabetic group II (Fig. 2).

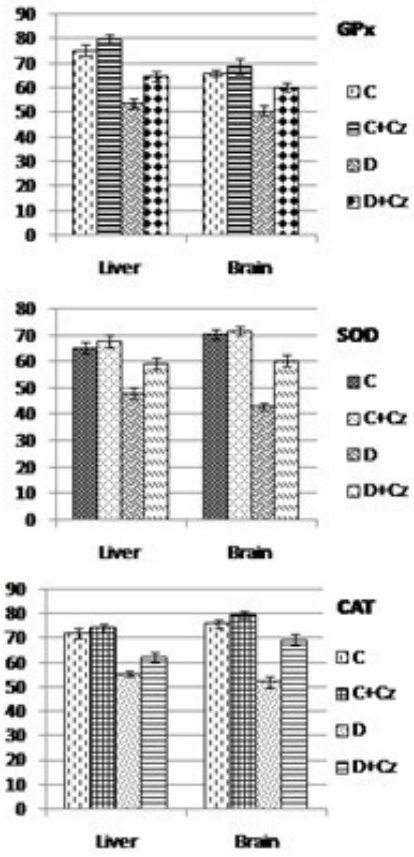

Figure 2
Densitometric analysis of expression pattern of mRNA of glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) in the liver and the brain of control and diabetic rats with and without Cinnamomum zeylonicum (Cz) treatment using quantity one software. Results are normalized by observing the expression of 18S RNA which is used as house keeping gene.

Effect of cinnamon on the level of DNA damage in the tissues of control and diabetic rats

The results of the presnt study showed that alloxan induced diabetes in rats caused increase single strand break in DNA, in the liver and the brain of the rats when comapared with control. Single strand DNA breaks were increased by 524% in the liver and 1292% in the brain, respectively, when compared with control (Table 5). When the diabetic rats were given cinnamon treatment for two weeks, the levels of single strand DNA breaks decreased 67.9% in the liver and 65.1% in the brain, respectively, were observed when compared with untreated diabetic group (Table 5).

Table 5
Effect of oral administration of cinnamon for two weeks on the DNA damage index in the liver and the brain of control and diabetic rats

Groups	DNA damage index[a]	
	Liver	Brain
Control	0.33 ± 0.04	0.14 ± 0.03
Control + *Cz*[b]	0.15 ± 0.04*	0.10 ± 0.03
Diabetic	2.06 ± 0.08***	1.95 ± 0.13**
Diabetic + *Cz*[b]	0.66 ± 0.02**, [cd]***	0.68 ± 0.09*, [cd]**

[a]Results are expressed as damage index calculated as # 0 + # I + # II + # III + # IV/ 50
Results are expressed as mean ± S.E. of six set of observation. * P < 0.05, ** P < 0.001, *** P < 0.0001 and # P > 0.05 when compared with respective control, [cd] comparison between diabetic and diabetic + treatment group.
[b]Control and diabetic rats were given aqueous suspension of powdered cinnamon bark orally, 200 mg/kg body weight, with the help of cannula, daily for two week.

DISCUSSION

Hyperglycemia induced oxidative stress is the main cause of micro and macro vascular complications in diabetes. Traditionally plant remedies have been used for the treatment of diabetes, but only a few have been scientifically evaluated.[20] The bark of *Cinnamomum zeylanicum* also known as Ceylon cinnamon has been used as spice and flavoring agent for centuries .The present study was planned to elucidate the effect of oral feeding of bark powder of cinnamon on the level of diabetes-induced oxidative stress by monitoring the

