ISSN: 2349-9141

Available online at http://www.ijirr.com

International Journal of Information Research and Review
Vol. 02, Issue, 05, pp.6924-6929, May, 2015

OPEN ACCESS JOURNAL

Full Length Research Paper

PREVENTIVE EFFECTS OF *CASSIA AURICULATA* ON BRAIN LIPID PEROXIDATION STREPTOZOTOCIN DIABETIC RATS

***Pidaran Murugan**

Department of Biochemistry, Faculty of Science, Bharathidhasn University constituent College, peramablure-621107, Tamil Nadu, India

***Corresponding Author**

Received 10th April 2015; Published 31th May 2015

Abstract

Oxidative damage has been suggested to be a contributory factor in development and complication of diabetes. To investigate the effect of *Cassia auriculata* L (CFEt) on the occurrence of oxidative stress in the brain of rats during diabetes we investigated the extent of oxidative damage as well as the status of the antioxidant defense system. Oral administration of CFEt (0.45 g/kg) to diabetic rats for 45 days resulted in a significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, CFEt caused significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione in brain of diabetic rats with significant decrease in lipid peroxidative markers namely: thiobarbituric acid reactive substances (TBARS) and hydroperoxides in brain, suggesting its role in protection against lipid peroxidation induced membrane damage. The effect of CFEt was better when compared with glibenclamide. Results of the present study suggest that CFEt showed antioxidant effect in addition to its antidiabetic effect in type 2 diabetic rats.

Keywords: Cassia Auriculata L , Lipid Peroxidation , Antioxidants, Streptozotocin Diabetes.

Copyright © Pidaran Murugan. *This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.*

To cite this paper*: Pidaran Murugan, 2015. "Preventive Effects of CASSIA AURICULATA on Brain Lipid peroxidation Streptozotocin Diabetic Rats", International Journal of Information Research and Review,* ***Vol. 2, Issue, 05, pp. 6924-6929****.*

INTRODUCTION

Diabetes mellitus is the most common human metabolic disease affecting about 200 million people in World. Changes in the concentrations of lipids including cholesterol, triglycerides are complications frequently observed with diabetes mellitus and certainly contribute to the development of vascular disease (Howard *et al*. 1978). In recent years, considerable focus has been given to an intensive search for novel type of antioxidants from numerous plant materials (Mc Call *et al*., 1997). Management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects (Halliwell and Gutteridge, 1989). Plants with antidiabetic activities provide useful sources for the development of drugs in the treatment of diabetes mellitus. Medicinal plants with hypoglycemic activity were used for centuries and some times as regular constituents of the diet, as they are free from side effects (Aragno *et al*., 1997). Phytochemicals isolated from plant source are used for the prevention and treatment of cancer, heart disease, diabetes and high blood pressure etc. (Li *et al*., 1998).

Cassia auriculata L. (Ceasalpiniaceae) is a shrub that has attractive yellow flowers, commonly used for the treatment of skin disorders and body odour. It is a native plant present in different parts of India. Indigenous people use various parts of the plant for diabetes mellitus. It is widely used in Ayurvedic medicine as a "Kalpa drug" which contains five parts of the shrub (roots, leaves, flowers, bark and unripe fruits) which are taken in equal quantity, dried and then powdered to give "Avarai Panchaga Choornam", for the control of sugar levels and reduction of symptoms such as polyuria and thirst in diabetes (Shrotri *et al*. 1963). A literature survey showed that a decoction of leaves, flowers, and seeds of the *Cassia auriculata* mediate an antidiabetic effect (Shrotri and Aiman, 1963). Thus, the available reports show that very little work has been done with respect to *Cassia auriculata* flowers, other than its hypoglycemic effects. In our previous study, we have demonstrated the antidiabetic effect of *Cassia auriculata* flower extract (CFEt) in streptozotocin (STZ) induced diabetic rats (Pari and Latha, 2002). To our knowledge, so far no other biochemical investigation has been carried out on the effect of CFEt in brain antioxidant status of experimental diabetic rats. The present investigation was carried out to study the effect of CFEt

on brain lipid peroxides and antioxidants in rats with STZ and nicotinamide induced diabetes.

