Antioxidant effect of *Cassia auriculata* in streptozotocin - nicotinamide induced diabetic rats

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Running title: Effect of *Cassia auriculata* flowers on diabetes

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Abstract

Introduction: The present study investigated the beneficial effect of Tanner's cassia *Cassia auriculta* flower extract (CFEt) and leaf extract (CLEt) antioxidants properties in streptozotocin (STZ) –induced diabetic rats.

Methods: The effects of an aqueous extract of CFEt (0.45 g/kg), CLEt (0.45 g/kg) and glybenclamide on plasma glucose, insulin, thiobarbituric acid reactive substances (TBARS) and hydroperoxides formation, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, vitamin C and vitamin E, histopathological examination of liver and kidney section of normal and experimental groups.

Results: Oral administration of CFEt and CLEt (0.45 g/kg) aqueous extract and glibenglamide to diabetic rats for 45 days significantly resulted in significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, CFEt and CLEt caused significant increase in the activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, reduced glutathione, vitamin C and vitamin E in liver and kidney of diabetic rats with significant decrease in thiobarbituric acid reactive substances (TBARS) and hydroperoxides formation in liver and kidney, suggesting its role in protection against lipid peroxidation induced membrane damage.

Conclusion: CFEt and CLEt 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 Streptozotocin (STZ) induced diabetes. The CFEt administration showed more effective than CLEt and glibencalamide.

Key words: antioxidants, *Cassia auriculta* flower extract, *Cassia auriculta* leaf extract, lipid peroxidation.

Introduction

Chronic hyperglycemia is the primer of a series of cascade reactions causing the over production of free radicals and increasing evidences indicate that these contributes to the development of diabetic complications [1]. Defense system against oxidative attacks is usually able to buffer most ROS produced during physiological and pathological metabolism. However, the imbalance in scavenging of free radicals, due to an increase in oxidative flux or a decrease in the antioxidant ability is responsible for cellular and tissue damage in diabetes mellitus [2].

STZ can result in the formation of ROS including H_2O_2, $O_2^{\bullet}$ and $OH^{\bullet}$. STZ-induced oxidative damage includes the induction of macromolecular damage, depletion of cellular thiol levels and increase in lipid peroxidation with disturbance of antioxidant defense system including alteration in the activities of SOD, CAT, GPx and impaired GSH metabolism [3]. Any compound natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this damage may have a significant role in treatment of diabetes mellitus. In our previous study, we have demonstrated the antidiabetic effect of CFEt in STZ induced diabetic rats [4].

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 [5,6]. A literature survey showed that a decoction of leaves, flowers, and seeds of the *Cassia auriculata* mediate an antidiabetic effect [6]. 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 STZ induced diabetic rats [7].

To our knowledge, so far no other biochemical investigations has been carried out on the effect of CFEt compared CLEt in tissue antioxidant status of experimental diabetic rats.

The present investigation was carried out to study the effect of CFEt compared CLEt on tissue lipid peroxides and antioxidants in rats with STZ and nicotinamide induced diabetes.

Materials and methods

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 [8].

Induction of diabetes

Non-Insulin dependent diabetes mellitus was induced [9] 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 procedure

In the experiment, a total of 30 rats (24 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 CLEt (0.45 g/kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

Group 5: Diabetic rats given glibenclamide (600 µg/ kg body weight) in 1 ml of aqueous solution daily using an intragastric tube for 45days.

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. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood. The tissues were weighed and 10% tissue homogenate was prepared with 0.025 M Tris - HCl buffer, pH 7.5. After centrifugation at 200 rpm for 10 min, the clear supernatant was used to measure thiobarbituric acid reactive substances and hydroperoxides. For the determinations of lipids the liver and kidney tissues were weighed and lipids were extracted from tissues by the method of Folch et al. (1957) using chloroform - methanol mixture ($CHCl_3$: MeOH)(2:1 v/v). The liver and kidney were also dissected out and placed into ice-cold containers for various biochemical estimations and for histopathology examination.

Preparation of tissue homogenate

The liver and kidney was dissected out, weighed and washed using chilled saline solution. Tissue was minced and homogenized (10 % w/v) in appropriate buffer (pH 7.4) and centrifuged and the resulting supernatant was used for enzyme assays.

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.[31] Plasma insulin was assayed by the enzyme- linked immunosorbent assay method using a Boehringer-Mannheim kit with an ES300 Boehringer analyzer (Mannheim, Germany) [10].

Estimation of Lipid peroxidation

Lipid peroxidation in brain was estimated colorimetrically by measuring TBARS and hydroperoxides using the methods of Fraga et al.[11] and Jiang et al.[12] 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 mM/100g – tissue.

