— Regular Article —

The Efficacy of *Costus igneus* Rhizome on Carbohydrate Metabolic, Hepatoproductive and Antioxidative Enzymes in Streptozotocin-induced Diabetic Rats

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In this study, effect of an ethanolic extract from rhizome of *Costus igneus* were investigated on activity of following enzyme in liver, kidney, pancreas of streptozotocin (STZ) induced diabetic rats: carbohydrate metabolic enzymes such as glucokinase glucose-6-phosphatase, and fructose-1,6-bisphosphatase in the liver; hepatoproductive enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in plasma and liver; and antioxidative enzymes such as superoxide dimutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione content (GSH), total sulfhydryl groups (TSH), lipid peroxides (LPO) in liver, kidney and pancreas. An ethanol extract from *Costus igneus* rhizome was administrated orally at a single dose of 100 or 200 mg/kg per day to diabetes induced rats for 30 days. This study demonstrated that glucose-6-phosphatase fructose-1,6-bisphosphatase, AST, ALT, ALP and LPO levels were significantly increased ($p < 0.05$), whereas glycolytic enzyme glucokinase, SOD, CAT, GPx, GSH and TSH levels were significantly decreased ($p < 0.05$) in STZ induced diabetic rats. Oral administration of *Costus igneus* rhizome ethanol extract [*CiREE*, 100 or 200 mg/kg per body weight (bw)] and glibenclamide to diabetic rats for 30 days significantly ($p < 0.05$) reversed levels of these enzymes to normal. Bioactive compound quercetin and diosgenin were isolated from *Costus igneus* rhizome by high performance thin layer chromatography (HPTLC). These results suggest that, components of *CiREE* quercetin and diosgenin exhibit antioxidant activity, which was sufficient to reverse oxidative stress in the liver, pancreas and kidney of diabetic rats as well as to stimulate glycolytic enzymes and control gluconeogenesis in diabetic animals.

Key words —— *Costus igneus*, quercetin, diosgenin, glycolytic enzyme

INTRODUCTION

Diabetes is one of the most prevalent metabolic disorders and is characterized increased blood sugar levels and improper primary metabolism. Furthermore, in diabetes mellitus, hyperglycemia can lead to the depression of the natural antioxidant system.[1] Several reports have demonstrated that elevated oxidative stress is common in chronic hyperglycemia[2] as well as in both experimental models and human diabetes mellitus.[3]

In diabetes, free radicals are formed adversely through the following mechanisms: glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins.[4,5] Increased oxidative stress resulting from abnormally high levels of free radicals and the depression of the antioxidant defense system may lead to the damage of enzymes within different cellular organelles,[6] increased lipid peroxidation, and the development of insulin resistance, all of which promote the development of complications associated with diabetes mellitus. Recent experimental and clinical studies have uncovered new insights into the role of oxidative stress in diabetic complications, and these reports have demonstrated differ-

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ent and innovative approaches to employ natural, antioxidant therapies, *e.g.*, flavonoids and saponin. Flavonoids possess several antioxidant mechanisms including scavenging or quenching free radicals, chelating metal ions, and inhibiting enzymatic systems responsible for free radical generation. The potent antioxidant activity of flavonoids may provide the best protection against elevated oxidative stress.[7,8] In the present study, we examined the effect of oral administration of *Costus igneus* rhizome ethanol extract (*CiREE*) on the levels of gluconeogenic, glycolytic, and liver-specific enzymes as well as its effect on the activity levels of antioxidative enzymes in the plasma, liver, pancreas and kidney of streptozotocin (STZ)-induced diabetic melitus (DM) in rats.

MATERIALS AND METHODS

Animals—— Albino Wister rats (150–200 g) were obtained at age 7 weeks from Sri Venkateshwara Enterprises, Bangalore, India (237/CPCSEA). The animals were housed in an air-conditioned room with controlled temperature and humidity, and they were fed with standard rat feed pellets that were supplied by Kamadhenu agencies (Bangalore, India) and filtered water *ad libitum*. Animals that underwent fasting were deprived of food for > 16 hr, but they were allowed free access to water. Ethical clearance for the handling of experimental animals was obtained from the Institute of Animal Ethics Committee (265/CPCSEA).

Plant Materials—— *Costus igneus* (N.E.Br.) is a species of herbaceous plant in the Costaceae family and fresh rhizome was collected from the Periyar Maniammai University nursery in April of 2008. The plant was identified, confirmed and authenticated by Rapinant Herbarium, St. Joseph's College, Trichy, Tamil Nadu, South India. All chemicals and reagents were purchased from Qualigens, Himedia and Loba Chemicals at the certified analytical grade.

Extraction—— Coarse powder from air-dried rhizome was treated by a successive solvent extraction method using solvents of increasing polarity (petroleum ether, hexane, and methanol, followed by ethanol). The solvent was then distilled, evaporated and vacuum dried.

