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EFFECT OF *CASSIA AURICULATA* L ON PLASMA ANTIOXIDANTS IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED EXPERIMENTAL DIABETES

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Abstract

Clinical research has confirmed the efficacy of several photochemicals in the modulation of oxidative stress associated with diabetes mellitus. In the present study, we have investigated the effect of *Cassia auriculata L* (CFEt), on antioxidants status in streptozotocin - nicotinamide induced diabetic rats. A single dose of streptozotocin (65 mg/kg body weight) produced decrease in insulin, hyperglycemia, increased lipid peroxidation (thiobarbituric reactive substances [TBARS] and lipid hydroperoxides) and decreased antioxidant levels (vitamin C, vitamin E, reduced glutathione, ceruloplasmin). Oral administration of CFEt ((0.45 g/kg)) for 45 days to diabetic rats significantly increased the plasma insulin and plasma antioxidants and significantly decreased the lipid peroxidation. The effect of CFEt was better when compared with glibenclamide. Results of the present study indicated that CFEt showed antioxidant effect in addition to its antidiabetic effect in type 2 diabetic rats.

Keywords: *Cassia Auriculata* , Plasma Lipid Peroxidation, Plasma Antioxidants, Diabetes Mellitus.

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INTRODUCTION

Diabetes is a major health problem affecting major populations worldwide. Epidemiological studies and clinical trials strongly support the notion that hyperglycaemia is the principal cause of complications. Erythrocyte membrane hyper polarization was found in diabetic cells as a result of constant oxidative stress that can be responsible for long term complications in diabetes (Augustyniak et al., 1996). Changes in membrane lipid composition and enzymatic properties of membrane bound enzymes are shown to occur in diabetes. The enhancement of non-enzymatic glycosylation diabetic extends beyond haemoglobin to the proteins of the of the erythrocyte membranes. Erythrocytes are highly sensitive to peroxidative damage probably due to the high content of unsaturated fatty acid in their membrane. Therefore the fair degree of ATPases activities in erythrocyte membrane could be serving as simple, safe and useful marker of intracellular damage (Ohta et al. 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 (Halim Eshrat and Hussain, 2002). Phytochemicals isolated from plant source are used for the prevention and treatment of cancer, heart disease, diabetes and high blood pressure etc. (Mary et al., 2002). *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 (Brahmachari and Augusti, 1961; 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 investigations has been carried out on the effect of CFEt in plasma antioxidant status of experimental diabetic rats. The present investigation was carried out to study the effect of CFEt on plasma 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^{o} 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: Stereptozotocin was obtained from Himedia Laboratory Limited, Mumbai, India. All other reagents used were of analytical grade.

Plant Material: *Cassia auriculata* 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: Five hundred g of *Cassia auriculata* 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 (Jain, 1968).

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 (Siddique et al. 1987).

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) (Pari and Uma Maheswari, 2000) in 1 ml of aqueous solution daily using an intragastric tube for 45days. At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Plasma was separated for the estimation of glucose, insulin and glycoproteins.

Induction of diabetes: Non-Insulin dependent diabetes mellitus was induced /21/ in overnight fasted rats by a single intraperitonial injection (i.p) of 65 mg/kg body weight STZ, 15 min after the i.p administration of 110 mg/kg body weight of nicotinamide. 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 plasma, determined at 72 h and then on day 7 after injection. The animals with blood glucose concentration more than 200 mg/dl will be used for the study.

Experimental design: In the experiment, a total of 24 rats (18 diabetic surviving rats, 6 normal rats) were used. The rats were divided into four groups of six each, after the induction of STZ diabetes. The experimental period was 45 days. Group I: Normal rats. Group II: Diabetic control rats. Group III: Diabetic rats given CFEt (0.45 mg/kg body weight) in aqueous suspension daily using an intragastric tube for 45 days. Group IV: Diabetic rats given Glibenclamide ((600 µg/kg) in aqueous suspension daily using an intragastric tube for 45 days. At the end of 45 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin and other biochemical parameters.