Hydroperoxides were expressed as mM/dl. 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 mM 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 [13]. 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 μM 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. [14]. 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 μM PMS, 0.3 ml of 30 μM NBT, 0.2 ml of 780 μM 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. [15]. Briefly, the reaction mixture contained 0.2 ml 0.4M phosphate buffer (pH 7.0), 0.1 ml 10 mM 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 mM 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. [16]. The reaction mixture contained 1.0 ml 100 mM phosphate buffer (pH 6.5), 0.1 ml 30 mM 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 is expressed as mM of GSH-CDNB conjugate formed/min/mg protein using an extinction coefficient of 9.6/ mM /cm.

Estimation of ascorbic acid (vitamin C)

Vitamin C was estimated by the method of Omaye et al. [17]. 0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6% TCA and centrifuged for 20 minutes. After centrifusion, 0.5 ml of the supernatant was mixed with 0.5 ml of DNPH reagent and allowed to stand at room temperature for an additional 3 hours then 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 μM/mg tissue.

Estimation of vitamin E

Vitamin E was determined by the method of Baker et al. [18]. 0.1 ml of lipid extract, 1.5 ml of ethanol and 2 ml of petroleum ether were added, mixed and centrifuged. The supernatant was evaporated to dryness at 80°C then 0.2 ml of 2,2^{1}□□□dipyridyl solution and 0.2 ml of ferric chloride solution was added and mixed well. This was kept in dark for 5 min and added 2 ml of butanol. The intense red colour developed was read at 520nm. Standard α-tocopherol in the range of 10-100 μg were taken and treated similarly along with blank containing only the reagent. The values were expressed as μM/mg – tissue. Protein was determined by the method of Lowry et al. (1951).

Estimation of reduced glutathione (GSH)

GSH was determined by the method of Ellman [19]. 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

Histopathological study

The liver and kidney samples fixed for 48h in 10% formal-saline were dehydrated by passing successfully in different mixture of ethyl alcohol – water, cleaned in xylene and embedded in paraffin. Sections of liver and kidney (4-5 □m thick) were prepared and then stained with hematoxylin and eosin dye, which mounted in neutral deparaffinated xylene (DPX) medium for microscopic observations.

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 $p < 0.05$ [20].

Results

Changes in blood glucose and plasma insulin

Table 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, CLEt and glibenclamide to diabetic rats significantly reversed the above biochemical changes. The administration of CFEt, CLEt and glibenclamide to normal rats showed a significant effect on blood glucose and plasma insulin levels. The CFEt administration showed more effective than CLEt and glibenclamide.

Effect of tissue lipid peroxidation

Table 2 represents the concentration of TBARS and hydroperoxides in tissues of normal and experimental rats. There was a significant elevation in tissue TBARS and hydroperoxides during diabetes, when compared to the corresponding normal group. Administration of CFEt, CLEt and glibenclamide significantly decreased the lipid peroxidation in diabetic rats. The CLEt was more potent than CLEt and glibenclamide.

Effect on tissue enzymes and antioxidants

For studying the CFEt and CLEt on free radical production, the activities of SOD, CAT, GPx, GST, GSH, vitamin C and vitamin E were measured (table 3 and 4). They presented significant increases in CFEt and CLEt treatment when compared with diabetic control rats. The effect of CLEt was more prominent compared with CLEt and glibenclamide.

Histopathological observations in liver, kidney and pancreas of control and experimental rats

Liver

Pathological changes of liver include hepatic nuclear condensation portal triad with inflammation, and sinusoidal dilation in diabetic control rats. The above

pathological changes were remarkably reduced in rats treated with CFEt, CLEt and glibenclamide, with mild inflammation and mild sinusoidal dilatation.

Kidney

Diabetic control rat kidney showed glomeruli mesangial capillary proliferation with tubular epithelial damage. These changes were reduced in CFEt, CLEt and glibenclamide treated diabetic rats. Tissues of normal rats treated with CFEt and CLEt revealed near normal appearance with no significant changes.

Pancreas

Histopathological studies (compared to normal) show the atrophic acini and damaged islet cells in diabetic rat pancreas and these changes were markedly reduced in diabetic rats treated with CFEt, CLEt and glibenclamide.

Discussion

The involvement of free radicals in diabetes and the role of these toxic species in lipid peroxidation and the antioxidant defense system have been studied. STZ at a given dose preferentially destroys the pancreatic insulin secreting β-cells, which leaves less active pancreatic cells and results in diabetes mellitus [21]. STZ directly generates oxygen free radical induced lipid peroxidation [22,23]. This study was therefore undertaken to assess the antiperoxidative and antioxidant properties of *cassia auriculata* in STZ diabetic rats.

