Introduction

Free radicals may play an important role in the causation and complication of diabetes mellitus. The increased oxidative stress and accompanying decrease in antioxidants may be related to the causation of diabetes (1). In diabetes mellitus, alterations in the endogenous free radical scavenging defense mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury (2). It has been proposed that streptozotocin acts as a diabetogenic owing to its ability to destroy pancreatic β-islet cells, possibly by a free radical mechanism. The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger systems (3,4), the levels of which are altered in diabetes (5).

Alcoholic extract of the stems of Coscinium fenestratum, a medicinal plant indigenous to India and Sri Lanka used in ayurveda and siddha medicine for treating diabetes, was studied for its carbohydrate metabolism effect and antioxidant status in streptozotocin–nicotinamide induced type 2 diabetic rats. Oral administration of C. fenestratum stem extract in graded doses caused a significant increase in enzymatic antioxidants such as catalase, superoxide dismutase, glutathione synthetase, peroxidase, and glutathione peroxidase and in the nonenzymatic antioxidants ascorbic acid, ceruloplasmin and tocopherol. Effects of alcoholic extract on glycolytic enzymes such as glucose-6-phosphate dehydrogenase, lactate dehydrogenase and hexokinase showed a significant increase in their levels, whereas a significant decrease was observed in the levels of gluconeogenic enzyme, glucose-6-phosphatase and alanine aminotransferase in treated diabetic rats. Serum creatinine and urea levels also declined significantly. This investigation demonstrates significant antidiabetic activity of C. fenestratum.

Keywords: carbohydrate metabolism – Coscinium fenestratum – enzymatic antioxidants – nonenzymatic antioxidants – streptozotocin–nicotinamide induced diabetes

Alcoholic Stem Extract of Coscinium fenestratum Regulates Carbohydrate Metabolism and Improves Antioxidant Status in Streptozotocin–Nicotinamide Induced Diabetic Rats

I. S. R. Punitha1, K. Rajendran1, Arun Shirwaikar2 and Annie Shirwaikar1

1Department of Pharmacognosy and 2Department of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal 576104, India

For reprints and all correspondence: Dr Annie Shirwaikar, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka 576104, India. Tel: +91 820 2571201 22430; Fax: +91 820 2571998; E-mail: annieshirwaikar@yahoo.com

© The Author (2005). Published by Oxford University Press. All rights reserved.

The online version of this article has been published under an open access model. Users are entitled to use, reproduce, disseminate, or display the open access version of this article for non-commercial purposes provided that: the original authorship is properly and fully attributed; the Journal and Oxford University Press are attributed as the original place of publication with the correct citation details given; if an article is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated. For commercial re-use, please contact journals.permissions@oupjournals.org

© The Author (2005). Published by Oxford University Press. All rights reserved.

The online version of this article has been published under an open access model. Users are entitled to use, reproduce, disseminate, or display the open access version of this article for non-commercial purposes provided that: the original authorship is properly and fully attributed; the Journal and Oxford University Press are attributed as the original place of publication with the correct citation details given; if an article is subsequently reproduced or disseminated not in its entirety but only in part or as a derivative work this must be clearly indicated. For commercial re-use, please contact journals.permissions@oupjournals.org
Methods

Chemicals and Instruments Used

The following chemicals were used for the study: glucose-6-phosphate dehydrogenase, glucose-6-phosphate, lactate dehydrogenase, streptozotocin (Sigma Aldrich Co., Germany); ascorbic acid, meta phosphoric acid, O-phosphoric acid, magnesium chloride, EDTA, sodium citrate (NICE Chemicals Pvt. Ltd, Cochin, India); phenazine methosulfate, nitroblue tetrazolium chloride, NADH, NADPH, ATP, glutathione, 5, 5'-dithio nitro bis benzoic acid, tocopherol (Himedia Laboratories Ltd, Mumbai, India); disodium hydrogen phosphate, potassium hydrogen phosphate (E. Merck India Ltd, Mumbai, India); phenazine methosulfate, nitroblue tetrazolium chloride, NADH, NADPH, ATP, glutathione, 5, 5'-dithio nitro bis benzoic acid, tocopherol (Himedia Laboratories Ltd, Mumbai, India); nicotinamide (Qualigens Fine Chemicals, a division of Glaxo, Mumbai, India).

A UV spectrophotometer (Shimadzu 160 IPC), homogenizer, centrifuge and pH meter were the instruments used for the study.