MATERIALS AND METHODS

Animals: Adult male albino Wistar rats (8 weeks), weighing 180–200 g bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were used. All animal experiments were approved by the ethical committee, Annamalai University and were in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India. The animals were housed in polycarbonate cages in a room with a 12 h day-night cycle, temperature of 24 ± 2° C, humidity of 45–64%. During the whole experimental period, animals were fed with a balanced commercial diet (Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

Chemicals: STZ was obtained from Himedia Laboratory Limited, Mumbai, India. All other reagents used were of analytical grade.

Plant Material: Tanner's cassia flowers were collected freshly from Neyveli, Cuddalore District, Tamil Nadu, India. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No.231) was deposited in the Botany Department of Annamalai University.

Preparation of plant extract: 500 g of Tanner's cassia flowers were extracted with 1,500 ml of water by the method of continuous hot extraction at 60ºC for six hours and evaporated. The residual extract was dissolved in water and used in the study.

Induction of diabetes: Non-Insulin dependent diabetes mellitus was induced (Masiello *et al*., 1998) in overnight fasted rats by a single intraperitonial injection (i.p) of STZ (65 mg/kg body weight), 15 min after the i.p administration of nicotinamide (110 mg/kg body weight). STZ was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in blood, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl was used for the study (Pari and Murugan, 2005). To our knowledge, so far no other biochemical investigations has been carried out on the effect of CFEt in plasma and tissue glycoproteins of experimental diabetic rats. The present investigation was carried out to study the effect of CFEt on plasma and tissue glycoproteins in rats with STZ induced diabetes.

Induction of experimental diabetes: A freshly prepared solution of STZ (45 mg/kg i.p) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg. After 48 hours of STZ administration, rats with moderate diabetes having glycosuira and hyperglycaemia (i.e. with a blood glucose of 200- 300 mg/dl) were taken for the experiment.

Experimental procedure: In the experiment, a total of 36 rats (30 diabetic surviving rats, six normal rats) were used. The rats were divided in to six groups of six rats each.

Group 1: Normal untreated rats.

Group 2: Diabetic control rats given 1 ml of aqueous solution daily using an intragastric tube for 45 days.

Group 3: Diabetic rats given CFEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45 days.

Group 4: Diabetic rats given glibenclamide (600 µg/ kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

Animals were sacrificed at the end of 45 days by cervical dislocation. Blood was collected in tubes containing potassium oxalate and sodium fluoride solution for the estimation of blood glucose and plasma was separated for assay of insulin. The entire brain was perfused immediately with ice-cold 0.9% sodium chloride. Thiobarbituric acid reactive substances (TBARS), hydroperoxides, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and reduced glutathione were estimated in brain. Brain was selected as it continuously generates large amounts of free radicals from mitochondrial oxidative activity and catecholamine catabolism. At the same time brain contains high levels of polyunsaturated fatty acids, which are the preferred targets of free radical damage in cell (Masiello *et al*., 1998).

Analytical procedure

Measurement of blood glucose and plasma insulin: Blood glucose was estimated colorimetrically using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Baroda, India) Lott and Turner. Plasma insulin was assayed by the enzyme-linked immunosorbent assay method using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany).