Increase in hydroxyl radical formation in diabetic rats may be elucidated by two biochemical mechanisms. One mechanism is increased production of activated oxygen species such as $O_2^{\bullet -}$ or H_2O_2. $OH^{\bullet}$ radicals are generated from $O_2^{\bullet -}$ or H_2O_2 by the iron catalyzed Haber-weiss reaction or Fenton reaction respectively. Another mechanism is decrease in the activity of enzymes (SOD, CAT, glutathione dependent enzymes) to scavenge the activated oxygen species [24]. STZ-induced redox impairment could result from (a) an increase in the production of intracellular free radicals, either endogenous or exogenous (eg. STZ itself) (b) second from a STZ-induced decrease in the ability of the cell to maintain antioxidant mechanisms (eg. inability to maintain reduced GSH concentration or a reduction in SOD activity) (Robbins et al. 1980). Hyperglycemia in the STZ treated rats leads to the formation of H_2O_2, which subsequently generates other

free radicals. These reactive compounds can cause peroxidation of lipids resulting in the formation of hydroperoxy fatty acids and endoperoxides.

It has been generally reported that diabetic patients with vascular lesions have higher TBARS levels than their healthy counterpart. TBARS and hydroperoxides significantly increased in plasma of diabetic control rats. Previous studies have reported that there was an increased lipid peroxidation in plasma of diabetic rats [25,26]. Several studies have shown increased lipid peroxidation in clinical and experimental diabetes [27,28]. Lipid peroxide mediated tissue damages have been observed in the development of type 1 and type 2 diabetes mellitus [29].

Our results show increased lipid peroxidation in the tissues (liver, and kidney) of diabetic control rats. Previous studies have reported that there was an increased lipid peroxidation in liver and kidney of diabetic rats [25,30]. This may be because the tissues contain relatively high concentration of easily peroxidizable fatty acids. Liver during diabetes, showed a relatively severe impairment in antioxidant capacity than kidney. The kidney exhibits a characteristic pattern of changes during diabetes [31]. The Central Nervous System (CNS) is also susceptible to long term complications associated with diabetes [32]. Experimental models of diabetes including the STZ diabetic rats, have provided evidence for functional and morphological alterations in the brain [33]. Free radicals are formed in the CNS as part of the normal metabolic processes [34]. High oxygen uptake and low antioxidant defenses increase the vulnerability of the CNS to oxidative damage [35]. The increase in oxygen free radicals in diabetes could be primarily due to increase in blood glucose levels, which upon autoxidation generate free radicals and secondarily due to the effects of diabetogenic agent STZ [36]. Administration of CFEt, CLEt and glibenclamide reduced the lipid peroxidative markers in plasma and tissues of diabetic rats.

Associated with the changes in lipid peroxidation, the diabetic tissues showed decreased activity of key antioxidants SOD, CAT, GSH, GPx, GST, GSH, vitamin C and vitamin E, which are playing important role in scavenging the toxic intermediates 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 is low in diabetes mellitus [37]. Reduced activities of SOD and CAT in liver, kidney and brain

have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of $O_2^{\bullet}$ and H_2O_2 [38]. Administration of CFEt and CLEt significantly increased the activities of SOD and CAT. Therefore, removing $O_2^{\bullet}$ and $OH^{\bullet}$ is probably one of the most effective defenses against diseases [39]. The result of increased activities of SOD and CAT suggest that CFEt and CLEt contains a free radical scavenging activity, which could exert a beneficial effect against pathological alterations caused by the presence of $O_2^{\bullet}$ and $OH^{\bullet}$. The increased activity of SOD accelerates dismutaion of $O_2^{\bullet}$ to H_2O_2, which is removed by CAT [40]. This action could involve mechanisms related to scavenging activity of CFEt and CLEt.

Under *in vivo* conditions, GSH acts as an antioxidant and its decrease was reported in diabetes mellitus [41]. We have observed a significant decrease in GSH levels in liver, kidney and brain during diabetes. The decrease in GSH levels represents increased utilization due to oxidative stress [42]. The pathophysiological consequence owing to depletion of GSH has been well studied. The depletion of GSH, GPx and GST in tissues promotes generation of ROS and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes (Raza et al. 2000). It has been proposed that GPx is responsible for the detoxification of H_2O_2 in low concentration whereas CAT comes into play when GPx pathway is reaching saturation with the substrate [43]. Furthermore, the decreased lipid peroxidation is correlated well in accordance with the induction of antioxidant enzymes above basal level. Christopherson [44] reported that GPx has broader protective spectrum than CAT in catalyzing the reduction of both H_2O_2 and other hydroperoxides. Treatment with CFEt and CLEt increased the GSH, GPx and GST activities. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies ROS generation from exposure to STZ [45].