Phytochemical Analysis—— All extracts from the rhizome of *Costus igneus* (ethanol, methanol, petroleum ether and hexane) underwent preliminary phytochemical analysis to identify their active components by standard methods. The presence or absence of different phytoconstituents, *e.g.*, triterpenoids, steroids, alkaloids, sugars, tannins, glycosides and flavonoids, were detected.[9]

Isolation and Identification of Active Compounds—— Plant active constituents, which may contribute to its antidiabetic properties, were isolated by high performance thin layer chromatography (HPTLC). The vacuum dried, concentrated ethanol extract of *Costus igneus* was treated by acid hydrolysis to liberate any aglycones and to determine if any glycosides were present. The concentrates were spotted on activated TLC plates (silica gel F 254, 0.5-mm coating thickness). To elute quercetin, the plates (10×10 cm) were developed with toluene : ethyl acetate : acetic acid : methanol (2 : 7.5 : 0.25 : 0.25). The developed plates were air dried, sprayed with 20% antimony chloride in chloroform, and dried in a chromatographic oven at 105°C for 10 min. In a similar manner, diosgenin was eluted with hexane : ethyl acetate (7.5 : 2.5) and was detected by spraying with anisaldehyde and drying in an oven at 105°C for 10 min. The resolution bands were obtained and the retardation factor (Rf) values were calculated.

Induction of Diabetes Mellitus in Rats—— Diabetes was induced by injecting STZ (Sigma, St. Louis, MO, U.S.A.) at a dose of 40 mg/kg of body weight (bw), dissolved in 0.1 M cold citrate buffer (pH 4.5) interaperitoneally. STZ-injected animals exhibited severe glycosuria and hyperglycemia, and the rats were stabilized over a period of 7 days. The onset of diabetes was confirmed in the experimental rats by measuring the blood glucose concentration 96 hr after the administration of STZ. The rats with blood glucose levels above 250 mg/dl were considered to be diabetic and were used for these experiments. Control rats were injected with citrate buffer (0.1 M, pH 4.5) only.

Experimental Design—— Animals were separated into five groups with six animals in each group. Group I were normal control rats; Group II were STZ-treated and surviving diabetic rats; Group III were STZ-induced diabetic rats that were treated with *CiREE* (100 mg/kg bw per day), Group IV were STZ-induced diabetic rats that were treated with *CiREE* (200 mg/kg bw per day), and Group V were STZ-induced diabetic rats that treated with Glibenclamide (0.5 mg/kg bw per day) for 30 days. All drugs were given by oral administration. After 30 days, the rats were sacrificed, and the blood sam-

ples were collected to analyze the effect of *CiREE* on several biochemical parameters.

Collection of the Pancreas, Liver and Kidney and the Estimation of Biochemical Parameters——At the end of the experiment, blood samples were collected from the tail vein. Glucose levels were measured using the One Touch Horizone (Lifescan, Milpitas, CA, U.S.A.) glucometer in heparinized tubes, and HbA_1C was estimated according to previous methods described by Sudhakar Nayak and Pattabiraman.[10] The liver, pancreas and kidney were quickly removed, washed in ice-cold isotonic saline, blotted individually on ash-free filter paper, and weighed. The tissues were then homogenized in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was used for further measurements.

Estimation of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST)——Serum glutamate pyruvate transaminase (SGPT) substrate [0.5 ml, serum glutamate oxalate transaminase (SGOT) substrate was used for SGOT analysis] was added to two test tubes (experimental and blank), and the tubes were incubated at 27°C in a water bath for few min. Tissue sample (0.3 ml) was added to the "test" test tube only and both tubes were incubated at 37°C for 30 min. 2,4,dinitrophenylhydrazine (DNPH) reagent (0.5 ml) was added to both tubes, and they were mixed well and incubated at room temperature for 20 min. NaOH (5.0 ml, 0.4 N) was added to both tubes, and the mixture was incubated for 10 min. The optical density (OD) was measured using a green filter at 540 nm.[11]

Assay for Alkaline Phosphatase (ALP)——Buffered substrate (6.0 ml) was added to a test tube and placed in a water bath at 37°C for a few min. Tissue sample (0.3 ml) was added to the test tube, and the tubes were mixed well and incubated at 37°C in a water bath. Trichloroacetic acid (TCA, 1.2 ml, 20%) was added, and the tubes were centrifuged at 4000 rpm for 10 min. The supernatants (5.0 ml) from the test, blank and control tubes were withdrawn. Acid molybdate (0.8 ml) followed by 1-amino 2-napthol 4-sulphonic acid (ANSA, 0.2 ml) were added to the supernatants and the solutions were mixed well followed by a 10-min incubation. The OD was measured using a green filter at 540 nm.[11]