Albino Rats

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

Plant Material

The C. fenestratum plant material, purchased from Jogappa Shanbag Ayurvedic Store, Udupi, Karnataka, India during August 2003, was authenticated by Dr Gopalakrishna Bhat, Professor, 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.

Preparation of Alcoholic Stem Extract

Approximately 160 g of the stem powder was placed in a soxhlet extractor and extracted with ethanol for 72 h. The solvent was recovered by distillation in vacuo, and the residue (yield 18 g), stored in the desiccator, was used for subsequent experiments (12,13).

Induction of Experimental Diabetes

An rat model of type 2 diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM) was induced (14) in overnight-fasted rats by a single intraperitoneal injection of 60 mg kg⁻¹ streptozotocin 15 min after the intraperitoneal administration of 120 mg kg⁻¹ nicotinamide. Hyperglycemia was confirmed by elevated blood glucose levels determined at 72 h and then on day 7 after injection. Only rats confirmed to have permanent NIDDM were used for the antidiabetic study (15).

Experimental Design

Rats were divided into four groups (n = 6): normal rats administered with 2% gum acacia solution, diabetic rats administered with 2% gum acacia solution, diabetic rats administered with C. fenestratum alcoholic extract 250 mg kg⁻¹ and diabetic rats administered with C. fenestratum alcoholic extract 500 mg kg⁻¹, respectively, for 12 days orally (16).

Sample Collection

Blood Sample

At the end of day 12, blood samples were collected under light ether anesthesia retro-orbitally from the inner canthus of the eye using capillary tubes (Micro Hematocrit Capillaries, Mucaps). Blood was collected in fresh vials containing anticoagulant, and serum was separated in a centrifuge at 2000 r.p.m. for 2 min.

Collection of Organs

Rats were euthanized using an overdose of intraperitoneal anesthesia, and tissue samples collected for assessing the following parameters.

Estimation of Enzymes in Carbohydrate Metabolism

The following parameters were evaluated.

Hexokinase (EC 2.7.1.1)

The hexokinase assay is based on the reduction of NAD⁺ through a coupled reaction with glucose-6-phosphate dehydrogenase. The excised liver tissue homogenate was prepared in saline. To 0.1 ml of homogenate were added 2.28 ml of Tris buffer (200 mmol l⁻¹-MgCl₂ buffer (20 mol l⁻¹), pH 8. 0.5 ml of 0.67 M glucose, 0.1 ml of 16 mM ATP, 0.1 ml of 6.8 mM NAD and 0.01 ml of 300 U ml⁻¹ glucose-6-phosphate dehydrogenase. The solution was mixed thoroughly, and the absorbance was measured at 340 nm (17).

Glucose-6-Phosphate Dehydrogenase (EC 1.1.1.49)

The measure of glucose-6-phosphate dehydrogenase activity is the rate of increase in absorbance. Addition of maleimide inhibits oxidation of reaction products by 6-phospho gluconolactone. Liver tissue was excised and rinsed with saline solution and the homogenate prepared in saline solution. To 0.02 ml of homogenate were added 0.6 ml of distilled water, 0.1 ml of 3.8 mmol l⁻¹ NADP, 0.1 ml of 0.5 mol l⁻¹ Tris buffer (pH 7.5), 0.1 ml of 0.63 mol l⁻¹ MgCl₂ and 0.1 ml of 33 mol l⁻¹ glucose-6-phosphate. Then ~0.5 mg of maleimide was added, with gentle mixing to dissolve the maleimide. The absorbance was measured at 339 nm. One unit of enzyme
activity is defined as that quantity which catalyses the reduction of 1 μM of NADP per minute (18).

**Lactate Dehydrogenase (EC 1.1.1.27)**

Lactate dehydrogenase catalyzes the conversion of L-lactate to pyruvate with simultaneous reduction and oxidation of NAD to NADH. Change in absorbance with time as a result of converting NAD to NADH is directly proportional to LDH activity. The liver and kidney homogenate was prepared in saline. To 0.05 ml of tissue homogenate was added 2.5 ml of Tris (81.3 mmol, pH 7.2)/NaCl (203.3 mmol)/NADH 0.244 mmol l⁻¹, and the solution was mixed thoroughly. To this solution was added 0.5 ml of Tris (81.3 mmol, pH 7.2)/NaCl (203.3 mmol)/pyruvate (9.76 mmol l⁻¹). The solution was mixed well and the absorbance measured at 339 nm (19).