Ascorbic acid is a potent antioxidant, which widely acts on OFR as well as interact with vitamin E [46]. Vitamin E levels in platelets are reduced in diabetes. Both the vitamins-C and E significantly decreased in plasma of diabetic control rats. Low levels of plasma antioxidants have been implicated as a risk factor for the development of diabetes [47]. It has been suggested that vitamin E deficiency may be one of the factors in the pathogeneseis of abnormalities of diabetic microvascular flow [48]. Previous

studies demonstrate the reduced plasma concentration of vitamin C in diabetes [49]. Oxidative stress, increased polyol pathway, non-enzymic glycation of proteins and disturbed vitamin C metabolism may be important in the pathogenesis of diabetic microangiopathies [50]. Administration of CFEt and CLEt increased the vitamin C and E levels. This indicates that vitamin E is used in combating free radicals and if vitamin C is present, vitamin E levels are preserved. Frei [51] has previously shown the ability of vitamin C to preserve the levels of other antioxidants in human plasma. Also vitamin C regenerates vitamin E from its oxidized form. GSH is the first line of defense against pro-oxidant status and GSH was elevated after CFEt and CLEt 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 and CLEt significantly increased the GSH levels. Increase in GSH level may inturn activates the GSH dependent enzymes such as GPx and GST.

Reduced levels of vitamin C and vitamin E in liver, kidney and brain were observed during diabetes in our study. Both vitamin C and E are known to prevent detectable lipid peroxidation and under physiological conditions, it has been suggested that vitamin C helps to recycle vitamin E from its radical form [52]. Higuchi observed a decrease in hepatic vitamin E levels in rats with STZ diabetes [53]. Reduced levels of vitamin C and E in tissues were reported by Anuradha and Selvam [42] in diabetic rats. Treatment with CFEt and CLEt increased the vitamin C and E levels.

Ceruloplasmin is an important enzyme, which oxidizes iron from the ferrous to ferric state and it has been demonstrated that iron catalyzed 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 diseased conditions leading to the scavenging of oxygen products such as $O_2^{\bullet}$ and H_2O_2 [8]. The observed increase in the level of plasma ceruloplasmin in diabetic rats may be due to increased lipid peroxides. Prince and Menon [25] also reported increased level of ceruloplasmin in diabetic rats. Oral administration of CFEt and CLEt to diabetic rats restored the level of ceruloplasmin to near normal level.

Administration of CFEt and CLEt increased the activity of antioxidants and may help to control free radical, as *cassia auriculata* has been reported to be rich in

diterpenoids and flavonoids, well-known antioxidants [4], which scavenge the free radicals generated during diabetes. Since the study of induction of the antioxidant enzymes is considered to be a reliable marker for evaluating the antiperoxidative efficacy of the medicinal plant, these findings are suggestions of possible antiperoxidative role played by *cassia auriculata* plant extract.

Conclusion

The present investigation shows that CFEt and CLEt 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 induced diabetes.

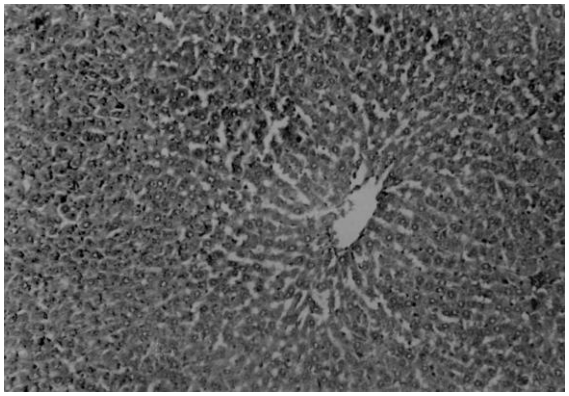

Figure1 A. Control rat liver.
Normal architecture of liver.

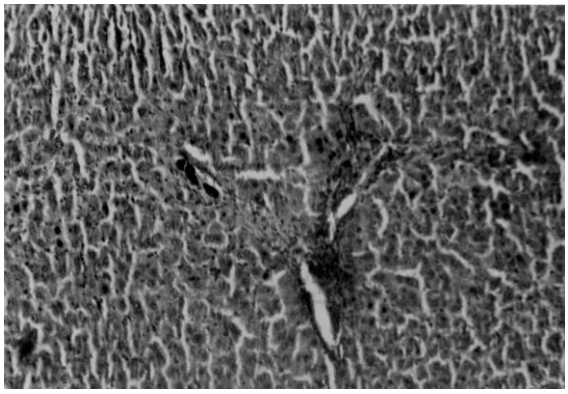

Figure 1B. Diabetic rat liver. Hepatocytic nuclear condensation portal triad with inflammation, sinusoidal dilation.