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) John and Lott Turner (1975. Plasma insulin was assayed by the enzyme- linked immunosorbent assay method using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany) Andersen et al., (1993).

Estimation of Lipid peroxidation: Lipid peroxidation in plasma was estimated colorimetrically by measuring thiobarbituric acid reactive substances (TBARS) and hydroperoxides using the methods of Fraga et al. (1988) and Jiang et al. (1992), respectively. In brief, 0.5 ml of plasma was treated with 2 ml of TBA-trichloroacetic acid (TCA)- HCl reagent (0.37% TBA, 0.25 N 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. Hydroperoxides were expressed as mmoles/dl. Plasma (0.5 ml) was treated with 0.9 ml of Fox reagent (88 mg of 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 ascorbic acid (Vitamin C: Vitamin C was estimated by the method of Omaye et al. (1979). To 0.5 ml with 1.5 ml of 6% TCA and centrifuged for 20 minutes. To 0.5

ml of the supernatant, 0.5 ml of DNPH reagent was added and mixed well, allowed to stand at room temperature for an additional 3 hours and added 2.5 ml of 85% sulphuric acid and allowed to stand for 30 minutes. A set of standards containing 10-50µg of ascorbic acid were taken and processed similarly along with a blank, read at 530 nm. Ascorbic acid values were expressed as mg/dl plasma.

Estimation of Vitamin E: Vitamin E was determined by the method of Baker et al. (1951). To 0.1 ml of plasma, 1.5ml ethanol and 2.0 ml of petroleum ether were added, mixed and centrifuged. The supernatant was evaporated to dryness at 80°C. To this was added 0.2 ml of 2, 2′ -dipyridyl solutions and 0.2 ml of ferric chloride solution. Mixed well and kept in dark for 5 minutes and added 2 ml of butanol. The intense red colour developed was read at 520 nm. Standard tocopherol in the range of 10-100µg were taken and treated similarly along with blank containing only the reagent. The amount of α-tocopherol was expressed as mg/dl plasma. Protein was determined by the method of Lowry et al. (1951).

Estimation of Ceruloplasmin: Plasma ceruloplasmin was estimated by the method of Ravin (1961). 0.05 ml of plasma was added. To control, 1 ml of sodium azide was added and mixed. To both the tubes 1.0 ml of p-phenylenediamine was added, mixed and kept at 37°C for 1 h. 1 ml of sodium azide was then added to the test. All the tubes were kept at 4 to 10°C for 30 min. The colour developed was then read at 540nm with control as blank. Ceruloplasmin values were expressed as mg/dl.

Estimation of reduced glutathione: Reduced glutathione (GSH) was determined by the method of Ellman (1959). An aliquot (1.0 ml) of the supernatant was treated with 0.5 ml Ellman's reagent and 3.0 ml phosphate buffer (0.2M, pH 8.0) and the absorbance was read at 412 nm. GPx activity was expressed as µg GSH consumed/ min/mg protein and GSH as mg / dl plasma.

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 /31/.

RESULTS

Changes in blood glucose and plasma insulin: Figure 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 level 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.

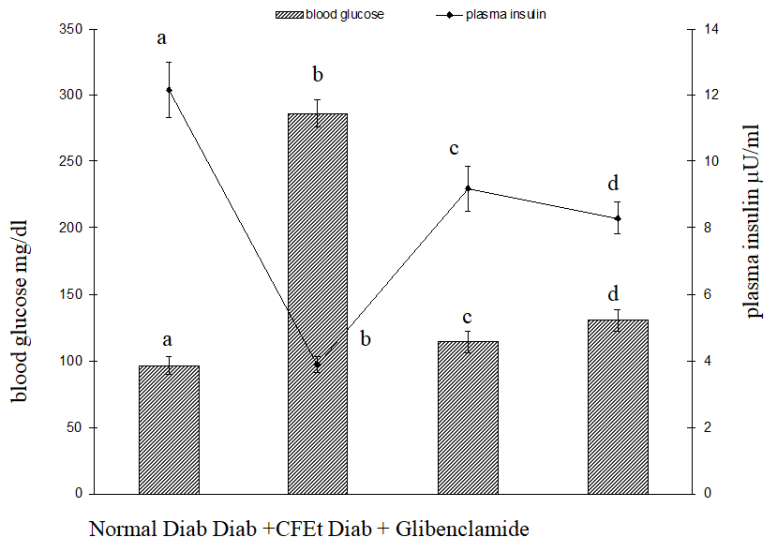
Effect of CFEt on plasma lipid peroxidation: Untreated diabetic rats showed a significant increase in the levels of lipid peroxide and TBARS in plasma (Table 1). Rats treated with CFEt and glibenclamide, however, had significantly lower plasma lipid peroxide and TBARS levels as compared with the untreated diabetic rats.