Assay for Superoxide Dimutase (SOD) Activity——To a 15-ml centrifuge tube, 1.15 ml of deionised distilled water (DIDW), 50 μl of enzyme source (homogenate), 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 100 μl of post mitochondria1 supernatant (PMS, 186 μM), 300 μl of nitroblue tetrazolium (NBT, 300 μM) and 200 μl of NADH (780 μM) were added. A control was also prepared by replacing the enzyme source with 50 μl of potassium phosphate buffer (pH 7.5, 0.1 M). Enzyme activity was calculated from an OD measurement for both control and test samples. Activity was expressed as unit enzyme per mg protein, where one unit of enzyme is defined as the amount of SOD required to give 50% maximal inhibition for the initial rate of NBT reduction.[12] The equation for this calculation is shown below:

$$\text{SOD activity (units of activity/mg protein)} = \frac{(\text{OD of control} - \text{OD of test}) \times 2 \times 10^6}{\text{OD of control} \times \mu\text{g of protein used}}$$

Assay for Catalase (CAT) Activity——The assay was performed as described by Sinha method in which the disappearance of peroxide was followed spectrophotometrically at 240 nm. Under the specified conditions, 25°C and pH 7.0, one unit of CAT should turnover one micromole of hydrogen peroxide per min. The spectrophotometer was set to 240 nm and 25°C, and 1.9 ml of Distilled water and 1.0 ml of hydrogen peroxide (0.059 M) were added to a cuvette. The mixture was incubated in spectrophotometer for 4–5 min to ensure temperature equilibration. Homogenate (0.1 ml) or sample was added to the cuvette and the decrease in absorbance at 240 nm was recorded for 2–3 min.[13] The change in absorbance at 240 nm per min was calculated from the initial linear portion of the curve using the following equation:

$$\text{CAT activity (units of activity/mg protein)} = \frac{\text{OD at 240 nm/min} \times 1000}{43.6 \times \text{mg of protein}}$$

Assay for Glutathione Peroxidase (GPx) Activity——The incubation mixture consisted of 1.5 ml of phosphate buffer, 0.2 ml of EDTA (9 mM), 0.1 ml of sodium azide (30 mM), 0.2 ml of reduced glutathione (8 mM), and 0.2 ml of homogenate containing approximately 200 μg of protein. The solution was mixed well and placed in a water bath for 10 min at 37°C. The reaction was induced by the rapid addition of 1.0 ml of H_2O_2 (18 mM). Samples were collected exactly one min after the addition of H_2O_2, and the reaction was stopped immediately by the addition of 1.0 ml of 10% TCA. Nonenzymatic oxidation of reduced glutathione content

(GSH) was also measured in a blank solution containing the above reagents except that the enzyme source was replaced with buffer. Under these conditions, the non-enzymatic oxidation appeared to be minimal. The residual GSH was then estimated by adding 1 mM 5,5-dithiobis(2-nitrobenzolc acid) (DTNB).[14)]

Assay for GSH —— Homogenate or sample containing 200 μg of protein was diluted to 0.2 ml and was precipitated with 0.8 ml of 5% TCA. The precipitate was removed by centrifugation at 10000 rpm for 10 min. DTNB (6 mM) was added to the supernatant to bring the total volume to 3.0 ml. The absorbance was measured at 412 nm against a blank containing TCA in replace of the sample. The GSH content was expressed as mM of GSH/mg protein.[15)]

Assay for Sulfhydryl Groups —— Sulfhydryl groups were estimated as described by Sedlack *et al.*, method with slight modification. Phosphate buffer (0.3 ml, 1.0 M) and DTNB reagent (0.2 ml, 3 mM) were added to 1.0 ml of sample containing 200 μg of protein. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 412 nm against a blank containing the reagent mixture without DTNB. The concentration of the colored thiol anion, 3-carboxylato-4-nitrothio phenolate (CNTP) that was released into the reaction mixture was calculated using its molar extinction coefficient (1.36×10^4). The values were expressed as μg/mg of protein.[16)]

Assay for Lipid Peroxides (LPO) —— The LPO concentration was determined by thiobarbutyric acid reaction.[17)] Homogenate or sample containing approximately 200 μg of protein was diluted to 0.2 ml. To this homogenate, 1.5 ml of 20% acetic acid with 0.8% TBA and 0.2 ml of 0.1% sodium dodecyl sulfate (SDS) were added. The mixture was then heated for 30 min at 95°C. After cooling, the mixture was centrifuged at 4000 rpm for 10 min. The organic layer was taken and the absorbance was read at 532 nm in a spectrophotometer. The LPO concentration was expressed as nM of malondialdehyde (MDA)/mg of protein.

Determination of Vitamin A (Vit A) and Vitamin E (Vit E) —— Vit A[18)] and vit E[19)] were also measured.

Statistical Analysis —— Statistical analysis was performed using Origin lab, version 7.5 (Northampton, MA, U.S.A.). Values were analyzed by one-way analysis of variance (ANOVA) and means comparisons using the Bonferroni test. All of the results were expressed as mean ± S.D. for six rats in each group, and $p < 0.05$ was considered to be statistically significant.

