

## Antidiabetic Effect of *Costus Speciosus* Rhizome Extract in Alloxan Induced Albino Rats

J. Revathy<sup>1</sup>

Dr. S. Sheik Abdullah<sup>2</sup>

Dr. P. Sampath Kumar<sup>2</sup>

### Abstract

*The antidiabetic effect of ethanolic extract of Costus speciosus rhizome was determined. Various secondary metabolites were also identified and quantified by HPLC analysis. The antioxidant activity is determined by hydrogen peroxide and phosphomolybdate method. The animals were divided into four groups such as normal, diabetic untreated, diabetic treated with ethanolic extract and diabetic treated with reference drug Glibenclamide. The ethanolic extract of Costus speciosus rhizome extract (200mg/kg body weight/rat/day) was given to the rats for 15 days orally. Blood samples were collected by retro orbital puncture and various biochemical parameters were measured using autoanalyser. The ethanolic extract shows significant reduction in blood glucose, glycosylated haemoglobin, blood urea, serum uric acid, serum creatinine, triglycerides, total cholesterol, phospholipids, low density lipoprotein (LDL), very low density lipoprotein (VLDL), and increase in liver glycogen, insulin and lactate dehydrogenase (LDH). Our experimental findings with respect to the mechanism of action of extract in alloxan induced diabetic rats suggest that it enhances insulin secretion by the islets of langerhans, enhances peripheral glucose utilization and increases serum protein levels.*

**Key Words:** Thiobarbituric acid (TBA), Superoxide dismutase (SOD), *Costus speciosus*, Alloxan, Glibenclamide, Glutathione (GSH), Catalase

Diabetes is a metabolic disorder characterized by impaired glucose utilization and is the underlying factor for both hypoglycaemia and hyperglycaemia. Chronic hyperglycaemia results in impaired function or failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Despite advances in medicine, diabetes as a major health complication seems to be growing at an alarming proportion world over and in India, in particular. By the end of 2030, 79.4 million Indians are expected to be affected by this metabolic disorder and this accounts for nearly one-sixth of the world's diabetics<sup>1</sup>. Plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones. In the traditional system of Indian medicine plant formulation and in several cases, combined extracts of plants are used as the drug of choice rather than individual. Many of these have shown promising effects. Various herbal formulations like D-400 and Trasina are well known for their antidiabetic effects<sup>2</sup>. Nearly 100 polysaccharides from plants have been reported to have hypoglycemic activity.

<sup>1</sup> Research Scholar, Department of Biochemistry, Bharathiar University, Coimbatore.

<sup>2</sup> Biochemistry Faculty, Department of Chemistry and Biosciences, Sastra University, Srinivasa Ramanujan Centre, Kumbakonam – 612 001 (India).

*Costus speciosus* is a spiral, crepe or wild ginger belongs to the family costaceae. It is an important source of diosogenin and is used in many human and veterinary medicines. It is bitter, astringent, cooling, digestive, stimulant and good for the heart. It cures kapha and pitta disorders, dyspepsia, fever, cough and other respiratory diseases, diabetes, edema, blood diseases, leprosy, and other skin ailments. An aqueous extract of *C.speciosus* rhizome part showed significant hypoglycemic effects when it was administered orally with a simultaneous glucose load<sup>3</sup>. The present study aimed to analyse the phytoconstituents and to determine the antioxidant activity and to assess the influence of oral administration of ethanolic extract of *C.speciosus* rhizome on the levels of biochemical parameters and activities of enzymes in alloxan induced diabetes rats.

## **Materials and Methods**

### **Collection of Plant Material**

The plant material for the present investigation was collected from the field areas of Kumbakonam, Thanjavur District, Tamilnadu, India.

### **Plant extraction**

Soxhlet apparatus is a method to extract a soluble fraction from a solid medium. This apparatus consists of condenser, an extraction unit and a round bottom flask. It has a standard paper round glass joints. The extraction unit and control unit plays a vital role in this apparatus.

The sundried coarsely rhizome of *Costus speciosus* about 100g was weighed and placed inside the extraction unit. The flask was filled with solvent (100% ethanol) about 250ml was switched on, and after few hours (approximately four cycles) it was switched off, the extract was collected from the flask. Then the evaporated final content was used for the phytochemical work and for animal treatment.