**Glucose-6-Phosphatase (EC 3.1.3.9)**

Glucose-6-phosphatase catalyzes the conversion of glucose-6-phosphate to glucose. The liver was homogenized in ice-cold sucrose (250 mM) solution. To 0.1 ml of sucrose/EDTA buffer were added 0.1 ml of glucose-6-phosphate (100 mM), 0.1 ml of imidazole buffer (100 mM, pH 6.5) and 0.1 ml of homogenate, with thorough mixing. The tubes were incubated at 37°C for 15 min. The enzymatic activity was terminated by the addition of 2 ml of TCA/ascorbate (10%/2%, w/v), and the solution was centrifuged at 3000 r.p.m. for 10 min. To 1 ml of clear supernatant were added 0.5 ml of ammonium molybdate (1%, w/v) and 1 ml of sodium citrate (2%, w/v). The absorbance was measured at 700 nm. The enzyme activity was expressed as unit per gram per minute in tissue (20).

**Alanine Amino Transferase (EC 2.6.1.2)**

Alanine amino transferase (ALT) catalyzes the transamination of L-alanine to 2-oxo glutarate, forming a glutamate and a pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase with simultaneous oxidation of reduced NADH to NAD. The change in absorbance with time as a result of the conversion of NADH to NAD is directly proportional to the ALT activity. The kidney homogenate was prepared in liver homogenate. To 0.2 ml homogenate were added 2.3 ml L-alanine (610 mmol) in Tris buffer (0.10 mol l⁻¹, pH 7.15), 0.1 ml of NADH (4.2 mmol l⁻¹), 0.1 ml of pyridoxal 6 phosphate (3.4 mmol l⁻¹) and 0.1 ml of lactate dehydrogenase 72 000 U l⁻¹. The reaction was initiated by the addition of 0.2 ml of 2-oxo glutarate solution. The absorbance was measured at 339 nm (21).

**Urea and Creatinine**

Serum urea level was determined using a Urease enzyme kit modified Berthlot method (Agappe Diagnostics, Thane, India). The creatinine level in serum was estimated using the alkaline picrate-modified Jaffe’s method with a creatinine kit (Agappe Diagnostics).

**Estimation of Antioxidant Parameters**

Enzymatic antioxidants [glutathione synthetase and glutathione peroxidase (22), catalase (EC 1.11.1.6) (23,24), peroxidase (EC 1.11.1.7) (25), superoxide dismutase (EC 1.15.1.1) (26)] and nonenzymatic antioxidants [ceruloplasmin (27), tocopherol (28) and ascorbic acid (29)] were determined. The protein content in the tissue homogenate was estimated following the standard methodology (30).

**Statistical Analysis**

Data were statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe’s test using the SPSS computer software, version 7.5. The values were considered significant when P < 0.05.
Results and Discussion

Carbohydrate Metabolism

The activities of different key enzymes (hexokinase, glucose-6-phosphate dehydrogenase and lactate dehydrogenase) are represented in Figs 1, 2 and 3. Diabetic animals treated with 500 mg kg\(^{-1}\) of the alcoholic extract of *C. fenestratum* showed better enzyme activity than those treated with the lower (250 mg kg\(^{-1}\)) dose of the alcoholic extract. The significant increase in the levels of hexokinase, a key glycolytic enzyme known to decrease in the diabetic state (31), may be due to the direct stimulation of glycolysis in tissues with increased glucose removal from the blood. The significant reversal of diabetes induced decreased levels of glucose-6-phosphate dehydrogenase and lactate dehydrogenase may be attributed to an increase in glucose utilization through the pentose phosphate pathway (32), interfering with the mitochondrial respiratory chain and promoting the peripheral glucose utilization by enhancing anaerobic glycolysis (33).

Treated groups exhibited a significant decrease in the levels of glucose-6-phosphatase (Fig. 4) and alanine amino transferase (Fig. 5). Glucose-6-phosphatase, a key enzyme in gluconeogenesis, plays an important role in glucose homeostasis in the liver and kidney (34). The decreased levels observed in treated diabetic animals may be because of the suppression of hepatic gluconeogenesis and glucose output from liver. The elevation in alanine amino transferase in liver and kidney observed in streptozotocin induced diabetic rats corroborates earlier findings (35,36) which attribute the increased gluconeogenesis and ketogenesis observed in diabetes to the high activity of transaminases. In our study, supplementation with the alcoholic extract decreased the enhanced transaminase activity significantly.

Serum urea (Fig. 6) and creatinine levels (Fig. 7) were also decreased significantly compared with the diabetic control.