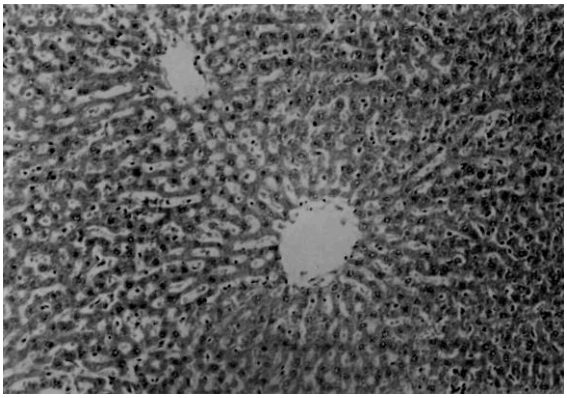

Figure 1C.Diabetic + CFEt (0.45 g/kg) treated rat liver. Normal appearance of liver cells.

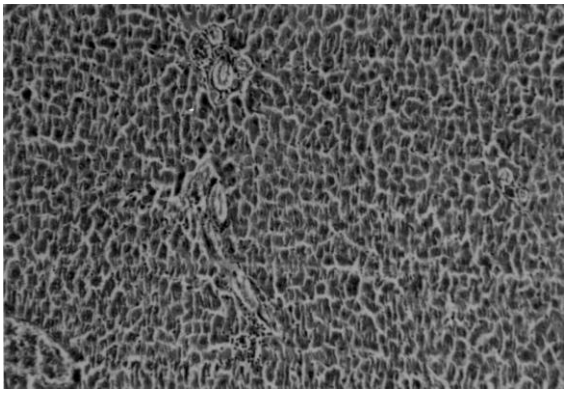

Figure 1D. Diabetic+ + CLEt (0.45 g/kg) treated rat liver. Normal hepatocytes with mild inflammation.

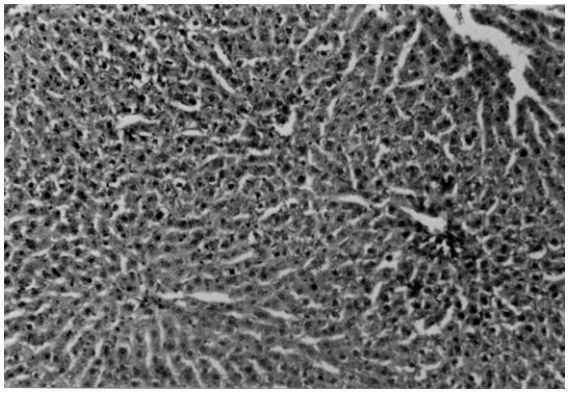

Figure 1E. Diabetic+ glibenclamide treated rat liver. Normal hepatocytes with mild inflammation and mild sinusoidal dilation.

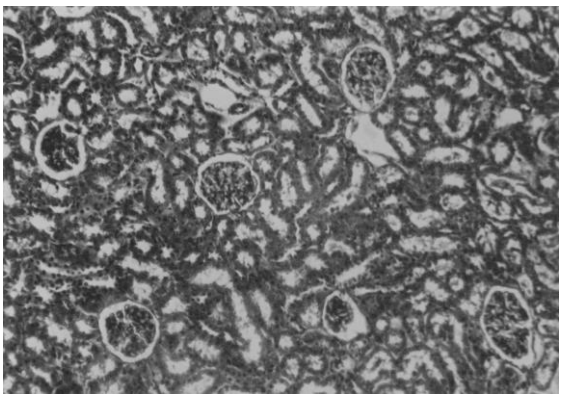

Figure 2A. Control rat kidney. **Normal architecture of kidney.**

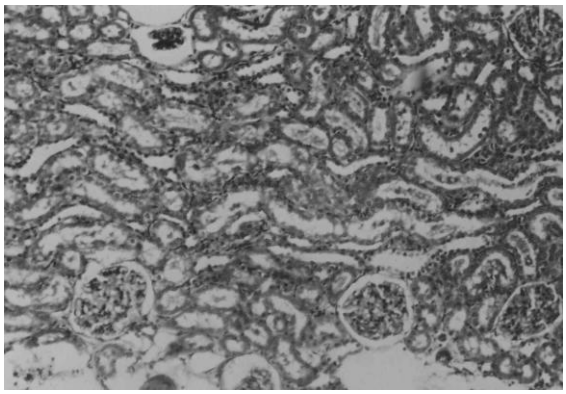

Figure 2B. Diabetic rat Kidney. Glomeruli mesangial capillary proliferation with tubular epithelial damage.