### **Phytochemical Analysis**

Phytochemical analysis was carried out qualitatively to identify the presence of various secondary metabolites such as alkaloids, flavanoids, tannins, phenols, steroids, glycosides, carbohydrates, aminoacids, proteins, saponins, terpenoids, ascorbic acid, coumarin, quinones and sulphur<sup>4</sup>.

Animal management Wistar Albino rats (150-180gms) were selected for these studies. Six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment (obtained from Periyar pharmaceutical institute, Trichy).The animals were maintained in a control environment. They were provided with food and water adlibitum.

### **Source of chemicals**

Alloxan chemical analytical grade was purchased from SD fine Chemicals Pvt. Ltd., Biosar. All other chemicals used, were obtained from Ranbaxy Research Laboratories, Glaxo Laboratories and Nice Pharmaceutical Company, India.

### **Induction of hyperglycemia by alloxan**

Hyperglycemia was induced by intra peritoneal injection of freshly prepared aqueous solution of alloxan monohydrate (SD fine Chemicals Pvt. Ltd., Biosar) 150mg /kg, to overnight fasted rats. Control rats receive similar volume of vehicle, normal saline (2 ml/kg body weight) alone. Animals that did not develop hyperglycaemia after 48 hrs of alloxan injection were rejected and new animals were used. Immediately after confirmation of diabetes, rats were classified into four groups of five rats each. Treatment group protocol

The animals were divided into four groups and each consists of five animals.

Group I - received normal saline and served as control.

Group II- treated with alloxan monohydrate 150 mg/kg served as diabetic control.

Group III –treated with ethanolic rhizome extract (200mg/kg)

Group IV- treated with glibenclamide (2.5mg/kg) and served as reference standard.

### **Drug Administration**

Hyperglycemic rats were treated using ethanol extract of *Costus speciosus* dissolved in Tween 40, through oral administration.

### **Collection of Blood Sample**

At the end of the experimental periods, the rats were sacrificed. Plasma and serum were separated. Treatment continued for 14 consecutive days. Before the treatment (0 day) and at the end of 7<sup>th</sup> and 14<sup>th</sup> day plasma levels were estimated using the glucose oxidase method. At the end of the experimental periods, the rats were sacrificed. Plasma and serum were separated from blood by centrifuging the samples at 5000 rpm for 10 min and stored in a refrigerator until analysed.

### **Biochemical Analysis**

Blood samples were examined to determine plasma glucose<sup>6</sup> using radioimmunoassay kit and the serum concentrations of triglycerides (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), total cholesterol (TC), protein, urea were determined using commercial kits. The liver cytosolic contents of thiobarbituric acid reactive substance (TBA), Glutathione peroxide and ROS scavenging enzymes such as catalase and superoxide dismutase (SOD) were also determined using autoanalyser.

#### **Histopathological studies**

On the 14<sup>th</sup> day, pancreatic tissues were taken from animals, which were fasted overnight, under ether anaesthesia. The whole pancreas from each animal was removed after killing the animals, was placed in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5µm thickness were cut and stained by haematoxylin and Eosin (H & E) for histological examination. The photomicrographs of histological studies are taken.

### **Statistical Analysis**

Results were statistically analysed by mean  $\pm$  standard error. Significance between groups was estimated using student 't' test<sup>7</sup>.

### **Results and Discussion**

Various phytochemicals such as alkaloids, flavanoids, tannins, phenols, steroids, glycosides, carbohydrates, aminoacids, proteins, saponins, terpenoids, ascorbic acid, coumarin, quinones, sulphur shows positive inference. They may help to prevent diseases like cancer and heart diseases besides their role to inhibit the microorganisms causing many diseases in human beings.

Flavanoids and other phenolic compounds have been reported as scavengers of free radicals and reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent the damage to lipids, proteins, enzymes, carbohydrates and DNA.