Antioxidant Status

Enzymatic Antioxidants

Antioxidants are of two types: enzymatic and nonenzymatic antioxidants. Catalase, superoxide dismutase, peroxidase, glutathione synthetase and glutathione peroxidase are examples...
of enzymatic antioxidants. Superoxide dismutase and catalase are considered primary enzymes since they are involved in the direct elimination of reactive oxygen species (37). Superoxide dismutase is an important defense enzyme which catalyzes the dismutation of superoxide radicals (38), and catalase is a hemoprotein which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals (39). The reduced activity of superoxide dismutase and catalase in the liver and kidney observed during diabetes may result in deleterious effects as a result of the accumulation of superoxide anion radicals and hydrogen peroxide (40). Glutathione synthetase, the most important biomolecule protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydroperoxide in the presence of glutathione peroxidase (41,42). The decreased level of glutathione synthetase observed in the diabetic animals represents increased utilization resulting from oxidative stress (43). Glutathione peroxidase, a selenium-containing enzyme present in significant concentrations, detoxifies $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$ through the oxidation of reduced glutathione (44). Depression of glutathione peroxidase activity, observed in diabetic liver and kidney, has been shown to be an important adaptive response to increased peroxidative stress (45). The activity of enzymatic antioxidants [catalase, glutathione peroxidase, glutathione synthetase, peroxidase and superoxide dismutase (Figs 8–12)] increased significantly in extract-treated animals ($P < 0.05$).

### Nonenzymatic Antioxidants

Tocopherol, ceruloplasmin and ascorbic acid are nonenzymatic antioxidants. Whereas $\alpha$-tocopherol reduces lipid

---

![Figure 7](image1)

**Figure 7.** Alcoholic extract of *C. fenestratum* significantly decreases creatinine levels in diabetic rats. Each value represents mean ± SE, $n = 6$; a, statistical significance versus control ($P < 0.05$); b, statistical significance versus normal ($P < 0.05$).

![Figure 8](image2)

**Figure 8.** Alcoholic extract of *C. fenestratum* significantly increases catalase levels in diabetic rats. Each value represents mean ± SE, $n = 6$; a, statistical significance versus control ($P < 0.05$); b, statistical significance versus normal ($P < 0.05$); U, $\mu$mol of hydrogen peroxide consumed per minute.

![Figure 9](image3)

**Figure 9.** Alcoholic extract of *C. fenestratum* significantly increases glutathione peroxidase levels in diabetic rats. Each value represents mean ± SE, $n = 6$; a, statistical significance versus control ($P < 0.05$); b, statistical significance versus normal ($P < 0.05$); U, $\mu$mol of glutathione consumed per minute.

![Figure 10](image4)

**Figure 10.** Alcoholic extract of *C. fenestratum* significantly increases glutathione synthetase levels in diabetic rats. Each value represents mean ± SE, $n = 6$; a, statistical significance versus control ($P < 0.05$); b, statistical significance versus normal ($P < 0.05$); U, $\mu$mol of 1-Chloro-2,4-dinitrobenzene (CDNB)–glutathione synthetase conjugate formed per minute.

![Figure 11](image5)

**Figure 11.** Alcoholic extract of *C. fenestratum* significantly increases peroxidase levels in diabetic rats. Each value represents mean ± SE, $n = 6$; a, statistical significance versus control ($P < 0.05$); b, statistical significance versus normal ($P < 0.05$); U, $\mu$mol of hydrogen peroxide consumed per minute.
The tetrahydro protoberberine analogue of berberine has been reported to inhibit lipid peroxidation and to scavenge hydroxyl free radicals (51). Preliminary phytochemical screening of C. fenestratum showed the presence of phenolic compounds (11), which have been reported to have antioxidant activity (52). Hence in our study the antioxidant activity of C. fenestratum may be due to the presence of berberine and phenolic compounds.

The present investigation draws out a sequential metabolic correlation between increased glycolysis and decreased gluconeogenesis stimulated by C. fenestratum, which may have been the biochemical mechanism through which glucose homeostasis was regulated. The enhanced effect and the protective action on cellular antioxidant defense of C. fenestratum suggest protection against oxidative damage in streptozotocin–nicotinamide induced diabetes.

References
1. Mohamed AK, Bierhaus A, Schiekofer S, Trichtshet H, Ziegler H, Nawroth PP. The role of oxidative stress and NF (B) activation in late diabetic complications. Biofactors 1999;10:175-9.
2. Oberley LW. Free radicals and diabetes. Free Radic Biol Med 1988;5:113–24.
3. Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. Lancet 1994;344:1396–7.
4. Simmons KJ. Defense against free radicals has therapeutic implications. *J Am Med Assoc* 1984;251:2187–92.

