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ANTIOXIDANT EFFECT OF DIFFERENT PARTS OF *CASSIA AURICULATA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract
Diabetes is a common chronic disease where therapeutics innovation is much needed. The search for novel antidiabetic molecules can be greatly facilitated by high throughput metabolomic characterization of herbal medicines. Cassia auriculata is a shrub used in ayurvedic medicine and native to India. Oral administration of *Cassia auriculta* flower buds extract (CFBEt), leaf extract (CLEt) and seeds extract (CSEt) of diabetic rats for 45 days resulted in significant reduction in blood glucose and significant increase in plasma insulin levels. In addition, CFBEt, CLEt and CSEt 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. The antidiabetic and antioxidant effects of CFBEt are more potent than those of CLEt, CSEt and glibenclamide.

Keywords: antioxidants, *Cassia auriculta* flower buds extract, *Cassia auriculta* leaf extract, lipid peroxidation, antioxidants.

Diabetes is a chronic metabolic disorder affecting people of all age groups. It is a metabolic disorder which is characterized with high blood glucose, high insulin production, high insulin resistance and glucose or insulin intolerance. Out of the various types of diabetes, the development of Type 2 Diabetes Mellitus (T2DM) is very high and common with the detection of about 90% of cases. T2DM is very common metabolic disorder affecting people of all age groups [1]. The change in life style and environmental factors are the considerable factors which are involved in the development of the disorder. The different parts of medicinal plants vary in their composition of bioactive compounds. There are reports on antidiabetic activity of *Cassia auriculata* L. flower and leaves.

The prevalence of diabetes among adults has been increased significantly worldwide. It has been predicted that the number of adults with diabetes will increase from 135 million in 1995 to 30 million in 2025. Diabetes mellitus and impaired glucose tolerance increase cardiovascular disease risk up to 8-fold . Furthermore, new blood vessel growth is impaired in response to ischemia in diabetic patients, resulting in decreased collateral vessel formation in ischemic hearts and in non-healing foot ulcers {2].

Herbal medicine and omics systems science offer significant synergy to aid drug discovery and development. *Cassia auriculata*, a Caesalpiniaceae shrub, is native to India and Sri Lanka, present in Indo-Malaysia, and cultivated in Myanmar. In Ayurvedic medicine, C. auriculata is one of the notable medicinal herbs. The individual parts of the C. auriculata plant, including the flowers, flower buds, root, leaves, seeds, and bark, are used in traditional herbal medicine practices with various indications for each [2].

Cassia auriculata L a member of genus Cassia belonging to family Caesalpiniaceae is commonly known as Tanner's Cassia. It is a shrub found throughout southern, western and central India. The various parts of the plant has been reported to posses a number of therapeutic activities to manage disease states like leprosy, asthma, gout, rheumatism and diabetes. The flower, buds, leaves, stem, root, and unripe fruit are used for treatment, especially in Ayurvedic medicine [3,4]. People use *Cassia auriculata* for diabetes, pink eye, joint and muscle pain (rheumatism), constipation, and other conditions, but there is no good scientific evidence to support any use. It is also used as antipyretic, antiulcer and in the treatment of skin infection. The flower has been reported to contain flavonoids, proanthocyanidins and β-sitosterol [5,6]. A literature survey showed that a decoction of leaves, flowers, and seeds of the *Cassia auriculata* mediate an antidiabetic effect [6]. In folk remedies, flowers of *Cassia auriculata* are proposed to have antidiabetic activity. From literature survey, it was evident that the aqueous extract of flowers has been reported for its antidiabetic activity in streptozotocin-diabetic rats at a dose of 0.45 g/kg body weight [7].

To our knowledge, so far no other biochemical investigations has been carried out on the effect of CFBEt, CLEt and CSEt in tissue antioxidant status of experimental diabetic rats. The present investigation was carried out to study the effect of CFBEt, CLEt and CSEt 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 (Flower, leaves and seeds) extract

Five hundred g of *Cassia auriculata* flowers and leaves 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].

Seeds cleaned off adhering dust and unwanted plant material, shade dried, cut and pulverized (powdered). Further Seeds (500 g) were extracted with successive extraction at room temperature, filtered and concentrated under reduced pressure on rotary evaporator. The dried

extract was successively fractionated in Petroleum Ether (40.5 gm) [CA-PE], n-butanol, (5.8g) [CA-NB] acetone: methanol 1:1 (26.8g) [CA-AM] and methanol: water 1:1 (21.23g) [CA-MW]. Also, separately seeds extracted with methanol by Soxhlet extraction at 60°C [CA-TS]. The solvents were chosen for larger delivery of bioactive compounds which are polar and mid-polar

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 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 CFBEt (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 CSEt (0.45 g/kg body weight) in 1 ml of ethanolic extract daily using an intragastric tube for 45days.