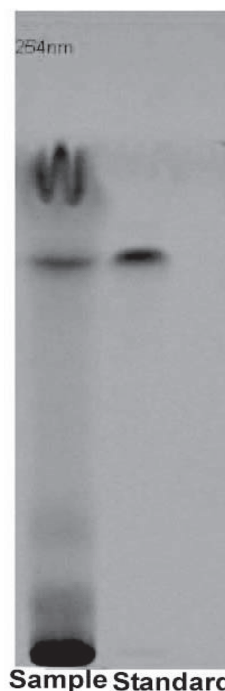

Fig. 1. Isolation of Quercetin from *Costus igneus* Rhizome

RESULTS

Phytochemical Analysis

Costus igneus rhizome dried powder was extracted with ethanol, and these extracts underwent preliminary phytochemical screening to identify different chemical groups such as tannins, phlobatanin, saponin, flavonoids, steroids, terpenoids, cardiac glycosides and naturally occurring phenolic compounds, such as quercetin. Quercetin was isolated by HPTLC (Rf 0.72) and was confirmed against standard quercetin (Rf 0.72, Fig. 1). Diosgenin a steroidal sapogenin, was isolated by HPTLC (Rf 0.92, Fig. 2) and was confirmed against standard diosgenin.

Biochemical Analysis

Serum glucose and HbA_1c levels for rats in different groups are shown in Table 1. These levels were significantly higher ($p < 0.05$) in the diabetic control (group II) compared to the normal control (group I). At the end of the 30th day of oral administration of *CiREE* (100 or 200 mg/kg per bw),

Table 1. The Effect of *CiREE* on Glucose and HbA_1C Levels in STZ-induced Diabetic Productive Rats on the 30th Consecutive Day of Administration

Groups	Glucose	HbA_1C
Group I (Normal control)	91.55 ± 1.269	5.38 ± 0.35
Group II (Diabetic control)	285.13 ± 1.315^{a}	7.275 ± 0.189^{a}
Group III (*CiREE* 100 mg/Kg)	$104.63 \pm 0.479^{a,b}$	$5.8 \pm 0.816^{a,b}$
Group IV (*CiREE* 200 mg/Kg)	$90.5 \pm 1.291^{b,c}$	$5.075 \pm 0.275^{b,c}$
Group V (Glibenclamide)	$106.5 \pm 1.291^{a,b,d}$	$5.85 \pm 0.129^{a,b,d}$

Values represent mean ± S.D. ($n = 6$); comparisons between groups are as follows: *a*: Group I *vs*. Groups II, III, IV, V; *b*: Group II *vs*. Groups III, IV, V; *c*: Group III *vs*. Groups IV, V; *d*: Group IV *vs*. Group V. Statistical significance was considered to be $^{a}p < 0.05$, $^{b}p < 0.05$, $^{c}p < 0.05$, $^{d}p < 0.05$.

Fig. 2. Isolation of Diosgenin from *Costus igneus* Rhizome

these levels were significantly reversed ($p < 0.05$) to achieve normal control (group I) levels for animals in groups III and IV. There was no difference between group I and IV, thereby suggesting that *CiREE* has an antidiabetic effect but does not lead to hypoglycemic activity at the tested dose.

Table 2 illustrates the effect of oral administration of *CiREE* on carbohydrate metabolic enzymes in the liver of normal and diabetic control rats. Diabetic control (group II) showed decreased activity of glucokinase and increased activity of glucose-6-phosphatase and fructose-1,6-bisphosphatase in the liver. Administration of *CiREE* (100 or 200 mg/kg per bw, group III and IV) or glibenclamide (group V) reversed these enzyme activity levels significantly ($p < 0.05$) toward normal levels. Hepatoproductive enzyme levels for AST, ALT and ALP were significantly increased ($p < 0.05$) in the plasma and liver of diabetic control (group II). Oral administration of *CiREE* (100 or 200 mg/kg per bw, group III and IV) for 30 days lowered the activity of AST, ALT and ALP to normal levels in the plasma and liver of these treated diabetic rats.

Table 3 showed that after inducing diabetes, the level of hepatic, pancreatic and renal LPO content increased significantly ($p < 0.05$) in the diabetic control (group II), compared to the normal control (group I). Administration of *CiREE* and glibenclamide for 30 days lowered the high values back to normal levels. The level of enzymatic antioxidants such as SOD, CAT and GPx in the liver, kidney and pancreas of diabetic control (group II) rats were significantly lower ($p < 0.05$) compared to normal control (group I). Oral administration of *CiREE* [100 or 200 mg/kg per bw, (100 or 200 mg/kg per bw, group III and IV)] for 30 days elevated these enzyme activity levels to normal level. Table 4 illustrates that the levels of non-enzymatic antioxidants *viz.*, glutathione (GSH), vit A, vit E and thiol groups such as total sulfhydryl groups (TSH) in the liver, pancreas and kidney of diabetic control (group II) were significantly reduced ($p < 0.05$) compared to normal control (group I). Oral administration of *CiREE* [100 or 200 mg/kg per bw, (100 or 200 mg/kg per bw, group III and IV)] or glibenclamide (group V) to diabetic rats for 30 days significantly reversed ($p < 0.05$) these values back to normal.

