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Antioxidant Activity of Aqueous Extract of *Coscinium fenestratum* in STZ-Nicotinamide Induced Diabetic Rats

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Abstract – The aqueous extract of *Coscinium fenestratum* was studied for its antioxidant status in STZ-nicotinamide induced type 2 diabetic rats at two dose levels of 250 mg/kg and 500 mg/kg. At the end of the experimental period, diabetic rats treated with aqueous extract at both dose levels showed a significant increase in the levels of enzymatic antioxidants such as glutathione peroxidase, glutathione synthetase, peroxidase, superoxide dismutase and catalase as compared to the untreated control. Similarly, a significant increase was also observed in the levels of the non enzymatic antioxidants ceruloplasmin, ascorbic acid and tocopherol. The results suggest that the aqueous stem extract of *C. fenestratum* prevents type 2 diabetes mellitus induced oxidative stress.
Keywords – *Coscinium fenestratum,* Aqueous extract, Enzymatic antioxidants, Non enzymatic antioxidants, Diabetes.

Introduction

Free radicals are chemical species of atoms or molecules that possess an unpaired electron in their outermost orbital and may be produced as a result of normal or pathological metabolism. Reactive oxygen species react easily with free radicals to form radicals themselves. Antioxidants can interfere with the oxidation process by reacting with the free radicals, chelating free catalytic metals and also by acting as oxygen scavengers. In diseases like diabetes as well as hypocholesterolemia/hyperlipidemia, there is an increased production of free radicals leading to accelerated lipid peroxidation (Stefano *et al.*, 1997). The elevated level of lipid peroxidation products, in turn may be responsible for some of the pathological effects in the above-mentioned diseases (Slatter *et al.*, 2000). In addition, there are changes in enzymatic as well as non-enzymatic antioxidant defense suggesting reduced resistance to free radical mediated damage (Jones *et al.*, 1988). Streptozotocin has been proposed to act as a diabetogenic due to its ability to destroy pancreatic β islet cells possibly by a free radical mechanism (Garg *et al.*, 1996).

The plant *Coscinium fenestratum* Colebr. (Menispermaceae) commonly known as tree turmeric is widely used in the treatment of diabetes mellitus in the Ayurveda and Siddha system of medicine (Varier, 1994). Berberine, ceryl alcohol, hentriacontane, sitosterol, palmitic acid, oleic acid and saponin together with some resinous material are the important constituents of stem (The Wealth of India, 1950). Isolation of tertiary alkaloids, berlambine, dihydroberlambine and nor oxyhydratsinine from the roots has been reported (Datta *et al.*, 1988). The stem extract has been reported for its antihypertensive activity (Singh *et al.*, 1990) and hepatoprotective activity (Venukumar *et al.*, 2004). The significant antidiabetic activity of *C. fenestratum* stem extract was established by the authors in earlier studies (Shirwaikar *et al.*, 2005). The present investigation is an attempt to study the effect of the aqueous stem extract of *C. fenestratum* for its antioxidant status in STZ-nicotinamide induced type 2 diabetic rats.

Materials and Methods

Chemicals and instruments – The following chemicals were used for the study. Streptozotocin (Sigma Aldrich Co., Germany). Hydrogen peroxide, Ascorbic acid, Meta phosphoric acid, Copper sulfate, O-phosphoric acid, EDTA, Sodium citrate (NICE Chemicals Pvt. Ltd., Cochin, India). Phenazine methosulfate, Nitroblue tetrazolium chloride, NADH, NADPH, ATP, Glutathione, 5, 5' Dithio

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nitro bis benzoic acid, Tocopherol (Himedia Laboratories Ltd., Mumbai, India). Disodium hydrogen phosphate, Potassium hydrogen phosphate, Sodium hydroxide (E.Merck-India ltd., Mumbai, India). 2,4 di nitro phenyl hydrazine (Sarabhai M. Chemicals, Baroda, India). Sodium pyruvate (S.D fine-chem Ltd., Mumbai). Tris buffer (SISCO Research Laboratories Ltd., Mumbai, India). Sodium pyro phosphate (Thomas Baker Chemicals Ltd., Mumbai, India). Nicotinamide (Qualigens Fine Chemicals, Division of Glaxo, Mumbai, India).

UV spectrophotometer (Shimadzu 160 IPC), Homogenizer, Centrifuge (Remi, New Delhi, India) and pH meter (Elico Ltd., Hyderabad, India) were the instruments used for the study.