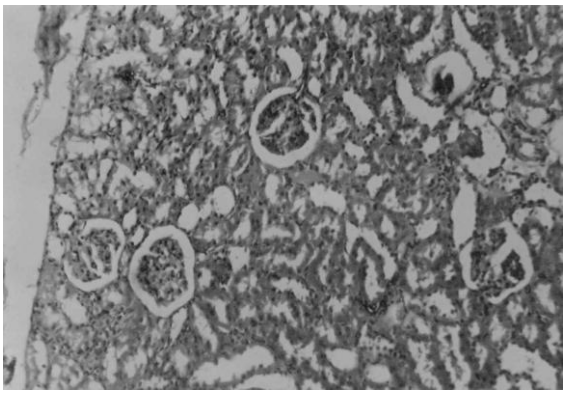

Figure 2C. Diabetic + CFEt (0.45 g/kg) treated rat kidney. Normal glomeruli with tubules.

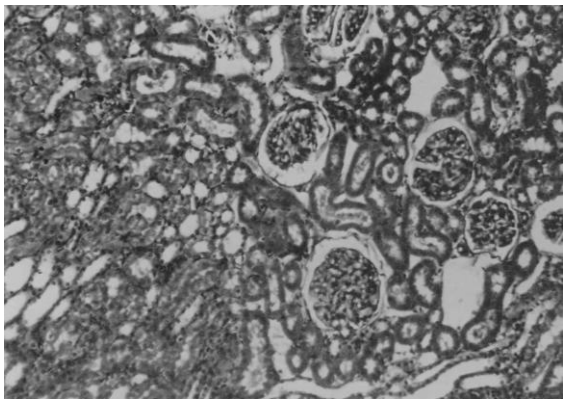

Figure 2D. . Diabetic+ + CLEt (0.45 g/kg) treated rat kidney. Normal tubules with focal glomerular changes.

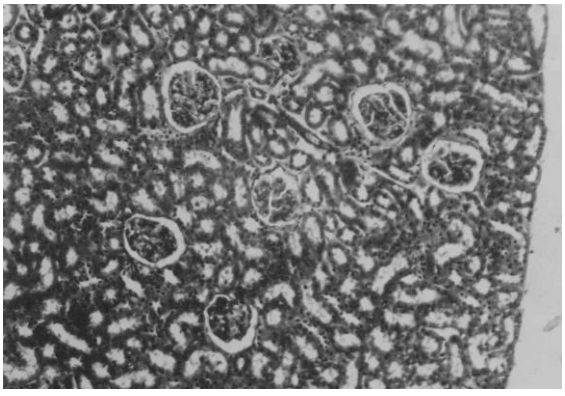

Figure 2E. Diabetic+ glibenclamide treated rat kidney. Normal tubules with focal glomerular changes.

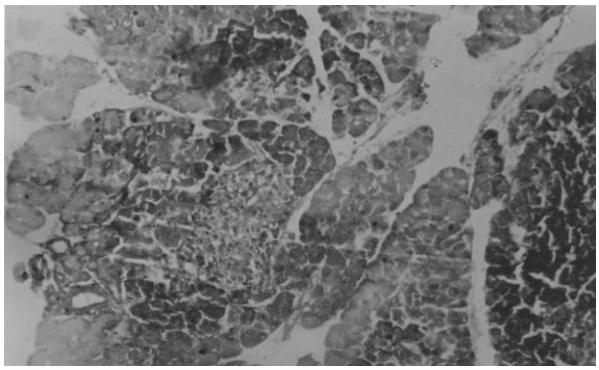

Figure 3A. Control rat pancreas. **Normal pancrease with islets of langarhans cells**

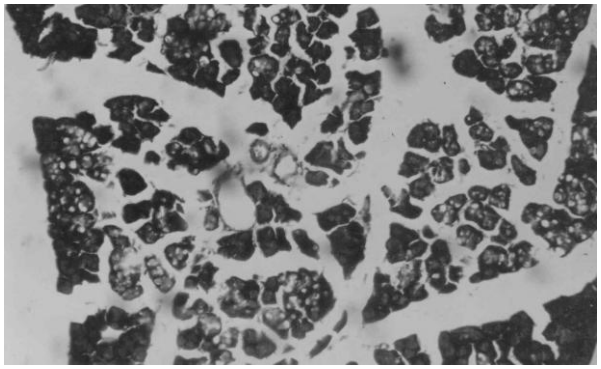

Figure 3B Diabetic rat pancreas.