Estimation of lipid peroxidation: Lipid peroxidation in brain was estimated colorimetrically by measuring TBARS and hydroperoxides using the methods of Fraga *et al.*(1988). and Jiang *et al*.,(1992) respectively. In brief, 0.1 ml of tissue homogenate was treated with 2 ml of TBA-trichloroacetic acid (TCA)- HCl reagent (0.37% TBA, 0.25 M HCl and 15% TCA, 1:1:1 ratio), placed for 15 min in a water bath and then cooled and centrifuged for 10 min (1000 rpm) at room temperature, the clear supernatant was measured at 535 nm against a reference blank. Values were expressed as mmoles/100g – tissue. Hydroperoxides were expressed as mmoles/100g – tissue. Tissue homogenate (0.1 ml) was treated with 0.9 ml of Fox reagent (88 mg of Butylated hydroxy toluene (BHT), 7.6 mg of xylenol orange and 0.8 mg of ammonium iron sulphate were added to 90 ml of methanol and 10 ml of 250 mmoles sulphuric acid) and incubated at 37°C for 30 min. The color development was read at 560 nm.

Estimation of catalase activity: *Catalase (CAT)* was estimated by the method of Sinha, 1972. The reaction mixture (1.5 ml, vol) contained 1.0 ml of 0.01M-pH 7.0-phosphate buffer, 0.1 ml of tissue homogenate and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). It was read at 620 nm and expressed as µmoles of H_2O_2 consumed/min/mg protein.

Estimation of superoxide dismutase (SOD) activity: The activity of *SOD* was assayed by the method of Kakkar *et al.,1984*. Pipetted out 0.5 ml of tissue homogenate was diluted with 1 ml of water. In this mixture, 2.5 ml of ethanol and 1.5 ml of chloroform (all reagents chilled) were added and shaken for 1 min at 4°C then centrifuged. The enzyme activity in the supernatant was determined. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.1 ml of 186 μmoles PMS, 0.3 ml of 30 μmoles NBT, 0.2 ml of 780 μmoles NADH, appropriately diluted enzyme preparation and water in a total volume of 3 ml. Reaction was started by the addition of NADH. After incubation at 30°C for 90 sec the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The intensity of the chromogen in the butanol layer was measured at 560nm against butanol blank. A system devoid of enzyme served as control. One unit of the enzyme activity is defined as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute under the assay conditions and expressed as specific activity in units/mg protein.

Estimation of glutathione peroxides (GPx) activity: GPX activity was measured by the method described by Rotruck *et al*., 1973 Briefly, the reaction mixture contained 0.2 ml 0.4M phosphate buffer (pH 7.0), 0.1 ml 10 mmoles sodium azide, 0.2 ml tissue homogenized in 0.4M, phosphate buffer, pH 7.0, 0.2 ml glutathione, and 0.1 ml 0.2 mmoles hydrogen peroxide. The contents were incubated for 10 min at 37 °C, 0.4 ml 10% TCA was added to stop the reaction and centrifuged. The supernatant was assayed for glutathione content using Ellman's reagent (19.8 mg 5,5'-dithiobisnitrobenzoic acid (DTNB) in 100 ml 0.1% sodium nitrate). The activities were expressed as g of GSH consumed/min/mg protein.

Estimation of glutathione-S-transferase(GST) activity: *GST* activity was determined spectrophotometrically by the method of Habig *et al., 1974* . The reaction mixture contained 1.0 ml 100 mmoles phosphate buffer (pH 6.5), 0.1 ml 30 mmoles 1-chloro-2, 4-dinitrobenzene (CDNB), and 0.7 ml double distilled water. After pre-incubating the reaction mixture for 5 min at 37 °C, the reaction was started by the addition of 0.1 ml tissue homogenate and 0.1 ml of glutathione as substrate. After 5 min the absorbance was read at 340 nm. Reaction mixture without the enzyme was used as a blank. The activity of GST was expressed as μmoles of CDNB-GSH conjugate formed/min/mg protein.

Estimation of reduced glutathione (GSH): GSH was determined by the method of Ellman., 1959. A known weight of tissue was homogenized in phosphate buffer. From this 0.5 ml was pipetted out and precipitated with 2 ml of 5% TCA. 1 ml of the supernatant was taken after centrifugation and added to it 0.5 ml of Ellman's reagent and 3 ml of phosphate buffer. The yellow colour developed was read at 412nm. A series of standards were treated in a similar manner along with a blank containing 3.5 ml of buffer. The values were expressed as mg/100g – tissue

Estimation of protein: Protein was determined by the method of Lowry *et al.,* 1951 using bovine serum albumin as the standard, at 660nm.