Effect of CFEt on plasma antioxidants: Plasma GSH and vitamin C levels were significantly lower in diabetic rats than in normal rats and plasma ceruloplasmin and α-tocopherol levels were significantly higher in diabetic rats than in normal rats. In contrast, diabetic rats treated with CFEt and glibenclamide had near normal levels of plasma antioxidants (Table 2)

DISCUSSION

The number of patients with type 2 diabetes is markedly increasing worldwide, and nowadays type 2 diabetes is recognized as the most prevalent and serious metabolic disease. The overall prevalence of diabetes is approximately six percent of the population, of which 90 percent is type 2 (Maiti et al., 2004). 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*. Novelli et al., (2001) reported that following STZ and nicotinamide administration a partial loss of □-cell mass occurs by necrosis and/or apoptosis. The residual □ cells (about 60% of the original mass) are most likely those, which escaped from irreversible damage and maintained the differentiation of mature β – cells. 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 level of plasma lipid peroxide is raised in STZ-induced diabetes. This rise in lipid peroxide is generally thought to be a consequence of due to pathological changes to tissues which increase production and liberation of lipid peroxides into the circulation (Selvam and Anuradha, 1990). Previous studies have reported that there was an increased lipid peroxidation in plasma of diabetic rats (Venkateswaran and Pari, 2002). It has been also supported by previous report of CFEt increase hepatic GSH levels and induce certain forms of GSH transferase important in preventing lipid peroxidation and detoxification of toxic lipid aldehydes in diabetic cataract rats (Osawa and Kato, 2005). This indicates the antiperoxidative effect of CFEt. In our study, the increase in plasma ceruloplasmin and α-tocopherol in diabetic rats is most likely a reaction of the body to increased lipid peroxides. Ceruloplasmin is known to be a powerful free radical scavenger that inhibits lipid peroxidation (Halliwell and Gutteridge, 1990). Administration of CFEt to the STZ-induced diabetic rats decreased measured plasma levels of TBARS, hydroperoxides, ceruloplasmin, and α-tocopherol, demonstrating an antiperoxidative effect of CFEt. Several studies have shown increased lipid peroxidation in clinical and experimental diabetes (Kakkar et al., 1998). The results showed elevation of lipid peroxidation in the tissues of diabetic group. The increase in oxygen free radicals in diabetes could be due to rise in blood glucose levels, which upon autoxidation generate free radicals. STZ has been shown to produce oxygen free radicals (Ivorra a et al., 1989). Lipid peroxide mediated tissue damages have been observed in the development of type I and type II diabetes mellitus (Feillet-Coudray et al., 1999).



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

Table 1. Changes in the levels of plasma TBARS and hydroperoxides in normal and experimental animals

Groups	TBARS (mmoles/dl)	Hydroperoxides (x 10^{-5} mM/100ml)
Normal	0.22 ± 0.02^{a}	11.02 ± 0.52^{a}
Diabetic control	0.48 ± 0.03^{b}	20.85 ± 1.25^{b}
Diabetic+ CFEt (0.45 g/kg)	0.26 ± 0.02^{c}	12.79 ± 0.72^{c}
Diabetic + Glibenclamide ((600 µg/kg)	0.29 ± 0.02^{d}	14.23 ± 0.72^{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).