level of ROS and end products of lipid Peroxidation, activities of antioxidant enzymes, expression as well as oxidative stress induced DNA damage. The results of the present work confirmed that the bark powder of cinnamon demonstrated hypoglycemic potential as it decreased the blood sugar level on alloxan-induced experimental diabetes in rats. Reactive oxygen species (ROS) interact with the lipid bilayer of the cell membrane resulting in the generation of MDA and 4HNE as end products of lipid peroxidation. These products of lipid Peroxidation are associated with a variety of anomalies, including diabetes mellitus. Most studies have shown increased lipid peroxidation in diabetic condition.[12, 35, 36] In the present study, MDA and 4HNE levels were significantly increased in the diabetic group and were decreased significantly in cinnamon treated diabetic groups relative to the untreated diabetic group. It is known that free radicals are formed in cells by both cellular metabolism and exogenous agents. These species react with biomolecules in cells, including DNA, causing oxidative stress and DNA damage.[37-39] Oxidative stress occurs when ROS are not adequately removed by the cellular antioxidant system. Results of the present study clearly showed that the enzymatic antioxidant machinery comprising of GPx, SOD, and CAT is severely inhibited in the liver and the brain of diabetic rats. Feeding of cinnamon bark powder for 15 days considerably decresed the level of lipid peroxidation, levels of hydrogen peroxide and increased the activities of antioxidant enzymes. The level of MDA and 4HNE was measured in the tissues of diabetic rats as an index of oxidative stress and redox imbalance. A moderate increase in tissue MDA and 4HNE in alloxan-treated rats was found to be reduced after cinnamon feeding for 15 days. The observed increase in lipid peroxidation levels in the liver and the brain are in agreement with previously reported findings.[40] Lipid peroxidation may bring about protein damage and inactivation of membrane-bound enzymes either through direct attack by free radicals or through chemical modification by its end products, MDA and 4HNE. The decreased activities of SOD, CAT, and GPx may be a response to increased production of ROS, for example, hydrogen peroxide, by the auto oxidation of the excess of glucose and nonenzymatic glycation of protein.[41] The decreased activities of antioxidant enzymes may be due to the decreased protein expression levels in the diabetic conditions as seen in the present study. RT-PCR analysis showed a corresponding decrease in the level of transcription of these enzymes. In diabetic rats, however, the effect of oxidative stress on hepatic and brain CAT activities reported in literature varied significantly from no change,[12] decreased[13, 42, 43] and increased[35] depending on the experimental conditions such as age of the animal and duration of diabetes. DNA damage is caused by multiple factors including oxidative stress, vitamin B_{12} deficiency and ischemia-reperfusion injury.[39, 44] The comet assay has been shown to be a very sensitive method for the evaluation of DNA damage in individual cells. Studies using the comet assay have shown increased DNA damage in diabetic patients with hyperglycemia.[37, 45-49] A significant positive correlation was observed between hyperglycemia and DNA damage index in alloxan induced diabetic rats. Our results confirm previous studies showing that DNA damage in diabetes can be provoked by an oxidative event, since hyperglycemia leads to high free radicals production, attacking essential molecules like as DNA proteins and lipid. Our findings have shown that DNA damage index is increased in alloxan induced diabetic rats and treatment with cinnamon decreased this DNA damage when compared with untreated diabetic rats. The effect of cinnamon bark can be explained by the decrease in blood glucose. Oxidative stress produces DNA base modifications, strand breakage, and other DNA damages.[50] Cinnamon bark treatment significantly improves activities of antioxidant enzymes, decreases the level of hydrogen peroxide and lipid perxidation as compared to that of control.

CONCLUSION

From the outcome of the present study, it can be concluded that cinnamon bark extract provides protection against diabetes induced oxidative stress and macromolecular damages. The levels of reactive oxygen species, and damaged molecules were reduced in cinnamon treated diabetic rats when compared with untreated rats. The levels of antioxidant machinery were also improved on cinnamon treatment. Further studies are required to find out the active compound in the bark of *Cinnamon zeylanicum* and to understand the mechanism of its action.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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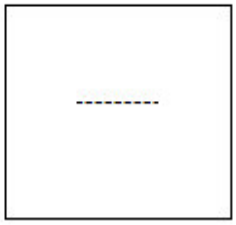

Dr. Manish Raj Kulshrestha M.D

Assistant Professor, Dept. of Biochemistry, Dr. R.M.L.I.M.S., Vibhuti khand, Gomathi Nagar, Lucknow - 226010.

Dr. S. Usha, Ph.D. (Chemistry)

Assistant Professor, Sri Sairam Engineering College, Affiliated to Anna University, Chennai, India.

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