Plant material – The plant material of C. fenestratum was purchased from Jogappa Shanbag Ayurvedic Store, Udupi, Karnataka, India in August 2003. The plant was authenticated by Dr.Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP 526) has been deposited at the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

Preliminary phytochemical screening – Preliminary phytochemical screening (Harborne, 1998) of the aqueous extract revealed the presence of alkaloids, saponins, phenolic substances and carbohydrates.

Preparation of stem extract – The aqueous extract was prepared (Kokate, 1994) by cold maceration of 125 g of the dried stem powder in 500 ml of chloroform water for 7 days. The extract was filtered, concentrated, dried in vacuo and the residue (yield 34 g) was stored in the refrigerator at 2-8°C for use in subsequent experiments.

Animals – Healthy adult male Wistar albino rats between 2 and 3 months of age weighing about 250-300 g were used for the study. The animals housed in polypropylene cages, maintained under standard conditions (12 h light/12 h dark cycle; 25 ± 3°C temperature; 35-60% humidity) were fed with standard rat pellet diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The study was approved by The Institutional Animal Ethical Committee of KMC, Manipal, India (IAEC/KMC/03/2003-04).

Induction of experimental diabetes – NIDDM was induced (Masiello *et al.*, 1998) in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg STZ, 15 min after the i.p. administration of 120 mg/kg nicotinamide. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 h and then on day 7 after injection. Only rats found with permanent NIDDM were used for the antidiabetic study.

Experimental design – The animals were divided into four groups (n = 6) i.e., normal rats administered with 2% gum acacia solution, diabetic rats administered with gum acacia 2% solution, diabetic rats administered with *C. fenestratum* aqueous extract 250 mg/kg and diabetic rats administered with *C. fenestratum* aqueous extract 500 mg/kg., respectively for 12 days.

Sample collection – *Blood sample*: At the end of day 12, blood samples were collected from the inner canthus of the eye under light ether anesthesia using capillary tubes (Micro Hemocrit Capillaries, Mucaps). Blood was collected in fresh vials containing anticoagulant and serum was separated in a centrifuge at 2000 rpm for 2 min.

Collection of organs: The animals were euthanized by an overdose of intraperitoneal anesthesia and tissue samples were collected for the assessment of antioxidant parameters.

Estimation of enzymatic antioxidant parameters – Glutathione synthetase and Glutathione peroxidase estimation was based on the reduction of 5,5'-dithio bis 2-nitro benzoic acid by sulfhydryl compounds (Rotruck et al., 1973). Catalase estimation was based on the decomposition of hydrogen peroxide (Aebi, 1983; Sinha, 1972). The amount of degradation hydrogen peroxide was determined in the peroxidase estimation (Johann and Reinhild, 1983). In superoxide dismutase, method was based on the inhibition of NADH dependent nitro blue tetrazolium reduction by dismutase (Kakkar et al., 1984).

Estimation of non enzymatic antioxidant parameters – Ceruloplasmin estimation was based on colour reaction of ceruloplasmin with p-phenylene diamine (Ravin, 1961). The tocopherol levels were estimated based on the reduction of ferric ions to ferrous ions in the presence of tocopherols (Desai, 1984). Ascorbic acid estimation was based on the oxidation of ascorbic acid by Cu^{+2} to form dehydro ascorbic acid, which reacts with acidic 2,4 dinitro phenyl hydrazone to form a red bis hydrazone (Omaye et al., 1979). The protein content in the tissue homogenate was estimated by the method of Lowry et al, 1951.

Statistical Analysis – Data were statistically evaluated by using one way ANOVA, followed by post hoc Scheffe's test using 7.5 version of SPSS computer software. The values were considered significant when $p < 0.05$.

Results

Table 1 demonstrates the levels of non enzymatic antioxidants such as ceruloplasmin, ascorbic acid and tocopherol. Decrease in the levels of non enzymatic antioxidants was observed in the diabetic rats as compared to normal rats, with the treated groups showing

Table 1. Effect of *C. fenestratum* aqueous stem extract on non-enzymatic antioxidants

Treatment	Ascorbic acid	Tocopherol		Ceruloplasmin
	Plasma (mg/dl)	Liver (mg/mg)	Kidney (mg/mg)	Plasma (mg/dl)
Normal (Positive Control)	1.5 ± 0.3	4.3 ± 0.3	2.1 ± 0.1	5.2 ± 0.3
Diabetic control	0.3 ± 0.08	1.2 ± 0.1	0.5 ± 0.06	2.7 ± 0.1
Aq. Extract (250 mg/kg)	0.4 ± 0.04	1.8 ± 0.6 [a,b]	0.9 ± 0.03 [a,b]	2.9 ± 0.4 [a,b]
Aq. Extract (500 mg/kg)	0.5 ± 0.09 [a,b]	2.2 ± 0.4 [a,b]	1.2 ± 0.4 [a,b]	3.1 ± 0.1 [a,b]

[a] $p < 0.05$ Vs Control; [b] $p < 0.05$ Vs Normal; One way Anova followed by post hoc Scheffe's test; (Mean ± S.E, n = 6). The levels of non enzymatic antioxidants were increased in the treated diabetic rats as compared to diabetic rats.