Shows atrophic acini, no islet cells

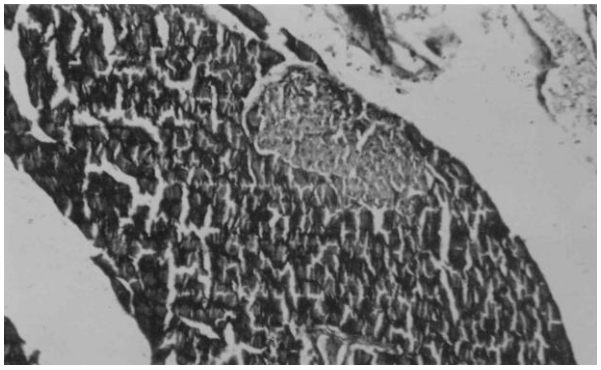

Figure 3C. Diabetic + CFEt (0.45 g/kg) treated rat liver. Normal architecture of pancreatic cells.

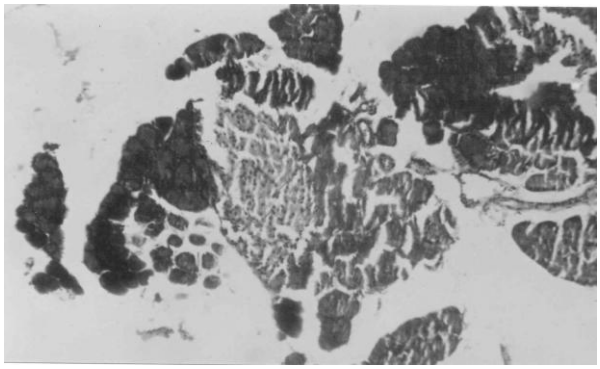

Figure 3D Diabetic+ + CLEt (0.45 g/kg) treated rat pancreas. Preservation of islet cells with few atrophic acini.

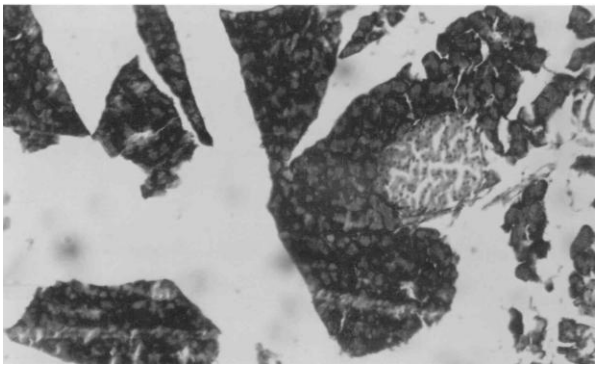

Figure 3 E. Diabetic+ glibenclamide treated rat pancreas. Preservation of islet cells in the pancreas.

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Table 1. Effect of CFEt and CLEt on the levels of blood glucose, plasma insulin in normal and experimental rats

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)
Normal	96.38 ± 6.56^{a}	12.14 ± 0.83^{a}
Diabetic control	286.41 ± 9.94^{b}	3.90 ± 0.23^{b}
Diabetic + CFEt (0.45 g/kg)	114.68 ± 7.87^{c}	9.18 ± 0.67^{c}
Diabetic + CLEt (0.45 g/kg)	131.11 ± 8.33^{d}	8.29 ± 0.47^{d}
Diabetic+ Glibencalamide (600 μg/ kg)	137.21 ± 7.25^{d}	8.12 ± 0.47^{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. Influence of CFEt and CLEt on the content of TBARS and hydroperoxides in rats liver and kidney.

Groups	Normal	Diabetic control	Diabetic + CFEt (0.45 g/kg)	Diabetic + CLEt (0.45 g/kg)	Diabetic+ Glibencalamide (600 μg/ kg)
TBARS					
Liver (mM/100g tissue)	0.77 ± 0.04^{a}	1.85 ± 0.13^{b}	1.39 ± 0.06^{c}	1.10 ± 0.05^{d}	1.20 ± 0.03^{d}
Kidney (mM/100g tissue)	$1.79+ \pm 0.13^{a}$	3.91 ± 0.21^{b}	2.28 ± 0.11^{c}	1.99 ± 0.12^{d}	2.15 ± 0.02^{e}
Hydroperoxides					
Liver (mM/100g tissue)	81.21 ± 5.31^{a}	102.31 ± 5.81^{b}	91.77 ± 4.15^{c}	88.12 ± 4.12^{ac}	85.25 ± 0.03^{d}
Kidney (mM/100g tissue)	57.01 ± 3.02^{a}	80.53 ± 4.58^{b}	67.12 ± 3.11^{c}	62.31 ± 4.12^{c}	71.18 ± 4.35^{d}

Values are given as mean ± S.D for 6 rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.05$ (Duncan's Multiple Range Test).