Group 6: 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^{l}□□□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

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 CFBEt, CLEt, CSEt and glibenclamide to diabetic rats significantly reversed the above biochemical changes. The administration of CFBEt, CLEt, CSEt and glibenclamide to normal rats showed a significant effect on blood glucose and plasma insulin levels. The CFBEt administration showed more effective than CLEt, CSEt 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 CFBEt, CLEt, CSEt and glibenclamide significantly decreased the lipid peroxidation in diabetic rats. The CFBEt, was more potent than CLEt, CSEt and glibenclamide.

Effect on tissue enzymes and antioxidants

For studying the CFBEt, CLEt and CSEt 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 CFBEt, CLEt and CSEt treatment when compared with diabetic control rats. The effect of CFBt was more prominent compared with CLEt, CSEt and glibenclamide.

Discussion

Diabetes mellitus can damage the eyes, kidneys, nerves and heart. Microvascular and macrovascular disorders are the leading causes of morbidity and mortality in diabetic patients. Hyperglycemia can increase the indicators of lipid peroxidation and oxidative stress in which free radicals have the main role in the pathogenesis of these complications. Therefore, antioxidants which combat oxidative stress should be able to prevent and repair free radicals induced damages [20].

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. Several studies have shown increased lipid peroxidation in clinical and experimental diabetes [21]. Lipid peroxide mediated tissue damages have been observed in the development of type 1 and type 2 diabetes mellitus [22].

Studies have reported an increase in serum, hepatic and renal thiobarbituric acid reactive substances and hydroperoxides concentration in streptozotocin induced diabetic rats, when compared with the normal rats. In diabetes, hypoinsulinaemia increases the activity of the enzyme, fatty acyl coenzyme, coenzyme A oxidase, which initiates □-oxidation of fatty acids resulting in lipid peroxidation [23] Increased lipid peroxidation impairs membrane functions by decreasing membrane fluidity, and changing the activity of membrane-bound enzymes [24]. Its products (lipid radicals and lipid peroxide) are harmful to the cells in the body and are associated with athrosclerosis and brain damage [24]. Our study shows that administration of CFBEt, CLEt, CSEt and glibenclamide significantly decreased the liver and renal thiobarbituric acid reactive substances and hydroperoxides significantly normal.

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. Aim our study, the diabetic tissues showed a decrease in the activities of key antioxidants like SOD, CAT, GSH, GPx, GST, GSH, vitamin C and vitamin E, 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 is low in diabetes mellitus [25]. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion $O_2^{\bullet-}$ and hydrogen peroxide in biological systems, which inturn generate hydroxyl radicals, resulting in initiation and propagation of lipid peroxidation [26].

The depletion of GSH, GPx and GST promotes generation of ROS and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes [27]. As the alteration produced in the antioxidant activities indicate the involvement of deleterious oxidative changes, increased activities of the components of this defence system would therefore be important in protection against radical damage.

Vitamin E is a well-known physiological antioxidant and membrane stabilizer [28]. It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals, thus protecting the cell structures against damage [29]. 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 [30]. Oral administration of CFBEt, CLEt and CSEt 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 [31]. 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 [32].

GSH is an important inhibitor of free radical mediated lipid peroxidation [33]. 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 [34,35]. GSH is the first line of defense against proxidant status [36] and GSH was evaluated after CFBEt, CLEt and CSEt 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 CFBEt, CLEt and CSEt significantly increased the GSH levels. Increase in GSH level may inturn activates the GSH dependent enzymes such as glutathione peroxidase and glutathione-S-transferase.

Conclusion

The present investigation shows that CFBEt, CLEt and CSEt 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. The CFBEt administration showed more effective than CLEt, CSEt and glibenclamide.

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

Groups	Fasting blood glucose (mg/dl)	Plasma insulin (μU/ml)
Normal	97.39 ± 6.59^{a}	12.18 ± 0.73^{a}
Diabetic control	289.48 ± 9.25^{b}	3.93 ± 0.25^{b}
Diabetic + CBEt (0.45 g/kg)	115.58 ± 7.57^{c}	9.27 ± 0.57^{c}
Diabetic + CLEt (0.45 g/kg)	127.25 ± 8.25^{d}	8.89 ± 0.42^{d}
Diabetic + CSEt (0.45 g/kg)	132.21 ± 8.33^{d}	8.23 ± 0.47^{d}
Diabetic+ Glibencalamide (600 μg/ kg)	139.21 ± 7.25^{d}	8.10 ± 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 CFBEt, CLEt and CSEt on the content of TBARS and hydroperoxides in rats liver and kidney.