Statistical analysis: The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Duncan's multiple range test (DMRT). Values were considered statistically significant if $p < 0.05$ (Duncan 1957).

RESULTS

Changes in blood glucose and plasma insulin: Fig.1 shows the level of blood glucose and plasma insulin of different experimental groups. The diabetic control rats showed a significant increase in the level of blood glucose with significant decrease in the activity of plasma insulin. Oral administration of CFEt to diabetic rats significantly reversed the above biochemical changes The administration of CFEt and glibenclamide to normal rats showed a significant effect on blood glucose and plasma insulin levels.

***Effect on brain lipid peroxidation and antioxidants*:** Table 1 shows the changes in the levels of lipid peroxidation and the activities of antioxidant enzymes in normal and experimental rats. TBARS and hydroperoxides from brain homogenate were significantly decreased with CFEt treatment whereas; diabetic control rats showed significantly increased levels of lipid peroxidation products. The effect of CFEt was better than glibenclamide. For studying the effect of CFEt on free radical production, the activities of SOD, CAT, GPx, GST and GSH were measured. They presented significant increases in CFEt treatment when compared with diabetic control rats. The effect of CFEt was more prominent compared with glibenclamide.

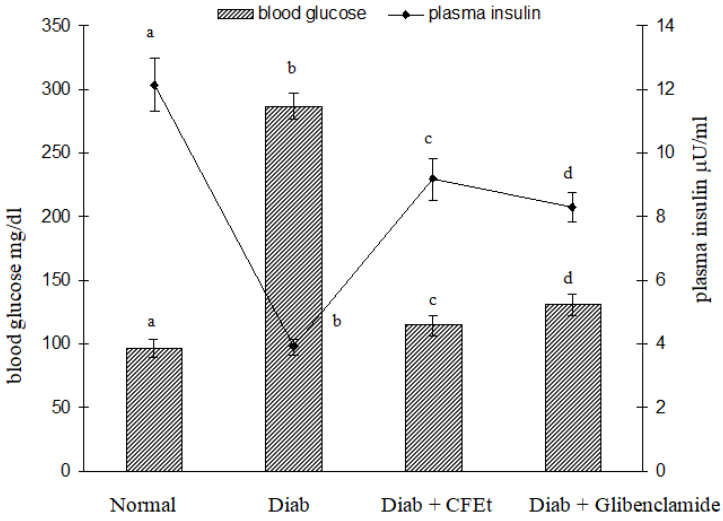
DISCUSSION

Diabetes mellitus comprises a group of chronic diseases characterized by hyperglycemia or diminished insulin secretion or both and profound effects on lipid metabolism. Hyperlipidaemia has an association with atherosclerosis and the incidence of atherosclerosis is vastly increased in diabetics. The capacity of CFEt to decrease the elevated blood sugar to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes (Pari and Latha, 2002). The possible mechanism by which CFEt exerts its hypoglycemic action in diabetic rats may be by potentiating the plasma insulin effect by increasing the pancreatic secretion of insulin from the existing β-cells as it is evidenced by the significant increase in the level of insulin by CFEt in diabetic rats. STZ induced diabetic animal is considered as an animal model of hyperlipidemia (Murugan, 2010). STZ induces oxygen free radicals induced lipid peroxidation by generating peroxynitrate, which is spontaneously formed when nitric oxide and superoxide co-exists (Bassirat and Khalil 2000). Masiello *et al*., 1998 described a new experimental diabetic model in adult rats by administering STZ and partially protected it with a suitable dose of nicotinamide. This syndrome shares a number of features with human type 2 diabetes, and is characterized by moderate stable hyperglycemia, glucose intolerance, altered but significant glucose-stimulated insulin secretion, *in vivo* and *in vitro*. STZ and nicotinamide administration a partial loss of β–cell mass occurs by necrosis and/or apoptosis. The residual β cells (about 60% of the original mass) are most likely those, which escaped from irreversible damage and maintained the differentiation of mature β – cells.