DISCUSSION

Diabetes mellitus is characterized by reduced capacity of the β-cells in the pancreas, whether

Table 2. Effect of *CiREE* on Carbohydrates Metabolic and Hepato-productive Enzymes in STZ-induced Diabetic Productive Rats on the 30th Consecutive Day of Administration

Enzymes	Group I (Normal control)	Group II (Diabetic control)	Group III (*CiREE* 100 mg/Kg)	Group IV (*CiREE* 200 mg/Kg)	Group V (Glibenclamide)
Glucokinase	0.25 ± 0.006	0.109 ± 0.002^{a}	0.216 ± 0.021^{a,b}	0.249 ± 0.002^{b,c}	0.226 ± 0.004^{a,b,d}
Glucose-6-phosphatase	0.17 ± 0.002	0.28 ± 0.00252^{a}	0.20 ± 0.0035^{a,b}	0.167 ± 0.002^{b,c}	0.186 ± 0.004^{a,b,d}
Fructose-1,6-bisphosphatase	0.68 ± 0.003	1.74 ± 0.004^{a}	1.18 ± 0.0036^{a,b}	0.647 ± 0.022^{b,c}	1.07 ± 0.003^{a,b,d}
ALP (liver)	0.122 ± 0.003	0.367 ± 0.002^{a}	0.23 ± 0.0038^{a,b}	0.122 ± 0.002^{b,c}	0.22 ± 0.02^{a,b,d}
ALP (Plasma)	74.75 ± 0.902	129.25 ± 0.902^{a}	97.43 ± 0.60^{a,b}	76.01 ± 2.716^{b,c}	98.07 ± 0.61^{a,b,d}
AST (Liver)	722 ± 2.0	975.08 ± 1.13^{a}	780.7 ± 2.08^{a,b}	728.3 ± 1.53^{b,c}	780.5 ± 1.5^{a,b,d}
AST (Plasma)	74.38 ± 0.77	113 ± 1.0^{a}	90.2 ± 0.265^{a,b}	75.67 ± 0.95^{b,c}	91 ± 0.5^{a,b,d}
ALT (Liver)	921.75 ± 2.53	1108.83 ± 1.61^{a}	993.2 ± 1.76^{a,b}	930.33 ± 1.53^{b,c}	994.33 ± 0.58^{a,b,d}
ALT (Plasma)	33.45 ± 1.0	63.1 ± 1.06^{a}	43.8 ± 0.52^{a,b}	34.25 ± 0.43^{b,c}	44.83 ± 0.76^{a,b,d}

Values represent mean ± S.D. ($n = 6$), comparisons between groups are as follows *a*: Group I *vs*. Group II, III, IV, V; *b*: Group II *vs*. Groups III, IV, V; *c*: Group III *vs*. Groups IV, V; *d*: Group IV *vs*. Group V. Statistical significance was considered to be $^{a}p < 0.05$, $^{b}p < 0.05$, $^{c}p < 0.05$, $^{d}p < 0.05$.

Expression units

Parameters	Units
Glucokinase	μM of glucose phospharylated/h per mg
Glucose-6-phosphatase	protein g/mg of protein
Fructose-1,6-bisphosphatase	μM of phosphate liberated/mg protein per min
ALP, AST, ALT	IU/l

Table 3. Effect of *CiREE* on Enzymatic Antioxidants and LPO in STZ-induced Diabetic Productive Rats at 30th Day

