

Antioxidant properties of *Coscinium fenestratum* stem extracts on Streptozotocin induced type 1 diabetic rats

Neethu P., Haseena P., ZevaluKezo, Sona Thomas R., Santhosh Wilson Goveas* and Asha Abraham

Father George Albuquerque Pai Cell and Molecular Biology laboratory, Dept. of Post Graduate Studies and Research in Biotechnology, St Aloysius College (Autonomous), Mangalore-575003, India.

ARTICLE INFO

Article history:

Received on: 25/11/2013

Revised on: 12/12/2013

Accepted on: 02/01/2014

Available online: 30/01/2014

Key words:

Coscinium fenestratum,
Methanolic fraction, Anti
diabetic, Antioxidants,
Glycogen content,
Hexokinase.

ABSTRACT

The aim of this investigation was to study the antioxidant status of Streptozotocin induced diabetic rats which were treated with methanolic stem extract of *Coscinium fenestratum*. Diabetes was induced by administering STZ by intra femoral vein injection at a dose of 40mg/kg body weight. Methanolic extract of *Coscinium fenestratum* was administered to the rats at a dose of 200 mg/kg body weight for a period of two weeks. The antioxidant status of the treated animals were assessed by measuring the activity of liver enzymes such as Catalase, Super oxide dismutase and Glutathione S Transferase. Levels of these antioxidant enzymes decreased in untreated diabetes compared to normal values. Administration of methanolic fractions of *Coscinium fenestratum* in STZ induced diabetic rats reversed these changes. Oxidative stress plays a major role in generation of free radicals in the pathogenesis of diabetes and its complications. Our findings suggest that *C. fenestratum* methanolic extract exerted anti-hyperglycemic activity by combating the oxidative stress, improving the glycogen content and by activating the carbohydrate metabolizing enzyme Hexokinase. The presence of Berberine and other phenolic compounds in the stem extracts may be responsible for this action.

INTRODUCTION

Diabetes is manifested by multiple etiologies characterized by chronic hyperglycemia with abnormalities in carbohydrate, fat and protein metabolism due to defect in insulin secretions or action or both. According to International Diabetes Federation there are currently more than 194 million diabetic patients worldwide. The number of diabetes is increasing in an alarming rate. The World Health Organization has pointed the prevention of diabetes and its complications not only a major challenge for future, but essential if health for all is to be an attainable target and strongly emphasize the optimal, rational use of traditional and natural indigenous medicines

Oxidative stress play a major role in the causation of diabetes and some of the antioxidants have a role in reducing the diabetes (John, 1991). Oxygen free radicals (OFRs) have been suggested to be a contributory factor in complications of diabetes mellitus. Mammalian cells are equipped with both enzymic and non- enzymic antioxidant defence mechanism to minimize the cellular damage caused by the interaction between cellular constituents and OFRs. The current therapy involves the use of

insulin as well as oral drugs like sulfonylurea drugs, biguanides, alpha glucosidase inhibitors, meglitinides and thiazolidinediones.

However these pose various side effects and are very expensive. In order to overcome this there is a need for an alternative therapy like, the use of herbal medicines. Herbal medicines are considered to less toxic and have less side effects (Pari and Umamaheshwari, 2000). *Coscinium fenestratum* belongs to the family of Menispermaceae. It is a well-known herb in Ayurvedic system of medicine. It grows well in Western Ghats (India) and Sri Lanka.

This plant is known to have hypoglycemic (Shirwarkar *et al.*, 2005), anti oxidative (Punitha *et al.*, 2005) and anti cancer effect (Tungpradit *et al.*, 2004). The stem of *C. fenestratum* contains berberine, which is the major active compound. There are a few reports about the anti-hyperglycemic effect of this plant. However the anti-hyperglycemic properties of the methanolic stem extract of the plant in STZ induced diabetic rats has not been reported. The aim of this study is to investigate the hypoglycemic activity of *C. fenestratum* stem extract in normal and STZ-induced diabetic rats by assaying the antioxidative enzymes, carbohydrate metabolizing enzymes, and estimating glycogen content.

* Corresponding Author

Email: swgoveas@gmail.com

MATERIALS AND METHODS

Animals

Male wistar rats were procured from local animal dealer. These rats were housed in groups of 2-3 in separate cages and fed lab chow and water *ad libitum*. They were maintained in twelve hour light and twelve hour dark cycles and maintained according to the guidelines of the ethical committee of the institution (SAC/IAEC/111/.dated 30/3/2011). The experiments were performed after the approval from the Institutional animal ethical committee.