Table 1. Change in the levels of tbars, hydroperoxides, catalase, superoxide dismutase, glutathione peroxidase, glutathione-s-transferase and reduced glutathione in brain of normal and experimental animals

Groups	Normal	Diabetic control	Diabetic + CFEt (0.45 g/kg)	Diabetic + Glibenclamide (600 (µg/kg)
TBARS Brain (mmoles /100g tissue)	1.17 ± 0.07[a]	1.91 ± 0.15[b]	1.27 ± 0.06[c]	1.38 ± 0.06[d]
Hydroperoxides Brain (mmoles /100g tissue)	112.04± 6.55[a]	135.89 ± 7.66[b]	126.52 ± 6.58[c]	123.09 ± 6.30[d]
Catalase Brain (Units[A] / mg protein)	3.24 ± 0.21[a]	0.75 ± 0.04[b]	2.69± 0.15 [c]	1.91 ± 0.11[d]
Superoxide dismutase Brain (Units[B] / mg protein)	7.79 ± 0.32[a]	5.01± 0.29[b]	7.01 ± 0.36[c]	6.22+ ± 0.29[d]
Glutathione peroxidase Brain (Units[C] / mg protein)	3.24± 0.19[a]	1.03 ± 0.05[b]	2.58 ± 0.16[c]	1.82 ± 0.12[d]
Glutathione-S-transferase Brain (Units[D] / mg protein)	5.35 ± 0.30[a]	0.81 ± 0.04[b]	2.78 ± 0.15[c]	2.12 ± 0.14[d]
Reduced Glutathione *Brain (mg / 100g tissue)*	33.24 ± 2.12[a]	14.88 ± 1.03[b]	29.24 ± 1.88[c]	23.29 ± 1.28[d]

Values are given as mean ± S.D for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p<0.05 (DMRT). A - µmoles of H2O2 consumed / minute. B – One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute. C - µg of GSH consumed / min. D - µmoles of CDNB – GSH conjugate formed / min.



Diab - Diabetic control, CFEt - *Cassia auriculata* L
Values are given as mean ± S.D for 6 rats in each group.
Values not sharing a common superscript letter differ significantly at p<0.05 (Duncan's Multiple Range Test).

Figure 1. Effect of cfet on the levels of blood glucose and plasma insulin in normal and experimental rats

Hence the possible mechanism by which CFEt brings about its antihyperglycemic action may be by stimulation of surviving β-cells to release more insulin. This was clearly evidenced by the increased level of insulin in diabetic rats treated with CFEt. The administration of CFEt and glibenclamide to decrease the increased blood glucose concentration to normal glycemic concentration is an essential trigger for the brain to revert its normal homeostasis during experimental diabetes. CFEt has the ability to trigger the proinsulin synthesis and also insulin release, which might be helpful to reduce the plasma glucose and increase insulin during diabetes. Several studies have shown increased lipid peroxidation in clinical and experimental diabetes (Novelli *et al*., 2001). TBARS and hydroperoxides (lipid peroxidative markers) showed high lipid peroxidation. This may be because; the brain contains relatively high concentration of easily peroxidizable fatty acids (Carney *et al*., 1991).