Table 2. Effects of C. fenestratum aqueous stem extract on enzymatic antioxidants Superoxide dismutase and Catalase

Treatment	Superoxide dismutase		Catalase	
	Liver (U/g/min)	Kidney (U/g/min)	Liver (U/g/min)	Kidney (U/g/min)
Normal (Positive control)	219.2 ± 18.4	136.1 ± 14.6	35.3 ± 1.3	12.8 ± 1.1
Diabetic control	86.6 ± 22.6	65.6 ± 14.8	15.9 ± 1.1	7.4 ± 0.4
Aq. Extract (250 mg/kg)	149.1 ± 23.7 [a,b]	85.3 ± 13.8 [b]	22.1 ± 1.1 [a,b]	7.8 ± 0.3 [b]
Aq. Extract (500 mg/kg)	160.1 ± 12.5 [a,b]	99.8 ± 1.21 [a,b]	25.3 ± 1.6 [a,b]	8.4 ± 0.2 [a,b]

[a] $p < 0.05$ Vs Control; [b] $p < 0.05$ Vs Normal; One way Anova followed by post hoc Scheffe's test; (Mean ± S.E, n = 6). The levels of superoxide dismutase and catalase were increased in the treated diabetic rats as compared to diabetic rats.

Table 3. Effects of C. fenestratum aqueous stem extract on enzymatic antioxidants Glutathione synthetase and Peroxidase

Treatment	Glutathione synthetase		Peroxidase	
	Liver (U/g/min)	Kidney (U/g/min)	Liver (U/g/min)	Kidney (U/g/min)
Normal (Positive control)	5.5 ± 0.3	2.1 ± 0.4	2.1 ± 0.1	0.6 ± 0.03
Diabetic control	2.2 ± 0.4	1.6 ± 0.3	1.1 ± 0.3	0.1 ± 0.02
Aq. Extract (250 mg/kg)	3.0 ± 0.3 [a,b]	1.7 ± 0.3 [b]	1.3 ± 0.2 [a,b]	0.2 ± 0.01 [a,b]
Aq. Extract (500 mg/kg)	3.5 ± 0.2 [a,b]	1.7 ± 0.4 [b]	1.5 ± 0.1 [a,b]	0.2 ± 0.04 [a,b]

[a] $p < 0.05$ Vs Control; [b] $p < 0.05$ Vs Normal; One way Anova followed by post hoc Scheffe's test; (Mean ± S.E, n = 6). The levels of glutathione synthetase and peroxidase were increased in the treated diabetic rats as compared to diabetic rats.

dose dependent activities significantly.

Table 2 illustrates the effect of the aqueous stem extract of C. fenestratum on enzymatic antioxidants viz., superoxide dismutase and catalase in the liver and kidney of normal and experimental diabetic rats. Administration of the aqueous extract to diabetic rats resulted in a significant increase in the level of catalase and superoxide dismutase as compared to the diabetic and normal rats.

Table 3 gives the concentration of the enzymatic antioxidants glutathione peroxidase and peroxidase levels in the tissues of normal and experimental rats. A significant increase was observed in the levels of glutathione peroxidase and peroxidase in the treated group.

Table 4 shows the effect of aqueous extract of C. fenestratum on the enzymatic antioxidant glutathione peroxidase. Reduction in the level of glutathione peroxidase in liver and kidney of diabetic rats was reversed on the administration of the extract.

Table 4. Effects of C. fenestratum aqueous stem extract on enzymatic antioxidant Glutathione peroxidase

Treatment	Glutathione peroxidase	
	Liver (U/g/min)	Kidney (U/g/min)
Normal (Positive control)	23.4 ± 1.5	7.2 ± 0.4
Diabetic control	14.2 ± 1.1	5.6 ± 0.1
Aq. Extract (250 mg/kg)	14.8 ± 1.3 [a,b]	5.7 ± 0.3 [b]
Aq. Extract (500 mg/kg)	15.9 ± 1.1 [a,b]	5.9 ± 0.4 [a,b]

[a] $p < 0.05$ Vs Control; [b] $p < 0.05$ Vs Normal; One way Anova followed by post hoc Scheffe's test; (Mean ± S.E, n = 6). The levels of glutathione peroxidase were increased in the treated diabetic rats as compared to diabetic rats.