Plant materials

The *Coscinium fenestratum* stems were collected from the foot hills of Western Ghats. The specimen was identified by Dr. Gopalkrishna Bhat, a taxonomist from Udupi. A herbarium sample of the plant is maintained in the Department of Botany, St Aloysius College (Autonomous), Mangalore. The material was brought to the laboratory and air dried at room temperature ($25 \pm 2^\circ\text{C}$). The dried samples were powdered using grinder mill and stored in desiccators.

Preparation of alcoholic stem extract

About 150grams of stem powder of *Coscinium fenestratum* was placed in soxhlet extractor. It was extracted with methanol for approximately 72 hours. After this extraction period the solvent collected from the extractor was evaporated using flash evaporator. The concentrated residue obtained which contains the plant extract was further concentrated by placing it in a incubator at 37°C for 24hours and then stored in a desiccator. Known amount (100mg) of this residue was dissolved in 5% Tween-80.

Induction of Diabetes Mellitus in Rats

Diabetes was induced in rats chemically by administering Streptozotocin (STZ) by intrafemoral vein injection at a dose of 40mg/kg body weight. The control rats were given 0.1M Citrate buffer injections (pH4.1). The diabetic rats were divided into diabetic, diabetic +stem extract. Four animals were maintained in each group.

Stem Extract Therapy

One group of diabetic rat was given methanolic stem extracts of *Coscinium fenestratum* orally at a dose of 200mg/kg of body weight for a period of 2 weeks. Control rats were only injected with citrate buffer.

Preparation of homogenate

After the end of the experiment, the animals were sacrificed by cervical decapitation. Liver was excised, washed with cold Phosphate buffered saline (PBS pH7.0). The tissue was weighed and tissue homogenate was prepared with TrisHCl buffer. The centrifugation was done at 10,000xg for 10min, the supernatant was collected and used for the various enzyme assays.

Estimation of Glutathione –S- transferase (GST)

GST assay was performed according to Habig (1974). Briefly, 3 ml reaction mixture, the final concentration in the mixture was 97 mM potassium phosphate, 0.97 mM EDTA, 5.0 mM glutathione, reduced p – nitrobenzyl chloride, 3.2% ethanol and 0.1 ml liver homogenate. The absorbance was read at 450nm.

Estimation of Catalase

Catalase assay was performed according to Aebi (1984). Briefly, 3 ml of the reaction mixture contains 50 mM potassium phosphate, 0.036% (w/w) H_2O_2 and 0.1 ml of tissue homogenate. The rate of disappearance of H_2O_2 is followed by the rate of decrease in the absorbance at 240 nm.

Estimation of Super Oxide Dismutase

Super oxide dismutase assay was performed according to Hassan (1978). Briefly, 3ml reaction mixture contains 5.6×10^{-5} NBT, methionine, riboflavin, 0.05M potassium phosphate buffer of pH 7.8 and 0.1ml of tissue homogenate. The absorbance was read at 560nm.

Estimation of enzymes in carbohydrate metabolism

Glucose 6 phosphate dehydrogenase

Glucose 6 phosphate dehydrogenase enzyme assay was performed as follows. The reaction mixture contained 1ml glucose -6- phosphate, 0.1ml NADP^+ , 0.025ml glycyl glycine buffer, 0.2ml magnesium chloride and 0.05ml tissue homogenate. The increase in absorbance was read at 340nm immediately.

Hexokinase

Hexokinase enzyme assay was performed according to Darrow (1962). Reaction cocktail was prepared by adding 5ml ATP, 6.60ml Cresol Red, 33.40 ml deionized water and 5ml glycyl glycine buffer. pH was set to 8.5. Titer determination was done by measuring the initial absorbance of a solution containing 2.5 ml reaction cocktail and 0.4 ml of glucose at 560 nm and by measuring the final absorbance of the solution after adding 0.1 ml cresol red at 560nm.

Estimation of liver glycogen

Glycogen content in liver was determined after digesting the tissue with hot concentrated potassium hydroxide, precipitation of glycogen with ethanol, hydrolyzing the extracted glycogen with acid to glucose, which was then estimated by DNS method. Pure glycogen processed similarly was used as the standard for quantification.

Statistical Analysis of Data

Statistical analyses were done by ANOVA test using graph pad INSTAT ver – 3.0 computer program.