**Table 1: Antidiabetic Activity of *Costus Speciosus* Rhizome Extract against Alloxan-Induced Diabetic Rats**

S.No	Groups	Blood Sugar Level (mg/dl)		
		Day 0	Day 7	Day 14
1	Control (normal saline) 2 ml/kg	90.3±4.6	90.8±3.6	90.8±6.6
2	Diabetic control(Alloxan) 150 mg/kg	263.3±18.4	262±19.2	262.3±17.7
3	Rhizome extract ( <i>Costus speciosus</i> ) 200 mg/kg	263.7±18.3	152.5±12.3*	109.7±7.3**
4	Glibenclamide 2.5 mg/kg	260±14.3	150±9.2*	101±6.2**

Values are expressed as mean ± S.E., n=6

\*P>0.01 Vs Control

\*\*P>0.001 Vs Control

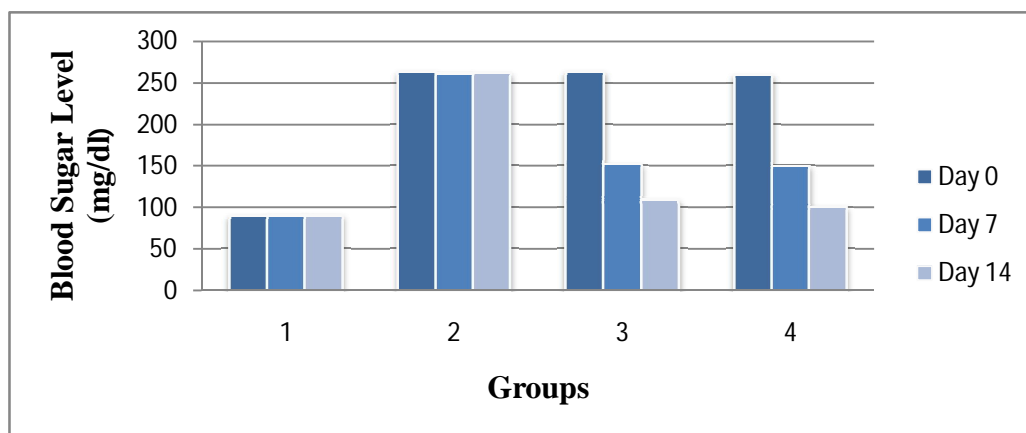
**Figure 1: Antidiabetic Activity of *Costus Speciosus* Rhizome Extract against Alloxan Induced Diabetic Rats**

Table and Fig 1: Reveals that diabetes mellitus increases the plasma glucose level, gradually which is due to insulin dysfunction. The effect of ethanolic extract of rhizome of *C.speciosus* on the blood glucose level of experimental animals was determined at various day intervals such as 0, 7 and 14. After oral administration (200mg/kg) of rhizome extract, there was a significant elevation in the plasma glucose level by 2-3 times during experimental time periods in alloxan induced diabetic rats, when compared to normal rats. Increased levels of plasma glucose in alloxan induced diabetic rats were lowered by the administration of *C.speciosus* ethanolic extract. The hypoglycemic effects of *C.speciosus* may result from the potentiation of insulin from existing  $\beta$  cells of the islets of Langerhans. The plasma glucose lowering effect was compared with glibenclamide, a standard hypoglycemic drug. The result indicates that overall blood glucose control is improved due to improvement in insulin secretion<sup>8</sup>.

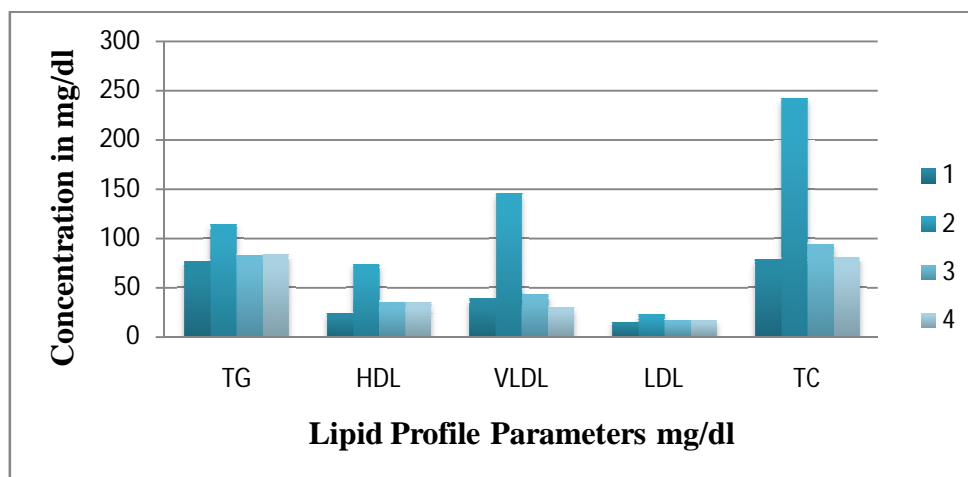
**Table 2: Effect of Ethanolic Extract of *Costus Speciosus* Rhizome on Lipid Profile in Normal and Alloxan Induced Diabetic Albino Rats**