5. Wohsieb SA, Godin DV. Alterations in free radical tissue defense mechanisms in STZ induced diabetes in rat, effects of insulin treatment. *Diabetes* 1987;36:1014–18.

6. Varier PS. *Coscinium fenestratum* In: Indian Medicinal Plants, Compendium of 500 species. Hyderabad, India: Orient Longman Ltd, Vol. 2, 1994, 191–193.

7. *Coscinium fenestratum* In: The Wealth of India, Publication Information and Directorate. India: CSIR, Vol. 2, 1950, 360.

8. Datta SC, Mathur RK, Bartaula JN. Minor alkaloids of *Coscinium fenestratum* root. *Indian Drugs* 1988;25:350.

9. Singh GB, Singh S, Bani S, Malhotra S. Hypotensive action of a *Coscinium fenestratum* stem extract. *J Ethnopharmacol* 1990;38:151–5.

10. Venkukumar MR, Latha MS. Effect of *Coscinium fenestratum* on hepatotoxicity in rats. *Indian J Exp Biol* 2004;42:792–7.

11. Shirwaikar A, Rajendran K, Punitha ISR. Antidiabetic activity of alcoholic stem extract of *C. fenestratum* in streptozotocin nicotinamide induced type 2 diabetic rats. *J Ethnopharmacol* 2005;97:369–74.

12. kokate CK, Purohit AP, Gokhale SB. Phytochemical Screening In: Practical Pharmacognosy. New Delhi: Vallabh Prakashan, 1994, 107–13.

13. Harborne JB. Methods of Extraction and Isolation In: Phytochemical Methods. London: Chapman & Hall, 1998, 60–6.

14. Masillo P, Broca C, Gross R, Royle M, Manteghetti M, Hillaire-Buys D et al. Development of a new model of type 2 diabetes in adult rats administered with streptozotocin and nicotinamide. *Diabetes* 1997;46:224.

15. Shirwaikar A, Rajendran K, Dinesh Kumar C, Oral anti-diabetic activity of *Annona squamosa* leaf alcohol extract in NIDDM rats. *Pharmacol Biopharm* 2004;42:50–5.

16. Shirwaikar A, Rajendran K, Dinesh Kumar C, Ram Gopal Bodla. Antidiabetic activity of aqueous leaf extract of *Annona squamosa* in streptozotocin nicotinamide type 2 diabetic rats. *J Ethnopharmacol* 2004;91:171–5.

17. Brandrup N, Kirk JE, Bruni C. Determination of hexokinase in tissues. *J Gerontol* 1957;12:166–71.

18. Deutsc J. Glucose 6 Phosphate dehydrogenase. In: Bergmeyer Methods of Enzymatic Analysis, Vol. III, 3rd edition. Deerfield Beach, FL: Verlag Chemie, 1983, 190.

19. Vassault A. Lactate dehydrogenase. In: Bergmeyer Methods of Enzymatic Analysis, Vol III, 3rd edition. Deerfield Beach, FL: Verlag Chemie, 1983, 118.

20. Baginski ES, Foa PP, Zad B. Glucose 6-phosphatase. In: Bergmeyer Methods of Enzymatic Analysis, Vol II, 2nd edition. New York: Academic Press, 1974, 788–92.

21. Horder M, Rej R. Alanine amino transferase. In: Bergmeyer Methods of Enzymatic analysis, Vol. III, 3rd edition. Deerfield Beach, FL: Verlag Chemie, 1983, 190.

22. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium biochemical role as a component of glutathione peroxide purification and assay. *Science* 1973;179: 588–90.

23. Aebi EH. Catalase. In: Bergmeyer Methods of Enzymatic analysis, Vol. III, 3rd edition. Deerfield Beach, FL: Verlag Chemie, 1983, 273.

24. Sinha KA. Colorimetric assay of catalase. *Anal Chem* 1972;47:389–94.

25. Putter J, Becker R. Peroxidase. In: Bergmeyer Methods of Enzymatic analysis, Vol. III, 3rd edition. Deerfield Beach, Florida: Verlag Chemie, 1983, 1986.

26. Kakkar P, Dos B, Viswanathan PN. A modified spectrophotometric assay for superoxide dismutase. *Ind J Biochem Biophys* 1984;21:130–2.

