

An Experimental Evaluation of the Antidiabetic and Antioxidant Effect of *Costus speciosus* Rhizome Extract in Alloxan Induced Albino rats

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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 methods. 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 (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 diabetics¹. 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 effects². Nearly 100 polysaccharides from plants have been reported to have hypoglycemic activity.

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

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for the heart. It cures kapha and pitta disorders, dyspepsia, fever, cough and other respiratory diseases, diabetes, oedema, 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 load³. The present study is 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.

MATERIAL 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 shade dried coarsely powder of 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 sulphur⁴.

Determination of antioxidant

The antioxidants are determined by using hydrogen peroxide scavenging assay and phosphomolybdate method⁵.

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 7th and 14th day plasma levels of glucose 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 glucose⁶ and the serum concentrations of triglycerides (TG), high density lipoprotein (HDL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), total cholesterol (TC), protein and urea were determined using commercial kits. The liver cytosolic contents of thiobarbituric acid reactive substance (TBA), Glutathione peroxidase and ROS scavenging enzymes such as catalase and superoxide dismutase (SOD) were also determined using autoanalyser.

Statistical analysis

Results were statistically analysed by mean \pm standard error. Significance between groups was estimated using student 't' test⁷.

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.

Radical scavenging effect of *Costus speciosus* of various concentrations are determined by using hydrogen peroxide method and phosphomolybdate method and the inhibition

concentrations are found to be greater. 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: Revealed 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 result indicates that overall blood glucose control is improved due to improvement in insulin secretion⁸.

Table 2: Shown high levels of total cholesterol, TG and more importantly LDL cholesterol in the blood are major coronary risk factors. 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

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 \pm 4.6 | 90.8 \pm 3.6 | 90.8 \pm 6.6 |
| 2 | Diabetic control (Alloxan) 150 mg/kg | 263.3 \pm 18.4 | 262 \pm 19.2 | 262.3 \pm 17.7 |
| 3 | Rhizome extract (<i>Costus speciosus</i>) 200 mg/kg | 263.7 \pm 18.3 | 152.5 \pm 12.3* | 109.7 \pm 7.3** |
| 4 | Glibenclamide 2.5 mg/kg body weight | 260 \pm 14.3 | 150 \pm 9.2* | 101 \pm 6.2** |

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

*P>0.01 Vs Control

**P>0.001 Vs Control

activity of lecithin cholesterol acyl transferase, which may contribute to the regulation of blood lipids⁹.

Table 3: From table 3 it is evident that hyperglycemia induces elevation of serum urea leading to renal dysfunction. The present study

found that the administration of the *C.speciosus* rhizome extract increased the total protein as compared with normal levels¹⁰.

Table 4: The concentrations of liver cytosolic enzymes were significantly increased after the induction of diabetes, whereas

Table 2. Effect of ethanolic extract of *Costus speciosus* rhizome on lipid profile in normal and Alloxan induced diabetic albino rats

| S. No | Groups | TG (mg/dl) | HDL (mg/dl) | VLDL (mg/dl) | LDL (mg/dl) | TC (mg/dl) |
|-------|---|-------------|--------------|--------------|-------------|------------|
| 1 | Control (normal saline) 2 ml/kg | 76.12±4.12 | 25.60±1.83 | 39.12±4.13 | 15.22±0.82 | 78.84±6.27 |
| 2 | Diabetic control (Alloxan) 150 mg/kg | 114.22±7.43 | 18*.0±2.6 | 145.41±1.2 | 22.8±1.48 | 242±7.38 |
| 3 | Rhizome extract (<i>Costus speciosus</i>) 200 mg/kg | 82.7*±5.6 | 24.93* ±6.52 | 42.96**±0.58 | 16.54±1.12 | 94.0 ±3.80 |
| 4 | Glibenclamide 2.5 mg/kg | 83.74*±4.9 | 33.82*±6.7 | 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

Table 3. Effect of administration of feeding the ethanolic extract of *Costus speciosus* on serum protein and urea

| S.No | Groups | Protein (mg/dl) | Urea (mg/dl) |
|------|---|-----------------|--------------|
| 1 | Control (normal saline) 2 ml/kg | 5.1±0.43 | 22.0±0.23 |
| 2 | Diabetic control (Alloxan) 150 mg/kg | 1.91±0.06 | 34.0±0.47 |
| 3 | Rhizome extract (<i>Costus speciosus</i>) 200 mg/kg | 3.6±0.13 | 21.0±1.2 |
| 4 | Glibenclamide 2.5 mg/kg | 3.8±0.28 | 22.8±1.1 |

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

Table 4. Effect of ethanolic extract of *Costus speciosus* rhizome on liver cytosolic contents and ROS scavenging enzymes

| S. No | Groups n moles of GSH | Glutathione peroxide n moles of TBA oxidized/min/mg protein | TBA n moles of H ₂ O ₂ formed/ mg protein | Catalase Units/mg decomposed mg protein | SOD protein /min/ |
|-------|---|--|--|--|-------------------------|
| 1 | Control (normal saline) 2 ml/kg | 0.961± 0.030 | 1.27±0.389 | 297.07±18.1 | 77.91± 6.21 |
| 2 | Diabetic control (Alloxan) 150 mg/kg | 0.747±0.053 | 1.79±0.14 | 165.75±6.78 | 32.31± 0.67 |
| 3 | Rhizome extract (<i>Costus speciosus</i>) 200 mg/kg | 0.975*±0.062 | 1.25*±0.08 | 259.47*±3.51 | 85.40*± 0.94 |
| 4 | Glibenclamide 2.5 mg/kg | 0.970*±0.055 | 1.10*±0.03 | 250.36*±2.48 | 83.20*±0.87 |

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

*P<0.01 Vs Control

administration *C. speciosus* rhizome extract decreased the level of TBA and reverted the GSH level to normal in diabetic rats, suggesting an increased protection against oxidative stress in these animals¹¹. 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 conditions¹². Selective damage of islet cells in type I diabetes could be due to low levels of antioxidant enzymes SOD and catalase in the pancreas.

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