Treatment	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	TC(mg/dl)
Normal 2 ml/kg	76.12 ± 4.12	24.50 ± 1.32	39.12± 4.13	15.22± 0.82	78.84 ± 6.27
Diabetic control(Alloxan) 150 mg/kg	114.22 ± 7.43	73.79 ± 4.7	145.41± 1.2	22.8± 1.48	242 ± 7.38
Rhizome extract ( <i>Costus speciosus</i> ) 200	82.7* ± 5.6	34.5* ± 2.1	42.96**± 0.58	16.54± 1.12	94.0 ± 3.80
Glibenclamide 2.5 mg/kg	83.74 * ± 4.9	34.4 * ± 2.8	29.8**± 1.2	16.74± 0.98	80.94 ± 4.98

Values are expressed as Mean ± S.E., n=6

\*P<0.01 Vs Control

\*\*P<0.001 Vs Control



**Figure 2: Effect of Ethanolic Extract of *Costus Speciosus* Rhizome on Lipid Profile in Normal and Alloxan Induced Diabetic Albino Rats**

Table and Fig 2: Shows high levels of total cholesterol, TG and more importantly LDL cholesterol in the blood are major coronary risk factors. The abnormally high concentration of serum lipids in the diabetic is mainly due to the increase in the metabolism of free fatty acids from the peripheral fat depots, since insulin inhibits hormone sensitive lipase. Insulin deficiency or insulin resistance may be responsible for dyslipidemia, because insulin has an inhibitory action on 3-hydroxy-3-methylglutaryl coenzyme A (HMG-coA) reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol rich LDL particles. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in increased production of cholesterol rich LDL particles. HDL is an antiatherogenic lipoprotein. It transports cholesterol from peripheral tissue into the liver and thereby acts as a protective factor against coronary heart disease. The level of HDL cholesterol, which increased after *C.speciosus* ethanolic extract administration, might be due to the increase in the activity of lecithin cholesterol acyl transferase, which may contribute to the regulation of blood lipids<sup>9</sup>.

**Table 3: Effect of Administration of Feeding the Ethanolic Extract of *Costus Speciosus* On Serum Protein And Urea**

Treatment	Protein (mg/dl)	Urea (mg/dl)
Normal	1.91± 0.06	22.0± 0.23
Diabetic control (Alloxan) (150)	7.1± 0.43	34.0± 0.47
Rhizome extract (200mg/kg)	3.8±0.28	21.0±1.2
Glibenclamide (2.5mg/kg)	3.6±0.13	22.8± 1.1

Values are expressed as Mean ± S.E., n=6

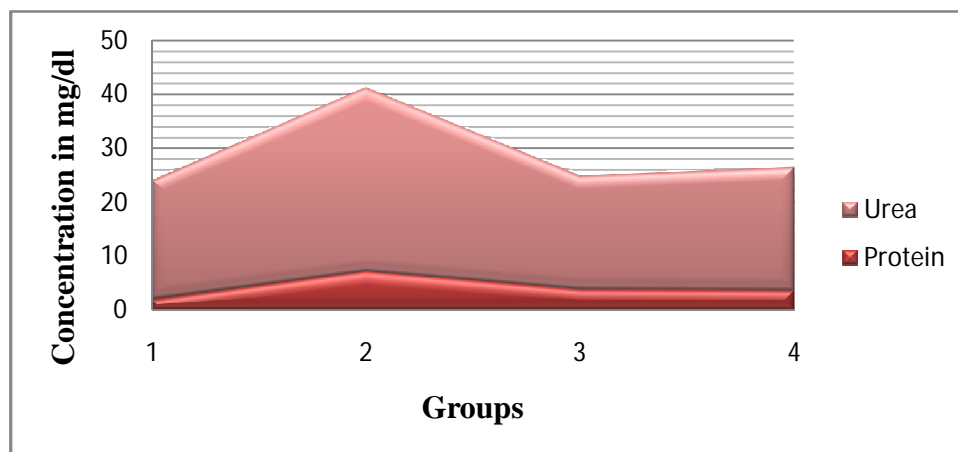
**Figure 3: Effect of Administration of Feeding the Ethanolic Extract of *Costus Speciosus* on Serum Protein and Urea**