Enzymes	Group I (Normal control)	Group II (Diabetic control)	Group III (*CiREE* 100 mg/Kg)	Group IV (*CiREE* 200 mg/Kg)	Group V (Glibenclamide)
CAT (liver)	6.09 ± 0.048	2.55 ± 0.029^{a}	5.057 ± 0.04^{a,b}	6.048 ± 0.005^{b,c}	4.95 ± 0.028^{a,b,d}
CAT (kidney)	4.67 ± 0.016	2.19 ± 0.003^{a}	3.95 ± 0.034^{a,b}	4.64 ± 0.151^{b,c}	3.91 ± 0.036^{a,b,d}
CAT (pancreas)	4.74 ± 0.0039	2.96 ± 0.018^{a}	4.027 ± 0.003^{a,b}	4.77 ± 0.051^{b,c}	3.77 ± 0.134^{a,b,d}
GPx (liver)	5.93 ± 0.022	3.34 ± 0.122^{a}	4.33 ± 0.016^{a,b}	5.77 ± 0.098^{b,c}	4.29 ± 0.013^{a,b,d}
GPx (kidney)	5.19 ± 0.013	2.81 ± 0.008^{a}	4.23 ± 0.007^{a,b}	5.03 ± 0.0029^{b,c}	4.19 ± 0.229^{a,b,d}
GPx (pancreas)	4.53 ± 0.23	2.27 ± 0.15^{a}	3.27 ± 0.027^{a,b}	4.26 ± 0.059^{b,c}	3.28 ± 0.025^{a,b,d}
SOD (liver)	11.49 ± 0.049	7.17 ± 0.03^{a}	10.18 ± 0.053^{a,b}	11.54 ± 0.053^{b,c}	10.11 ± 0.03^{a,b,d}
SOD (kidney)	7.08 ± 0.005	3.53 ± 0.0032^{a}	5.24 ± 0.015^{a,b}	6.93 ± 0.066^{b,c}	5.21 ± 0.024^{a,b,d}
SOD (pancreas)	3.89 ± 0.0167	0.159 ± 0.01^{a}	2.58 ± 0.016^{a,b}	3.63 ± 0.06^{b,c}	2.53 ± 0.01^{a,b,d}

Values represent mean ± S.D. ($n = 6$), comparisons between groups are as follows *a*: Group I *vs*. Group II, III, IV, V; *b*: Group II *vs*. Groups III, IV, V; *c*: Group III *vs*. Groups IV, V; *d*: Group IV *vs*. Group V. Statistical significance was considered to be $^{a}p < 0.05$, $^{b}p < 0.05$, $^{c}p < 0.05$, $^{d}p < 0.05$.

Expression units

Parameters	Units
CAT	units of activity/mg protein per min
GPX	μM of GSH consumed/mg protein per min
SOD	units of activity/mg protein

the cells are destroyed as in type 1 diabetes or intact as in type 2 diabetes, to release sufficient insulin to induce the activity of glucose metabolizing enzymes.[20] Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phosphofructokinase and pyruvate kinase.[21] One of the key enzymes in the catabolism of glucose is glucokinase, which phosphorylates glucose to glucose-6-phosphate. In our study, glucokinase activity was decreased significantly in the liver of diabetic rats which is consistent with previous reports.[22] Treatment with *CiREE* and glibenclamide decreased blood sugar levels, possibly by increasing

Table 4. Effect of *CiREE* on Non-enzymatic Antioxidant of STZ-induced Diabetic Productive Rats at 30th Day

Enzymes	Group I (Normal control)	Group II (Diabetic control)	Group III (*CiREE* 100 mg/Kg)	Group IV (*CiREE* 200 mg/Kg)	Group V (Glibenclamide)
GSH (liver)	8.38 ± 0.0265	4.1 ± 0.02[a]	6.57 ± 0.025[a,b]	8.11 ± 0.0066[b,c]	6.52 ± 0.0252[a,b,d]
GSH (Kidney)	6.21 ± 0.019	1.71 ± 0.005[a]	5.15 ± 0.03[a,b]	6.233 ± 0.153[b,c]	5.13 ± 0.008[a,b,d]
GSH (pancreas)	5.42 ± 0.025	2.427 ± 0.025[a]	4.47 ± 0.040[a,b]	5.22 ± 0.13[b,c]	4.25 ± 0.052[a,b,d]
LPO (liver)	0.214 ± 0.0032	1.61 ± 0.0015[a]	0.626 ± 0.003[a,b]	0.276 ± 0.019[b,c]	0.632 ± 0.01[a,b,d]
LPO (kidney)	0.403 ± 0.0007	1.54 ± 0.014[a]	0.83 ± 0.004[a,b]	0.475 ± 0.02[b,c]	0.857 ± 0.0007[a,b,d]
LPO (pancreas)	0.323 ± 0.0019	1.24 ± 0.01[a]	0.736 ± 0.013[a,b]	0.414 ± 0.027[b,c]	0.74 ± 0.0023[a,b,d]
TSH (liver)	6.179 ± 0.0013	3.65 ± 0.008[a]	4.9 ± 0.083[a,b]	5.97 ± 0.17[b,c]	4.74 ± 0.033[a,b,d]
TSH (kidney)	4.74 ± 0.08	2.47 ± 0.023[a]	3.77 ± 0.028[a,b]	4.41 ± 0.07[b,c]	3.76 ± 0.078[a,b,d]
TSH (pancreas)	4.24 ± 0.036	1.93 ± 0.015[a]	3.2 ± 0.074[a,b]	3.96 ± 0.037[b,c]	3.15 ± 0.05[a,b,d]
Vitamin A (liver)	15.27 ± 0.21	9.49 ± 0.103[a]	12.61 ± 0.166[a,b]	15.47 ± 0.4[b,c]	12.37 ± 0.023[a,b,d]
Vitamin A (kidney)	10.69 ± 0.034	4.52 ± 0.11[a]	7.68 ± 0.025[a,b]	10.02 ± 0.07[b,c]	7.5 ± 0.05[a,b,d]
Vitamin A (pancreas)	8.39 ± 0.023	3.31 ± 0.17[a]	6.36 ± 0.037[a,b]	8.41 ± 0.077[b,c]	6.28 ± 0.025[a,b,d]
Vitamin E (liver)	0.85 ± 0.025	0.25 ± 0.004[a]	0.663 ± 0.67[a,b]	0.83 ± 0.01[b,c]	0.635 ± 0.015[a,b,d]
Vitamin E (kidney)	1.29 ± 0.0013	0.18 ± 0.004[a]	0.8 ± 0.02[a,b]	1.07 ± 0.029[b,c]	0.71 ± 0.0076[a,b,d]
Vitamin E (pancreas)	0.78 ± 0.003	0.138 ± 0.002[a]	0.63 ± 0.017[a,b]	0.76 ± 0.05[b,c]	0.64 ± 0.043[a,b,d]