In addition, it was known that certain regions of the brain were highly enriched in iron, a metal that, in its free form, was catalytically involved in production of damaging oxygen free radical species (Nistico *et al*., 1992). Vulnerability of brain to oxidative stress induced by oxygen free radicals seems to be due to the fact that, on one hand, the brain utilizes about one fifth of the total oxygen demand of the body and on the other, that it is not particularly enriched, when compared with other organs, in any of the antioxidant enzymes. Relatively low levels of these enzymes may be responsible in part for the vulnerability of this tissue (Baynes and Thrope, 1999). Previous studies have reported that there was an increased lipid peroxidation in brain of diabetic rats.[49] Our study shows that administration of CFEt and glibenclamide significantly decreased the brain TBARS and hydroperoxides. It has been also supported by previous report of CFEt/ glibenclamide increased hepatic GSH levels and induced certain forms of

GSH transferase important in preventing lipid peroxidation and detoxification of toxic lipid aldehydes in diabetic cataract rats (Latha and Pari, 2003). This indicated the antiperoxidative effect of CFEt. Increased lipid peroxidation under diabetic conditions can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. Associated with the changes in lipid peroxidation the diabetic tissues showed decreased activities of key antioxidants SOD, CAT, GSH, GPx, GST and GSH, which play an important role in scavenging the toxic intermediate of incomplete oxidation. SOD and CAT are the two major scavenging enzymes that remove toxic free radicals *in vivo*. Previous studies have reported that the activity of SOD was low in diabetes mellitus (Osawa and Kato, 2005). The decreased activities of CAT, SOD may be as a response to increased production of H_2O_2 and O_2 by the autoxidation of glucose and non-enzymatic glycation. Treatment with CFEt increased the activity of antioxidant enzymes that scavenge the free radicals generated during diabetes. CFEt has been shows to increase the activity of SOD, which may protect CAT and GPx against inactivation by $O_2^{\cdot-}$ anions as these anions have been shown to inactivate CAT and GPx (Anuradha and Selvam, 1993). The GST catalysed the conjugation of GSH with a large number of electrophiles as a step to detoxify these species.

It has been proposed that GPx was responsible for the detoxification of H_2O_2 in low concentration whereas catalase comes in to play when GPx pathway was reaching saturation with the substrate (Lu, 1999). Administration of CFEt increased the GSH content. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species generated from exposure to STZ (Yu, 1994). The significant increases in GSH content and GSH dependent enzymes GPx and GST in diabetic rats treated with CFEt indicated an adaptive mechanism in response to oxidative stress. It may be concluded that during diabetes brain tissue was more vulnerable to oxidative stress and showed increased lipid peroxidation. The above observations showed that CFEt possesses antioxidant effect that may contribute to its protective action against lipid peroxidation and enhancement of cellular antioxidant defense. This activity contributes to the protection against oxidative damage in STZ induced diabetes.

REFERENCES

Anuradha CV, Selvam R: Effect of oral methionine on tissue lipid peroxidation and antioxidants in alloxan induced diabetic rats. J Nutrit Biochem 1993; 4: 212-217.

Aragno M, Brignardello E, Tamagno O, Boccuzzi G: Dehydroeppiandrosterone administration prevents the oxidative damage induced by acute hyperglycemia in rats. J Endocrinol 1997; 155: 233-240.

Bassirat M, Khalil Z.: Endothelin and free radicals modulate microvascular responses in streptozotocin diabetic rats. Microvasc. Res. 59: 88-98, 2000.

Baynes JW, Thrope SR: Role of oxidative stress in diabetic complications. Diabetes 1999; 48: 1-9.

Carney JM, Strake-Reed PE, Oliver CN, Landum RW, Chang MS, Wu JF, Floyd RA: Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity and loss in temporal and spatial memory by chronic administration of the spin trapping compound N-tert-buty-alfa-pheynitrone. PNAS 1991; 88: 3633-3636.

Duncan BD: Multiple range tests for correlated and heteroscedastic means. Biometrics 1957; 13: 359–364.

Ellman GL: Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82: 70-77.

Fraga, CG, Leibouitz BE, Toppel AL. Lipid peroxidation measured as TBARS in tissue slices: characterization and comparison with homogenates and microsomes. Free Radic Biol Med 1988; 4: 155–161.