RESULTS AND DISCUSSIONS

Antioxidant Enzymes

STZ is known to bring about destruction of pancreatic islet beta cells through reactive oxygen species and free radical generation. Oxidative stress is suggested as mechanism underlying diabetes and diabetic complications, which result from an imbalance between radical generating and radical scavenging systems. In the present study, we observed a significant decrease in the activities of key antioxidant enzymes such as of glutathione-S-transferase and catalase in the liver of diabetic rats. These were brought back to near normal range upon treatment with methanolic stem extract of *Coscinium fenestratum* in STZ induced diabetic rats.

Table. 1: Level of antioxidant enzymes in the liver.

Animals	Glutathione-S-transferase (Units/mg protein)	Catalase (Units/mg protein)	Superoxide dismutase (Units/mg protein)
Control	0.0626 ±0.008	0.369±0.0288	1.575±0.257
STZ induced diabetic	0.0399±0.0755	0.2485±0.015	1.5781±0.060
Diabetic + Extract	0.1295±0.0027*	0.536±0.0415**	1.5773±0.099

Values are Mean ±S.E.M of three independent observations.*P<0.05 when compared with control and **P<0.001 when compared with control.

The level of Glutathione-S-transferase (GST) in the case of diabetic rat was 0.0399±0.07 where as in case of diabetic with Methanolic stem extract of *Coscinium fenestratum* was increased to 0.1295±0.0027. The activity of catalase in the liver of diabetic animal was decreased to 0.2485±0.015 when compared with the control it was 0.369±0.0288. With the Methanolic stem extract of *Coscinium fenestratum* the activity of catalase in the liver was increased to 0.536±0.0415. The activity of catalase in the liver of diabetic animals is generally believed to decrease (Indran *et al.*, 2004). The decrease in the catalase activity might be due to glycation of the enzyme (Subbiah *et al.*, 2005). Superoxide dismutase (SOD) are class of closely related enzymes that catalyses the breakdown of the superoxide anion into oxygen and hydrogen peroxide. (Johnson *et al.*, 2005). Indarn *et al.*, 2004 reported that superoxide dismutase was significantly decreased in Insulin Dependant Diabetic Rats (IDDM) rats. However, in our studies we did not see any significant change in the activities of SOD. Our results showed that Glucose-6-Phosphate dehydrogenase increased in diabetic condition. Perhaps this would have prevented the accumulation of superoxide anions by providing reducing power. It was 1.575±0.257 in case of control and 1.5781±0.060 and 1.5773±0.099 in case of diabetic rats and diabetic + extract rats (Table.1.).

Table 2 indicates the level of hepatic glycogen content. Our results shows that in STZ diabetic rats (50.28 ±1.25), hepatic glycogen content was significantly lower than control rats (93.23 ±3.25 and it was reversed by stem extract treatment (80.34 ±2.38). Table 3. shows the level of Hexokinase activity decreased in the case of STZ induced diabetic rats (123.4±24.28) when compared

with control (290.6±15.88) and diabetic rats with stem extract treatment (283.6±18.83). Hexokinase reaction is the rate limiting step in glycogen synthesis (Mathieu *et al.*, 1998). Decreased glycolysis must have depressed glycogen synthesis and promoted its breakdown in diabetic rats and these were reversed by the improved hexokinase levels in stem extract treated diabetic rats. The level of Glucose -6-Phosphate dehydrogenase enzyme increased in the case of diabetic rats (165.82±11.56), when compared with control (103.43±10.24) and diabetic rats treated with stem extract (110.26±15.43).

Table . 2: Estimation of Liver Glycogen Content.

Control	93.23 ±3.25
Streptozotocin induced diabetes	50.28 ±1.25*
Diabetic + Extract	80.34 ±2.38*

Values are Mean±S.E.M. of three independent observations,*p< 0.05 when compared with controls, ** p<0.001 when compared with control

Table. 3: Estimation of Carbohydrate Metabolizing Enzymes.