Groups	Normal	Diabetic control	Diabetic + CBEt (0.45 g/kg)	Diabetic + CLEt (0.45 g/kg)	Diabetic + CSEt (0.45 g/kg)	Diabetic+ Glibencalamide (600 μg/ kg)
TBARS						
Liver (mM/100g tissue)	0.79 ± 0.05^{a}	1.83 ± 0.13^{b}	1.12 ± 0.06^{c}	1.21 ± 0.05^{d}	1.25 ± 0.05^{d}	1.31 ± 0.03^{d}
Kidney (mM/100g tissue)	$1.80+ \pm 0.10^{a}$	3.95 ± 0.21^{b}	2.10 ± 0.10^{c}	2.25 ± 0.13^{d}	2.29 ± 0.12^{d}	2.19 ± 0.02^{e}
Hydroperoxides						
Liver (mM/100g tissue)	80.27 ± 5.33^{a}	105.31 ± 5.81^{b}	89.77 ± 4.14^{c}	92.12 ± 4.12^{d}	94.12 ± 4.12^{d}	96.25 ± 0.03^{d}
Kidney (mM/100g tissue)	58.25 ± 3.05^{a}	83.59 ± 4.54^{b}	63.12 ± 3.11^{c}	64.31 ± 4.12^{c}	65.31 ± 4.12^{c}	66.18 ± 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 CFBEt, CLEt and CSEt on the CAT, SOD, GPx, and GST activities in rats liver and kidney

Groups	Normal	Diabetic control	Diabetic + CBEt (0.45 g/kg)	Diabetic + CLEt (0.45 g/kg)	Diabetic + CSEt (0.45 g/kg)	Diabetic+ Glibencalamide (600 μg/ kg)
CAT (units/mg of protein)						
Liver	76.26 ± 5.32^{a}	50.52 ± 3.11^{b}	62.23 ± 3.26^{c}	57.21 ± 4.21^{d}	69.21 ± 4.21^{d}	71.31 ± 4.21^{c}
Kidney	35.34 ± 2.14^{a}	98.31 ± 1.21^{b}	26.11 ± 1.31^{c}	30.31 ± 1.18^{d}	29.31 ± 1.18^{d}	30.41 ± 1.20^{c}
SOD (units/mg of protein)						
Liver	7.15 ± 0.20^{a}	3.51 ± 0.22^{b}	5.54 ± 0.31^{c}	5.89 ± 0.35^{d}	5.89 ± 0.35^{d}	6.97 ± 0.32^{c}
Kidney	15.55 ± 0.52^{a}	9.33 ± 0.51^{b}	13.25 ± 0.55^{c}	11.56 ± 0.52^{c}	11.55 ± 0.49^{c}	11.21 ± 0.39^{c}
GPx (units/mg of protein)						
Liver	6.08 ± 0.71^{a}	3.61 ± 0.31^{b}	5.59 ± 0.25^{c}	5.11 ± 0.37^{d}	5.25 ± 0.37^{d}	5.15 ± 0.29^{d}
Kidney	4.55 ± 0.55^{a}	2.25 ± 0.15^{b}	3.95 ± 0.15^{c}	4.31 ± 0.22^{d}	4.39 ± 0.22^{d}	4.55 ± 0.32^{d}
GST (units/mg of protein)						
Liver	6.77 ± 0.42^{a}	3.52 ± 0.20^{b}	5.77 ± 0.21^{c}	5.12 ± 0.32^{d}	5.25 ± 0.32^{d}	6.31 ± 0.30^{d}
Kidney	5.20 ± 0.30^{a}	2.21 ± 0.15^{b}	4.79 ± 0.19^{c}	4.48 ± 0.28^{d}	4.58 ± 0.28^{d}	4.61 ± 0.21^{d}

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 CFBEt, CLEt and CSEt on content of vitamin C, vitamin E, and GSH in rats liver and kidney

Groups	Normal	Diabetic control	Diabetic + CBEt (0.45 g/kg)	Diabetic + CLEt (0.45 g/kg)	Diabetic + CSEt (0.45 g/kg)	Diabetic+ Glibencalamide (600 μg/ kg)
Vitamin C (μM/mg of tissue)						
Liver	1.38 ± 0.02^{a}	0.88 ± 0.02^{b}	1.27 ± 0.02^{c}	1.11 ± 0.04^{d}	1.15 ± 0.04^{d}	1.19 ± 0.02^{d}
Kidney	1.05 ± 0.04^{a}	0.33 ± 0.02^{b}	0.89 ± 0.02^{c}	0.72 ± 0.03^{d}	0.71 ± 0.03^{d}	0.75 ± 0.01^{d}
Vitamin E (μM/mg of tissue)						
Liver	0.65 ± 0.03^{a}	0.16 ± 0.01^{b}	0.55 ± 0.02^{c}	0.47 ± 0.02^{d}	0.49 ± 0.02^{d}	0.46 ± 0.03^{d}
Kidney	0.46 ± 0.03^{a}	0.08 ± 0.01^{b}	0.35 ± 0.02^{c}	0.30 ± 0.03^{d}	0.32 ± 0.03^{d}	0.31 ± 0.03^{d}
GSH (mg/100 g of tissue)						
Liver	46.21 ± 2.74^{a}	22.50 ± 1.63^{b}	37.47 ± 1.55^{c}	31.21 ± 2.21^{d}	32.21 ± 2.21^{d}	31.27 ± 2.31^{d}
Kidney	35.21 ± 2.07^{a}	19.55 ± 1.33^{b}	28.21 ± 1.27^{c}	23.21 ± 1.85^{d}	25.21 ± 1.85^{d}	36.12 ± 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).