Discussion

Oxidative stress, suggested as a mechanism underlying diabetes and diabetic complications (Halliwell and Gutteri-

dge, 1989) results from an imbalance between radical generating and radical scavenging systems. The decoction of the plant *C. fenestratum* is used for the treatment of diabetes in rural people of Kanyakumari district, Tamilnadu, India (Kalavincela, 1998). Preliminary screening of this plant showed that the plant possessed significant antidiabetic activity (Mahapatra, 1997). Further studies were carried out to establish antidiabetic activity of this plant (Shirwaikar *et al.*, 2005). The present work is an attempt to study the protective action of the aqueous extract of *Coscinium fenestratum* in oxidative stress caused by diabetes mellitus.

Ceruloplasmin, the copper containing oxidase which serves to transport copper to tissues has been established as a chain breaking antioxidant with a potential to scavenge peroxyl radicals (Halliwell and Gutteridge, 1984). The decreased levels of ceruloplasmin in the plasma of diabetic rats were increased significantly ($p < 0.05$) in the diabetic rats treated with the aqueous stem extract. Vitamin C or ascorbic acid, an excellent water-soluble antioxidant, primarily scavenges oxygen radicals and has been reported to contribute up to 24% of the total peroxyl radical trapping antioxidant activity (Atanasiu *et al.*, 1998). The decreased level of ascorbic acid in diabetic rats could be due to the increased utilization of ascorbic acid in deactivation of the increased levels of reactive oxygen species or to the decrease in the glutathione level, since glutathione is required for recycling of ascorbic acid (Chatterjee *et al.*, 1991; Inefers *et al.*, 1988). The decreased ascorbic acid level in diabetic rats was increased on treatment with aqueous extract, 250 mg/kg and 500 mg/kg respectively. Alpha tocopherol is the most important antioxidant in the cell membrane. It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxyl radicals, thus protecting the cell structures against damage (Selvam and Anuradha, 1990; Takenaka *et al.*, 1991). In our investigation, the levels of non-enzymatic antioxidants were decreased in the STZ induced diabetic rats and it corroborates with the similar to the findings reported earlier (Stanley Mainzen Prince and Kamalakannan 2003). An increase of 33.3 & 45.5% in the liver and 44.4 & 58.3% in the kidney of diabetic rats treated with aqueous extract was observed in the tocopherol levels of treated groups.

Superoxide dismutase (SOD) and catalase (CAT) are the two major scavenging enzymes that remove the toxic free radicals *in vivo*. SOD protects the tissues against oxygen free radicals by catalyzing the removal of superoxide radical, which damages the membrane and biological structures (Wohaieb *et al.*, 1987; Saxena *et al.*, 1993). CAT has been shown to be responsible for the detoxification of significant amounts of hydrogen peroxide. The reduced activity of SOD and CAT in the liver and kidney observed during diabetes may result in deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide (Searle and Wilson, 1980). The levels of SOD and CAT in liver and kidney of diabetic rats treated with aqueous extract showed a significant increase. Glutathione synthetase has a multifaced role in antioxidant defense. It is a direct scavenger of free radicals as well as a co substrate for peroxide detoxification by glutathione peroxidases (Ewis and Abdel Rahman, 1995). Loven *et al*, (1986) suggested that the decrease in hepatic glutathione synthetase could be the result of decreased synthesis or increased degradation by oxidative stress in diabetes. The glutathione synthetase and peroxidase levels of liver and kidney of diabetic rats were found to increase significantly on treatment with the aqueous extract. Glutathione peroxidase, a selenium-containing enzyme present in significant concentrations detoxifies hydrogen peroxide to hydroperoxide through the oxidation of reduced glutathione (Bruce *et al.*, 1982). Reduction of glutathione peroxidase in diabetes may be due to the inactivation of the enzymes involved in the disposal of oxygen species and also the insufficient availability of GSH. A significant increase was observed in the glutathione peroxidase levels of treated rats.

In our study, the decreased levels of both enzymatic and non enzymatic antioxidants in STZ induced diabetic rats were found to increase in diabetic rats treated with aqueous stem extract of *C. fenestratum*. The overexpression of these antioxidant parameters in diabetic rats treated with the extract suggests its efficacy in protection against oxidative damage in STZ induced diabetic rats.

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