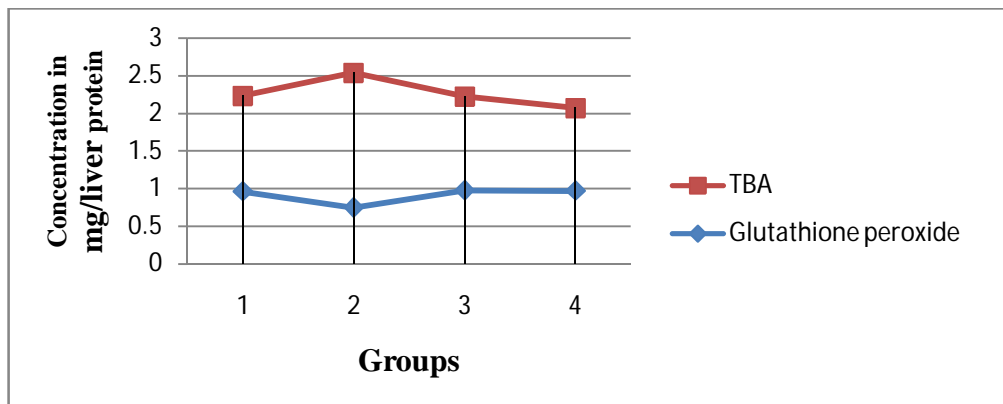
Table and Fig 3: It is evident that hyperglycemia induces elevation of serum urea leading to renal dysfunction. In the present study there was a significant ( $p < 0.05$ ) increase in the level of serum urea in diabetic rats when compared with control rats. When diabetic rats received the ethanolic extract of *C. speciosus* serum urea level decreases significantly ( $p > 0.05$ ). A marked ( $p < 0.05$ ) reduction in plasma total protein level was observed in diabetic rats due to increased protein catabolism. The present study found that the administration of the *C. speciosus* rhizome extract increased the total protein as compared with normal levels<sup>10</sup>.

**Table 4: Effect of Ethanolic Extract of *Costus Speciosus* Rhizome on Liver Cytosolic Contents of Thiobarbituric Acid Reactive Substance TBA, Glutathione Peroxide**

Treatment	Glutathione peroxide	TBA (mg/liver protein)
Normal	0.961± 0.030	1.27± 0.389
Diabetic control (Alloxan) (150)	0.747± 0.053	1.79± 0.14
Rhizome extract (200mg/kg)	0.975* ± 0.062	1.25*± 0.08
Glibenclamide (2.5mg/kg)	0.970*±0.055	1.10* ± 0.03

Values are expressed as mean ± S.E., n=6

\* $P < 0.01$  Vs Control



**Figure 4: Effect of ethanolic extract of *Costus speciosus* rhizome on liver cytosolic contents of Thiobarbituric acid reactive substance TBA, Glutathione peroxidase**

Table and Fig 4: Shows the capacity of *C.speciosus* to improve hyperglycemia is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. The concentrations of liver cytosolic enzymes were significantly increased after the induction of diabetes, whereas administration *C.speciosus* rhizome extract decreased the level of TBA.