Animals	Hexokinase (Units/mg of protein)	Glucose 6 phosphate dehydrogenase (Units/mg of protein)
Control	290.6±15.88	103.43±10.24
STZ induced diabetic	123.4±24.28*	165.82±11.56
Diabetic + Extract	283.6±18.83**	110.26±15.43*

Values are Mean±S.E.M. of three independent observations,*p< 0.05 when compared with controls, ** p<0.001 when compared with control

The most commonly recognized antioxidants are Superoxide dismutase (SOD), Catalase and Glutathione-S-Transferase (GST). SOD changes the structure of oxidants and breaks them into hydrogen peroxide. Catalase found in peroxisome in turn breaks down hydrogen peroxide into water and tiny oxygen particles. Alloxan induced diabetes resulted in decrease of glutathione-s-transferase and this was reversed to normal values by treatment with the methanol extracts of *Terminalia arjuna* barks (Raghavan and Krishnakumari, 2006), *B. diffusa* leaf extract (Satheesh *et al.*, 2004) and *A. augusta* and *A. indica* (Halim, 2003). Earlier studies (Punitha *et al.*, 2005) have shown that *Coscinium fenestratum* genus is rich in Vitamin C, alkaloids like berberine, flavonoids, and terpenoids. This might play an important role in improving the antioxidant status. These findings clearly indicate that the methanolic extract of *Coscinium fenestratum* improves the antioxidant status of STZ induced diabetic rats.

ACKNOWLEDGEMENT

The authors thank the UGC for providing financial assistance through Minor Research Project. Authors also thank the Principal and Management of St. Aloysius College (Autonomous), Mangalore for the facilities for carrying out the work.

REFERENCES

- Aebi H .Catalase *in Vitro*. Methods of Enzymol, 1984; 105: 121-126.
- Darrow, R. A., and S. P. Colowick. 1962. Hexokinase from baker's yeast. In: Methods in Enzymology No. 5. S. P. Colowick and N. O. Kaplan, ed. Academic Press Inc., New York. p.226-227.

Fridovich I. The biology of oxygen radicals. *Science*, 1978; 201: 875–880.

Habig WH, Pabst MJ, Jakoby WB . Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* ,1974; 246:7130-7139

Halim EM. Lowering of blood sugar by water extract of *Azadirachta indica* and *Abroma augusta* in diabetic rats. *Indian J. Exp.Bio*,2003; 41: 636-640.

John WB. Role of oxidative stress in development of complications in diabetes. *Diabetes* , 1991; 40: 405.

Johnson F and Giulivi C. Superoxide dismutase and their impact on human health. *Mol aspects Med*, 2005; 26 (4-5):340-52

Indran M, Rokiah P, Chan SP, Kuppusamy UR .Alteration of lipid peroxidation and antioxidant enzymes in young Malaysian IDDM patients. *Med J Malaysia*,2004;59:166-70.

Mathieu B, Stefaan K, Willy S. Specific features of glycogen metabolism in the liver. *Biochem.J* 1998; 336:19-31

Pari L. and Satheesh A.M. Antidiabetic effect of *Boerhavia diffusa* : effect on serum and tissue lipids in experimental diabetes. *J. Med. Food*, 2004; 7: 472-476.

Pari L, Umamaheswari J. 2000. Antihyperglycemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan diabetic rats. *Phytother Res* 14(2)136-138.

Punitha, ISR, Rajendran K, Shirwaikar A and Shirwaikar A. Alcoholic stem extract *Coscinium fenestratum* regulates carbohydrate metabolism and improves antioxidant status in streptozotocin-nicotinamide induced diabetic rats. *Evid. Based Complement. Alternat. Med*, 2005; 2: 375-381.

Raghavan B, Krishnakumari S. Antidiabetic Activity of T. Arjuna Bark Extract in Alloxan induced diabetic rats' *Ind. J. Of Clinical Biochemistry*, 2006;1(2):123-128

Shirwaikar A, Rajendran K and Punitha ISR. Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type 2 diabetic rats. *Journal of Ethnopharmacology*, 2005; 97(2):369-374

Subbaiah R, Kururan S and Subramanian S. Antioxidant effect of Aloe vera gel extract in streptozotocin induced diabetes in rats. *Pharmacological reports*,2005; 57:90-96.

Tungpradit R. Anti-Cancer Compound Screening and Isolation: *Coscinium fenestratum*, *Tinospora crispa* and *Tinospora cordifolia*. *Chiang Mai Journal of Science*,2010; 37(3): 476-488.

How to cite this article:

Neethu P., Haseena P., ZevaluKezo, Sona Thomas R., Santhosh Wilson Goveas and Asha Abraham., Antioxidant properties of *Coscinium fenestratum* stem extracts on Streptozotocin induced type 1 diabetic rats. *J App Pharm Sci*, 2014; 4 (01): 029-032.