Habig WR, Pbst MJ, Jakpoly WB: Glutathione transferase. A first enzymatic step in mercaturic acid formation. J Biol Chem 1974; 249: 7130-7139.

Halliwell B, Gutteridge JMC: Free radicals in biology and medicine, 2nd edition, Oxford, Clarendon Press 1989, pp. 1-20.

Howard, B.V., P.J. Savage, L.J. Bennion & P.H. Bennett: Lipoprotein composition in diabetes mellitus. Atheroscler. 1978, 30, 153-162.

Jiang ZY, Hunt JV, Wolff SD. Ferrous sulphate oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low-density lipoprotein. Anal Biochem 1992; 202: 384–389.

Kakkar P, Das B, Viswanathan PN: A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophy 1984; 21: 130-132.

Latha M, Pari L. Preventive effects of Cassia auriculata L. flowers on brain lipid peroxidation in rats treated with streptozotocin. Mole Cell Biochem 2003; 243: 23-28.

Li PA, Gisselsson J, Keuker J, Vogel ML, Kuschinsky SW, Siesjo K: Hyperglycemia-exaggerated ischemic brain damage following 30 min of middle cerebral artery occlusion is not due to capillary obstruction. Brain Res 1998; 804: 36–44.

Lowry OH, Roesborough MJ, Farr AL, Randall RJ: Protein measurement with Folin-Phenol reagent. J Biol Chem1951; 193: 265- 275.

Lu SC: Regulation of hepatic glutathione syntesis: Current concepts and controversies. FASEB 1999; 13: 1169-1183.

Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G: Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 1998; 47: 224-229.

Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G.: Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes. 47: 224-229, 1998.

Mc Call AL, Kong C, Collins J, Ellkeles R, Richmond W: Total antioxidant status, protein glycation, lipid hydroperoxides in non insulin dependent diabetes mellitus. Biochem Sco Transplant 1997; 25, 132-137.

Murugan P.Taners cassia (Cassia auriculata) Extracts prevents Hemoglobin glycation and tail tendon collagen properties in experimental diabetic rats.Journal of cell and tissue research vol. 10(1) 2109-2114. 2010

Nistico G, Cirilol HR, Fiskin K, Iannone M, Martino A, Rotilio G: NGF restores decrease in catalase activity and increases superoxide dismutase and glutathione peroxidase activity in the brain of aged rats. Free Radic Bio Med 1992; 12: 177-181.

Novelli M, Fabregat ME, Fernandez-Alvarez J, Gomis R, Masiello P. Metabolic and functional studies on isolated islets in a new rat model of type 2 diabetes. Molel Cell Endocrinol 2001;175: 57–66.

Osawa T, Kato Y: Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann NY Acad Sci 2005; 1043: 440-451.

Pari L, Latha M. Antidiabetic effect of Cassia auriculata flowers: Effect on Lipid Peroxidation in streptozotocin diabetes rats. Pharm. Biol. 40: 351-357, 2002.

Pari L, Murugan P: Effect of tetrahydrocurcumin on blood glucose, plasma insulin and hepatic key enzymes in streptozotocin induced diabetic rats. J Basic Clin Physiol Pharmacol 2005;16: 257-274.

Pushparaj P, Tan CH, Tan BKH.: Effects of Averrhoa bilimbi leaf extract on blood glucose and lipids in streptozotocin diabetic rats. J. Ethnopharmacol. 72: 69-76, 2000.

Rotruck JT, Pope AL, Ganther HE, Swanson AB: Selenium: Biochemical roles as a component of glutathione peroxidase. Science 1973; 179: 588 - 590.

Shrotri DS, Aiman R. The relationship of the post-absorptive state to the hypoglyceamic action. Ind. J. Med. Res. 48: 162 – 168, 1960.

Sinha KA: Colorimetric assay of catalase. Annal Biochem 1972; 47: 389-394.

Yu BP: Cellular defense against damage from reactive oxygen species. Physiol Review 1994; 74: 139 – 162.

****