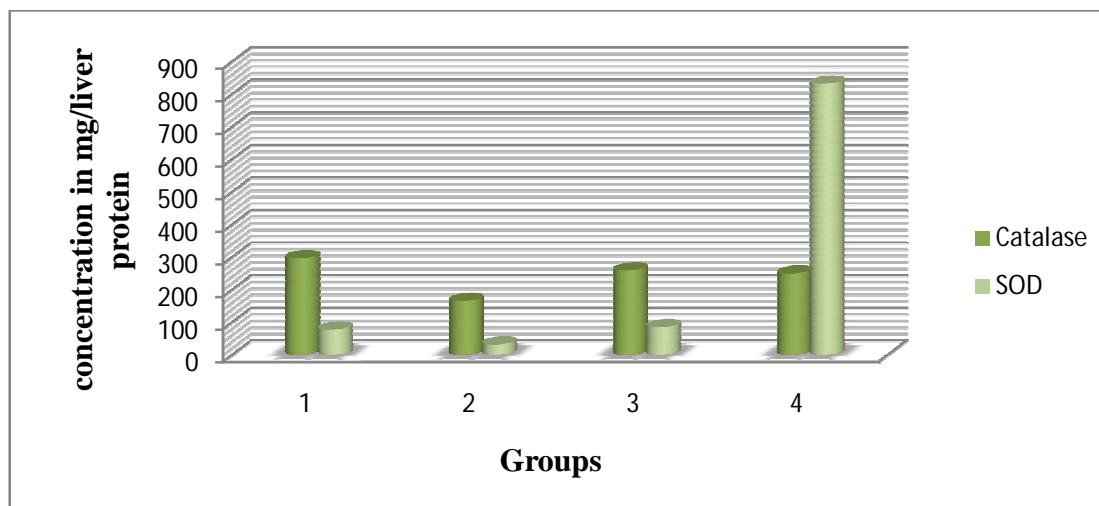
GSH is the most important biomolecule against chemically induced toxicity and can participate in the elimination of reactive intermediates by reducing hydroperoxides in the presence of GSH-Px. GSH also functions as free radical scavenger and in the repair of radical induced biological damage. The decrease in GSH level represents increased utilization due to oxidative stress. *C.speciosus* rhizome extract reverted the GSH level to normal in diabetic rats, suggesting an increased protection against oxidative stress in these animals<sup>11</sup>.

**Table 5: Effect of ethanolic extract of *Costus speciosus* rhizome on ROS scavenging enzymes**

Treatment	Catalase (mg/liver protein)	SOD (mg/liver protein)
Normal	297.07± 18.1	77.91± 6.21
Diabetic control (Alloxan) (150)	165.75± 6.78	32.31± 0.67
Rhizome extract (200mg/kg)	259.47* ± 3.51	85.40*± 0.94
Glibenclamide (2.5mg/kg)	250.36*±2.48	83.20*±0.87

Values are expressed as mean ± S.E., n=6

\*P<0.01 Vs Control



**Figure 5: Effect of ethanolic extract of *Costus speciosus* rhizome on ROS scavenging enzymes**

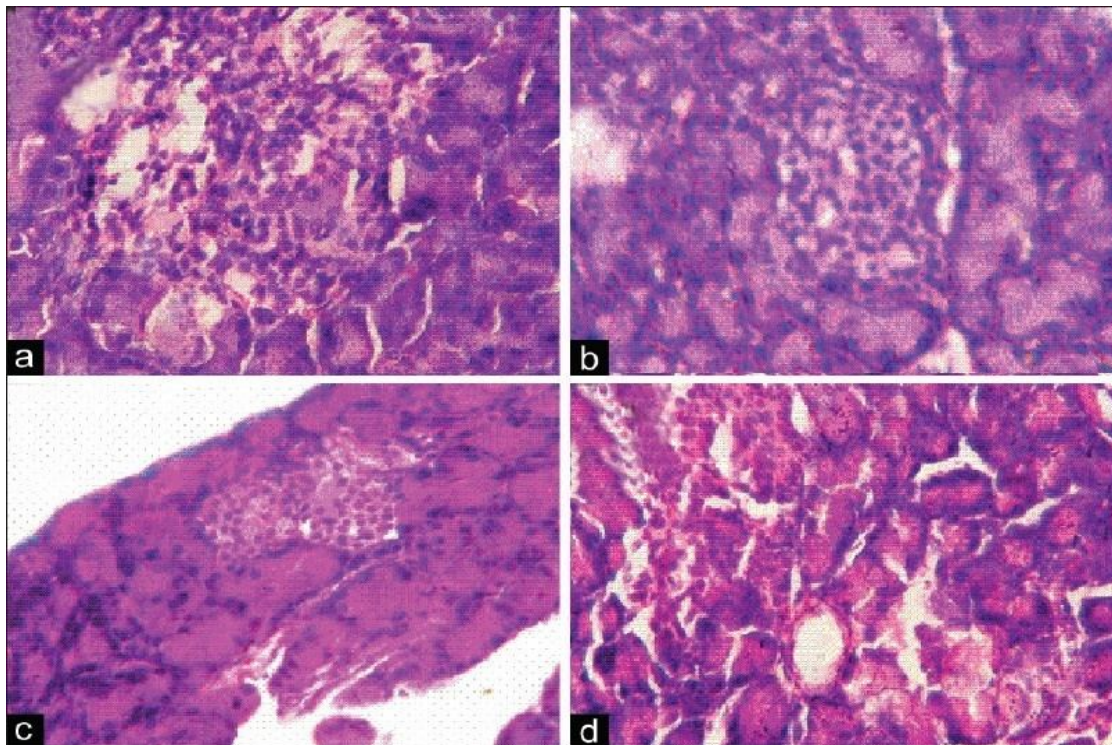
Table and Fig 5: Reveals that the ROS scavenging enzymes can respond to conditions of increased oxidative stress with a compensatory mechanism that increases the enzyme activity in diabetic rats. In the current study the hepatic SOD and catalase activity were significantly increased in diabetic rats compared with normal control rats probably to increase dismutation of superoxide anions. Increase in SOD activity could be due to its induction by increased production of superoxide radical.