Table 2. Changes in levels of vitamin C, vitamin E, ceruloplasmin and reduced glutathione (GSH) in plasma of normal and experimental animals

Groups	Normal	Diabetic control	Diabetic+ CFEt (0.45 g/kg)	Diabetic + Glibenclamide (600 µg/kg)
Vitamin C (mg/dl)	1.87 ± 0.14^{a}	0.79 ± 0.04^{b}	1.65 ± 0.10^{c}	1.39 ± 0.05^{d}
Vitamin E (mg/dl)	1.45 ± 0.07^{a}	0.69 ± 0.05^{b}	1.34 ± 0.08^{c}	1.24 ± 0.04^{d}
Ceruloplasmin (mg/dl)	18.25 ± 1.15^{a}	31.89 ± 2.11^{b}	21.05 ± 1.30^{c}	24.32 ± 1.25^{d}
Reduced Glutathione (mg / dl)	25.03 ± 1.44^{a}	12.78 ± 0.73^{b}	21.05 ± 1.31^{c}	18.26 ± 5.01^{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).

Previous studies have reported that there was an increased lipid peroxidation in liver and kidney of diabetic rats (Latha and Pari, 2003). Our study shows that administration of CFEt and glibenclamide significantly decreased the liver and kidney TBARS and hydroperoxides. It has been also supported by previous report of CFEt increase hepatic GSH levels and induce certain forms of GSH transferase important in preventing lipid peroxidation and detoxification of toxic lipid aldehydes in diabetic cataract rats (Murugan, 2010). This indicates the antiperoxidative effect of CFEt. Vitamin E is a well-known physiological antioxidant and membrane stabilizer (Machin and Bendich, 1987). It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals, thus protecting the cell structures against damage (Yoshito, 1991). The elevated level of vitamin E observed in the diabetic rats is compatible with the hypothesis that vitamin E excess in the plasma of diabetes plays a protective role against increased peroxidation. Oral administration of CFEt to diabetic rats restored the level of vitamin E to near normal.

We have also observed significant changes in the levels of plasma antioxidants in diabetic rats. Vitamin C is a hydrophilic antioxidant in plasma, because it disappears faster than other antioxidants when plasma is exposed to reactive oxygen species (Frei, 1991). The observed significant decrease in the level of plasma vitamin C could be due to the increased utilization of vitamin C as an antioxidant defense against reactive oxygen species or to a decrease in the GSH level, since GSH is required for the recycling of vitamin C (Infers Sies, 1988). GSH is an important inhibitor of free radical mediated lipid peroxidation (Meister and Anderson, 1987). The decreased levels of plasma GSH in diabetes may be due to increased utilization in trapping the oxyradicals. Several workers have also reported decreased levels of plasma GSH and vitamin C in experimental diabetic rats. GSH is the first line of defense against proxidant status (Ahmed et al., 2000) and GSH was evaluated after CFEt administration. GSH systems may have the ability to manage oxidative stress with adaptional changes in enzymes regulating GSH metabolism.

In the present study, treatment with CFEt significantly increased the GSH levels. Increase in GSH level may inturn activates the GSH dependent enzymes such as glutathione peroxidase and glutathione-S-transferase. Ceruloplasmin is an important enzyme which oxidizes iron from the ferrous to ferric state and it has been demonstrated that iron catalysed lipid peroxidation requires both Fe (ii) and Fe (iii) and the maximum rate occurs when the ratio is approximately one (Bucher et al., 1983). The level of Ceruloplasmin is reported to increase under conditions leading to the generation of oxygen products such as superoxide radical and hydrogen peroxide (Dormandy, 1980). The observed increase in the level of plasma ceruloplasmin in diabetic rats may be due to increased lipid peroxides. Prince and Menon (1999) also reported increased level of ceruloplasmin in diabetic rats. Administration of CFEt to diabetic rats restored the level of ceruloplasmin to near normal level. In conclusion, the present investigation shows that CFEt possesses antioxidant effect that may contribute to its protective action against lipid peroxidation and enhancing effect on cellular antioxidant defense. This activity contributes to the protection against oxidative damage in STZ and nicotinamide induced diabetes.

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