Table 3. Influence of CFEt and CLEt on the CAT, SOD, GPx, and GST activities in rats liver and kidney

Groups	Normal	Diabetic control	Diabetic + CFEt (0.45 g/kg)	Diabetic + CLEt (0.45 g/kg)	Diabetic+ Glibencalamide (600 μg/ kg)
CAT (units/mg of protein)					
Liver	75.21 ± 5.32^{a}	49.51 ± 3.11^{b}	59.23 ± 3.26^{c}	69.21 ± 4.21^{d}	71.31 ± 4.21^{c}
Kidney	34.31 ± 2.14^{a}	18.28 ± 1.21^{b}	24.11 ± 1.31^{c}	29.31 ± 1.18^{d}	30.41 ± 1.20^{c}
SOD (units/mg of protein)					
Liver	7.13 ± 0.21^{a}	3.41 ± 0.21^{b}	4.87 ± 0.31^{c}	5.89 ± 0.35^{d}	6.97 ± 0.32^{c}
Kidney	15.61 ± 0.77^{a}	9.31 ± 0.55^{b}	13.21 ± 0.55^{c}	11.52 ± 0.52^{c}	12.31 ± 0.41^{c}
GPx (units/mg of protein)					
Liver	6.58 ± 0.41^{a}	3.71 ± 0.31^{b}	4.35 ± 0.25^{c}	5.77 ± 0.37^{d}	6.02 ± 0.29^{c}
Kidney	4.84 ± 0.31^{a}	2.21 ± 0.13^{b}	3.58 ± 0.17^{c}	4.31 ± 0.22^{c}	4.92 ± 0.32^{c}
GST (units/mg of protein)					
Liver	6.71 ± 0.42^{a}	3.52 ± 0.21^{b}	5.65 ± 0.23^{c}	5.59 ± 0.32^{d}	6.12 ± 0.30^{c}
Kidney	5.21 ± 0.29^{a}	2.21 ± 0.13^{b}	3.58 ± 0.19^{c}	4.58 ± 0.28^{d}	4.91 ± 0.21^{c}

Data are mean ± SD values for six rats in each group. Units are as follows: CAT, μM of H_2O_2 consumed per minute; SOD, 1 unit of activity equals the enzyme reaction that gave 50% inhibition of nitro blue tetrazolium reduction in 1 minute; GSH, micrograms of GSH consumed per minute; GST, μM of 1-chloro-2, 4-dinitrobenzene-glutathione (CDNB–GSH) conjugate formed per minute. Values not sharing a common superscript letter differ significantly at $P < .05$ (Duncan's Multiple Range Test).

Table 4. Influence of CFEt and CLEt on content of vitamin C, vitamin E, and GSH in rats liver and kidney

Groups	Normal	Diabetic control	Diabetic + CFEt (0.45 g/kg)	Diabetic + CLEt (0.45 g/kg)	Diabetic+ Glibencalamide (600 µg/ kg)
Vitamin C (µM/mg of tissue)					
Liver	1.39 ±0.02^{a}	0.89 ± 0.02 ^{b}	1.13 ± 0.02^{c}	1.32 ± 0.04^{d}	1.41 ± 0.02^{d}
Kidney	1.05 ± 0.04^{a}	0.33 ± 0.02^{b}	0.72 ± 0.02 ^{c}	0.82 ± 0.03^{d}	0.85 ± 0.01^{d}
Vitamin E (µM/mg of tissue)					
Liver	0.67 ± 0.03^{a}	0.15 ± 0.01^{b}	0.43 ± 0.02 ^{c}	0.55 ± 0.02^{d}	0.57 ± 0.03^{d}
Kidney	0.46 ± 0.03^{a}	0.08 ± 0.01^{b}	0.30 ± 0.02^{c}	0.37 ± 0.03^{d}	0.37 ± 0.03^{d}
GSH (mg/100 g of tissue)					
Liver	46.21 ± 2.74^{a}	22.52 ± 1.63^{b}	33.78 ± 1.61^{c}	39.21 ± 2.21^{d}	41.27 ± 2.31^{d}
Kidney	35.21 ± 2.07^{a}	19.52 ± 1.33^{b}	24.21± 1.27^{c}	31.21 ± 1.85^{d}	32.12 ± 1.75^{d}

Data are mean ± SD values for six rats in each group.

Values not sharing a common superscript letter differ significantly at $P < .05$ (Duncan's Multiple Range Test).