Present study suggest that ethanolic extract *C.speciosus* could be used to improve the glucose and lipid metabolism as to reduce the imbalance between the generation of ROS and the scavenging enzyme activity in diabetic conditions<sup>12</sup>. Selective damage of islet cells in type I diabetes could be due to low levels of antioxidant enzymes (SOD, catalase) in the pancreas.

### ***Effect on Pancreas***

Alloxan induces extensive damage to the  $\beta$  cells of islets of langerhans. Restoration of normal cellular population and size of islets with hyperplasia were seen in extract treated groups. The partial restoration of normal cellular population and enlarged size of  $\beta$  cells with hyperplasia were indicative of the antidiabetic potential of the plant.





**Figure 6: Effect of *Costus speciosus* on histology of pancreas. Figure illustrates the photomicrographs of pancreas (haematoxylin and eosin staining) of untreated rats (a), alloxan treated rats (b), *Costus speciosus* (200 mg/kg) treated rats (c), and Glibenclamide(2.5mg/kg) treated rats (d). Microscope magnification ( $\times 100$ )**

### ***Acknowledgement***

The authors would like to thank the professors, lecturers and the lab technician of the Department of Chemistry and Biosciences, Sastra University, Srinivasa Ramanujan Centre, Kumbakonam for their support during the work.

## References

- Susheela, T., Padma Balaravi., Jane Theophilus., Narender Reddy T., and Reddy, P.U.M. Evaluation of hypoglycaemic and antidiabetic effect of *Meliadubai* CAV fruit in mice. *Current science*, vol.94, 1191-1195, (2008).
- Pari, L. and Saravanan, G. Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. *Comparative Biochemistry and Physiology Part C* 131 19-25 (2002).
- Pitchai Daisy, James Eliza, and Savarimuthu Ignacimuthu. Influence of *Costus speciosus* (Koen.) Sm. Rhizome extracts on biochemical parameters in streptozotocin induced diabetic rats. *Journal of Health science*, 54(6), 675-681, (2008).
- Gopiesh Khanna, V., and Kannabiran, K., Larvicidal effect of *Hemidesmus indicus*, *Gymnema sylvestre* and *Eclipta prostrata* against culex quinquefasciatus mosquito larvae. *African Journal of Biotechnology* Vol.6 (3) pp 307-311, (2006).
- Umamaheswari, M., and Chatterjee, T.K., In vitro antioxidant activities of the fractions of *coccinia grandis* L. Leaf extract. *African J.Traditional CAM* (000) 5(1): 61-73, (2008).
- Trinder, P. Determination of glucose in blood using oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.*, 6, 24-27, (1969).
- Snedecor G.W., and Cochran W.G., in "Statistical Methods", Oxford and IBH Publishing Co., New Delhi, 33 (1997).
- Kim, S.H., Hyun, S.H., and Choung, S.Y., Antidiabetic effect of *cinnamon* extract on blood glucose in db/db mice. *J.Ethnopharmacol.*, 104, 119-123, (2006).
- Colca, J.R., Insulin sensitizer may prevent metabolic inflammation. *Biochem.Pharmacol.*, 72, 125-131, (2006).
- Patil, U.K., Saraf, S., and Dixit, V.K., Hypolipidemic activity of seeds of *Cassia tora* Linn. *J.Ethnopharmacol.*, 90, 249-252, (2004).
- Venugopal, S.K., Devaraj, S., Yang, T., and Jialal I., *Diabetes*, 51, 3049-3054, (2002).
- Zhang, H., Matsuda, H., Kumahara, A., Ito, Y., Nakamura, S., and Yoshikawa, M., *Bioorg.Med.Chem.Lett.*, 17, 4972-4976, (2007).