Values represent mean ± S.D. ($n = 6$); Comparisons between groups are as follows *a*: Group I *vs*. Group II, III, IV, V; *b*: Group II *vs*. Groups III, IV, V; *c*: Group III *vs*. Groups IV, V; *d*: Group IV *vs*. Group V. Statistical significance is as follows $^{a}p < 0.05$, $^{b}p < 0.05$, $^{c}p < 0.05$, $^{d}p < 0.05$.

Expression units

Parameters	Units
GSH	µg/mg protein
LPO	nM of MDA formed/mg
TSH	µg/mg of protein
Vitamin A	µg/mg of protein
Vitamin E	µg/mg of protein

insulin secretion, which would activate the glucokinase and increase utilization of glucose. Glucose-6-phosphatase plays an important role in the homeostasis of blood glucose.[23] However, the activity of glucose-6-phosphatase is known to be inhibitory under hyperinsulinemic and hyperglycemic conditions.[24] Fructose-1,6-bisphosphatase is one of the key enzymes in the gluconeogenic pathway. This enzyme is expressed in the liver and kidney but is absent in the heart, muscle and smooth muscle. The activity levels of gluconeogenic enzymes such as glucose 6-phosphatase and fructose1, 6-bisphosphatase increased significantly ($p < 0.05$) in the liver of diabetic rats,[25] which may result from insulin deficiency. Diabetic rats treated with *CiREE* and glibenclamide exhibited depressed activity levels of these enzymes, which may have resulted from elevated secretion of insulin.

The increase in the activity levels of plasma AST, ALT and ALP suggest that diabetic mellitus may induce hepatic dysfunction. AST and ALT are directly associated with the conversion of amino acids to keto acids, which are found to exhibit increased activity levels in the diabetic condition. Begum and Shanmugasundaram also reported this increase in the activity levels of AST and ALT in the liver of diabetic animals.[11] Treatment with *CiREE* was sufficient to normalize these enzyme activity levels. Increased activity levels of AST and ALT in the diabetic liver were also reported by Jorda *et al*.[26] Increased protein catabolism during gluconeogenesis and urea formation, which has been reported in the diabetic state, might be responsible for the elevation of these tissue transaminases. The elevated activity of ALT has been shown to be caused by hepatocellular damage and is usually accompanied with a rise in AST.[27] This process may contribute to the elevated activity levels of these enzymes, which were reduced to near normal levels by the described *CiREE* treatment. These results demonstrate the antidiabetic effects of *CiREE* on hepatocellular damage and suppression of gluconeogenesis. Elevated activity of ALP was also observed in STZ-diabetic rats. In addition, Prince *et al.* have reported increased ALP activity in experimentally diabetic rats. The increased activity of this enzyme in the plasma may be a result of diabetes-induced damage to different tissues.[28]

The hyperphysiological burden of free radicals can cause an imbalance in the homeostatic phenomena between oxidants and antioxidants in the body, and this imbalance can lead to oxidative stress. Hyperglycemia promotes the liberation of oxygen radicals, which reduces the antioxidant potential in the body and has been shown to damage pancreatic β-cells. Insulin deficiency promotes beta-oxidation of fatty acids, which results in an increased formation of hydrogen peroxide.[29] The harmful effects of diabetes mellitus on metabolism in tissues of various organs are well known. Glucose control plays an important role in the pro-oxidant-antioxidant balance.[30] Antioxidants can scavenge free radicals against damage and decay, and they have an important role in biological systems, thereby providing a way to prevent or treat diabetes mellitus. Administration of *CiREE* which increase the number of beta cells and insulin level in serum and insulin has been reported to increase the capacity of cell to metabolize glucose and also it controls betaoxidation of fatty acids and formation of hydrogen peroxide. By this oral administration of *CiREE* which increase the antioxidant enzyme while decrease LPO levels in STZ-injected rats.