27. Ravin HA. An improved colorimetric assay of ceruloplasmin. *J Labor Clin Med* 1961;589:161–8.

28. Desai LD. Vitamin E analysis methods for animal tissues. Methods *Enzymol* 1984;105:135–42.

29. Omuyeh ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. In: McCormick DB, Wright CD (eds). Methods in Enzymology, Vol. 62. New York: Academic Press, 1979, 1–11.

30. Lowry OH, Rosebrugh MJ, Farr AL, Randall RJ. Protein measurement with folin–phenol reagent. *J Biol Chem* 1951;236:265–75.

31. Baquer NZ, Gupta D, Raju J. Regulation of metabolic pathways in liver and kidney during experimental diabetes: effects of antioxidant compounds. *Int J Clin Biochem* 1998;13:63–80.

32. Ugochukwu NH, Babady NE. Antihyperglycemic effect of aqueous and ethanol extract of *Glycine max* leaves in streptozotocin induced diabetic rats. *Arch Biochem Biophys* 1985, 1–27.

33. Vassault A. Lactate dehydrogenase. In: Bergmeyer Methods of Enzymatic Analysis, Vol III, 3rd edition. Deerfield Beach, FL: Verlag Chemie, 1983, 190.

34. Baginski ES, Foa PP, Zad B. Glucose 6-phosphatase. In: Bergmeyer Methods of Enzymatic Analysis, Vol II, 2nd edition. New York: Academic Press, 1974, 788–92.

35. Bruce A, Freeman D, James C. Biology of disease–free radicals and tissue injury. *Lab Invest* 1982;47:412–16.

36. Belliwell B, Halliwell B, Gutteridge JMC. Free Radicals and Toxicology In: Free Radicals in Biology and Medicine. Oxford: Clarendon Press, 1985, 1–27.

37. McCord JM, Keele BB, Fridovich I. An enzyme based theory of obligate anaerobiosis, the physiological functions of superoxide dismutase. *Proc Natl Acad Sci USA* 1976;68:1024–7.

38. Chance B, Greenstein DS, Roughton RJW. The mechanism of catalase action—steady state analysis. *Arch Biochem Biochem* 1952;37:301–39.

39. Searle AJ, Wilson R. Glutathione peroxidase effect of superoxide, hydroxyl and bromine free radicals on enzyme activity. *Int J Radiat Biol* 1980;37:213–17.

40. Meister A. New aspects of glutathione biochemistry and transport selective alterations of glutathione metabolism. *Nutr Rev* 1984;42:397–410.

41. Nicotera P, Orenius S. Role of thiols in protection against biological reactive intermediates. *Adv Exp Med Biol* 1986;197:41–9.

42. Anuradha CV, Selvan R. Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan induced diabetic rats. *J Nutr Biochem* 1993;4:212–17.

43. Bruce A, Freeman D, James C. Biology of disease–free radicals and tissue injury. *Lab Invest* 1982;47:412–16.

44. Kinalska M, Sledziewski A, Telejko B, Zarzycki W, Kinalska T. Lipid peroxidation and scavenging enzyme activity in streptozotocin induced diabetes. *Acta Diabetologia* 2000;37:179–83.

45. Halliwell B, Gutteridge JMC. The antioxidant of human extra cellular fluids. *Arch Biochem Biochem* 1990;280:1–8.

46. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. *Proc Natl Acad Sci USA* 1986;83:3777–81.

47. Inefers H, Sies H. The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. *Eur J Biochem* 1988;174:353–7.

48. Sajithal GB, Chitra P, Chandrakasan G. Effect of curcumin on the advanced glycation and cross linking of collagen in diabetic rats. *Biochem and Pharmacol* 1998;56:1607–14.

49. Jin XL, Shao Y, Wang MJ, Chen LJ, Jin GZ. Tetrahydro protoberberines inhibit lipid peroxidation and scavenge hydroxyl free radicals. *Acta Pharmacol Sin* 2000;21:477–80.

50. Auddy B, Ferreiru M, Blasina F, Lafon L, Arredondo F, Dajas F et al. Screening of antioxidant activity of three Indian medicinal plant traditionally used for the management of neurodegenerative diseases. *J Ethnopharmacol* 2003;84:131–8.

51. Hang CY, Yen GC. Antioxidant activity of phenolic compounds isolated from *Mesona procumbens* Hems. *J Agric Food Chem* 2002; 50:2993–7.

Received November 29, 2004; accepted June 12, 2005