A notable increase in lipid peroxidation in the liver, pancreas and kidney was observed in diabetic rats, which can contribute to chronic hyperglycemia and can cause an increased production of reactive oxygen species (ROS) from the autooxidation of monosaccharides, thereby leading to the production of superoxide and hydroxyl radicals. The production of these chemical species can cause tissue damage by reacting with polyunsaturated fatty acids in lipid membranes.[31,32] Several of the plant-derived phenolic compounds, such as quercetin and other flavonoids, may be successful target antioxidants to treat these conditions. Diabetic rats treated with *CiREE* reduced lipid peroxidation markers to near normal levels, which could be a result of the improved antioxidant status.

Reduced activity levels of SOD and CAT in the liver and kidney tissues have been observed in diabetic rats, and this activity may result in a number of deleterious effects caused by the accumulation of superoxide radicals ($O_2{\bullet}-$) and hydrogen peroxide (H_2O_2).[30] Administration of *CiREE* increased the activity levels of SOD and CAT in diabetic rats, thereby indicating that *CiREE* exhibited free radical scavenging activity, which could rescue pathological alterations caused by the presence of $O_2{\bullet}-$ and $OH{\bullet}-$. This effect may involve mechanism related to scavenging activity.

Glutathione is an important intracellular peptide that exhibits multiple functions ranging from antioxidant defense to modulation of cell proliferation.[33] The observed decrease in the level of erythrocyte GSH in type 2 diabetes is consistent with previous findings.[34] It has been suggested that the hyperglycemia leads to enhanced activity of the polyol pathway to promote GSH depletion, which creates a redox imbalance in diabetic hepatocytes, nephrons, pancreatic cells. This state can lead to a depressed defense against oxidative stress. Sustained oxidative insult causes lipid peroxidation in cellular membrane and leads to the accumulation of malondiadehyde, which is a stable end product of lipid peroxidation. These alterations have been implicated in the development of long term complications associated with diabetes.[35] Administration of *CiREE* which increase the β-cells and insulin level in serum and insulin has been reported to increase the capacity of cell to metabolize glucose leading to enhance production of lactic acid and CO_2.[35] This action of insulin on glucose metabolism may help in elevating intracellular NADPH/NADP ration thereby causing an increase in GSH/oxidized glutathione (GSSH) ration.

Vit A acts as a powerful free radical scavenger, for singlet oxygen, and as a chain breaking antioxidant.[36] The function of vit A as a radical scavenging antioxidant can provide protection from oxidative damage in cells.[37] Vit A likely contributes to the inhibition of lipid peroxidation by promoting the recycling the vit E.[38] Decreased levels of vit A in the liver, pancreas and kidney of diabetic rats may be due to an increased level of oxygen radicals in tissues of STZ-induced diabetic animals. Administration of *CiREE* (100 and 200 mg/kg per bw) rescued the depressed levels back to normal levels. This effect could be attributed to the regeneration of vit A from its radical.

TSH group levels decreased in the liver, pancreas and kidney of diabetic rats. The formation of this chemical group likely resulted from the oxidation of essential thiols.[39] The nonprotein sulfhydryl group is predominantly found on cysteine in glutathione and in some minor thiols. The liver plays a major role in glutathione homeostasis and is the main export organ for glutathione.[40] Administration of *CiREE* restored TSH levels in the liver, pancreas and kidney of diabetic rats, which was likely caused by increased glutathione export from muscles into the circulation.

These results indicate that quercetin, diosgenin and other flavonoids exhibit an antioxidant effect by elevating intracellular GSH, GPx, SOD, CAT, vit A and vit E content in both normal and diabetic hepatocytes, nephrons, pancreatic cells, is an interesting finding. Flavonoids are antioxidant, polyphenolic compounds ubiquitously found in Costaceae. Recent work with quercetin has been focused on its efficacy as a natural antioxidant. *In vitro*, the flavonoid quercetin has been show to possess antioxidant properties by inhibiting xanthine oxidase activity[41] and scavenging radical species such as superoxide anion, hydroxyl radical, and peroxynitrite.[42] When orally administered *in vivo*, quercetin is turned over, and its metabolites retain antioxidant properties.[43]

In conclusion, administration of *CiREE* provided antioxidant activity, which may result from its inhibition of lipid peroxidation and its augmentation of the cellular antioxidant defense. Administration of *CiREE* also increased glycolysis and decreased gluconeogenesis rates in the diabetic animals, which may result from the presence of tannins, phlobatanin, saponin (diosgenin), flavonoids (quercetin), steroids, terpenoids and cardiac glycosides in this extract. These results revealed that 200 mg/kg of *CiREE* induced a significant antioxidant effect, suggesting that *CiREE* may augment protection against oxidative damage in pancreas, renal and hepatic tissues that has been associated with diabetes.

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