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Research Article

Histopathological Assessment of the Kidney of STZ Induced Diabetic Rats Treated with Macerated *Costus Spicatus* Jacq. Rhizome Extract

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Abstract

Diabetes mellitus is a serious chronic metabolic disorder. Effective control of the blood glucose level is required in preventing or reversing diabetic complications and improving the quality of life in both types 1 and 2 diabetic patients. This study was undertaken to assess the nephro-protective and possible reversible effect of *Costus spicatus* rhizomes extract on cyto-architectural alterations observed following administration of STZ (150mg/kg) which was maintained over a given period of time. Normal control (NC) animals kidney tissues is stable, while diabetic control group showed high level cellular abnormalities including tubular necrosis, thickening of basement membrane, glomerular damages and edematous convulated tubules, atrophy and disarrangement of cytoartitectural component. The administration of macerated preparation of *Costus spicatus* (rhizomes extract) to diabetic rats restored the changes in the architecture of kidney tissues to near normal level.

Keywords: Kidney tissues, Tubular necrosis, Glomerular damages, Diabetic mellitus, *Costus spicatus*.

INTRODUCTION

Diabetes mellitus is a name given to a group of disorders characterized by Chronic hyperglycemia, Polyuria, Polydipsia, Polyphagia, emaciation, and weakness due to the disturbance in carbohydrate, fat, and protein metabolism associated with absolute or relative deficiency of insulin secretion or insulin action. Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025[1]. Unfortunately, apart from having a number of side effects, none of oral synthetic hypoglycemic agents has been successful of diabetes mellitus management and controlling long-term microvascular and macrovascular complications in diabetes mellitus [2-4]. The Society for Cardiovascular Pathology (SCVP) and the Association for European Cardiovascular Pathology (AECVP) created a working group of over a series of meetings held across many years, developed two consensus statements regarding by the inflammatory diseases and non inflammatory degenerative diseases of the ascending aorta [5,6]. Liver disease is one of the leading causes of death in persons with type 2 diabetes. The standardized mor-

tality rate for death from liver disease is greater than that of cardiovascular disease. The spectrum of liver disease in type 2 diabetes ranges from non alcoholic fatty liver disease to cirrhosis and hepatocellular carcinoma [7]. Epidemiologic studies and clinical trials strongly support the notion that hyperglycemia is the main cause of complications such as coronary artery diseases , renal failure, blindness, limb amputation, neurological complications and premature death[8]. STZ is used for experimental induction of diabetes mellitus in laboratory animals are considered as a potential compound for the clinical treatment of malignant diseases, there is an intensive search to establish the exact mechanisms underlying cytotoxicity by STZ. STZ is a potent alkylating agent which directly methylates DNA [9]. In vitro studies on insulin dependent diabetes mellitus secretory response to glucose is attenuated in STZ induced diabetic rats compared to control group of reduced β-cell mass of as well as metabolic defects. Results of numerous experiments revealed in the model of diabetes studies of different aspects of diabetes mellitus [10]. No further studies have been conducted to elucidate possible histopathological alterations induced by STZ and possible restorative effect of *Costus spicatus* (rhizomes extract) on the kidney as an excretory organ Injury to the kidney leads to electrolyte imbalance and urinary dysfunctions and other morphological abnormalities. This study therefore explores the ameliorative effect of *Costus spicatus* (rhizomes extract) in STZ induced diabetic groups, keeping in view of histopathological alterations in diabetic treated and untreated groups by highlighting the nephro-protective role. The Diabetes mellitus, has drawn attention in the number of occurrences. The World Health Organization estimates the annual occurrence of 3 million deaths caused by diabetes. Among that 1million amputations, 500,000 cases of kidney disease, 300,000 cases of blindness. 285 million people worldwide with diabetes, and in 2030 will be 435 million diabetics in most developing countries is an estimate of disease problems generate annual spending of $ 150 billion [11].

Materials and Methods

Animals

The present study, albino Wistar male rats; 10-weeks old, body weight (BW) 170-240 g, were used. Animals were housed under standard conditions temperature (24±2°C) and relative humidity (30-70%) with a 12:12 (light:dark) conditions. The animals were fed with standard pellet diet. They were maintained on standard pellets (guinea feed) and water *ad libitum.* Animal handling was performed according to Good Laboratory Practice (GLP). Ethical clearance was obtained from Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of the experimental animals.

Sourcing of Plant material

Plant materials Fresh plant material of *Costus spicatus* collected from the Saliyamangalam, Thanjavur District, Tamil Nadu, India.

Preparation of *Costus spicatus* (rhizomes extract)

The fresh bulbs of *Costus spicatus* (rhizomes extract) which weighed (350g) were washed and air dried for 10 minutes. The bulb plants were macerated mechanically with a piston and mortar. The preparation was stored in a refrigerator at 10°C until used for the experiments reported in this study.

Induction of Diabetes

The animals were fasted overnight and diabetes was induced by a single intra-peritoneal injection of a freshly prepared solution of STZ (150mg/kg body weight) in 0.9% NaCl saline solution into all the animals in group 2,3,4 and 5.while group I containing Normal control rats were not given anything except their standard pellet (Guinea feed) and water *ad-libitum*. After 72 hours for the development of diabetes, the rats with moderate diabetes having glucosuria and hyperglycemia (blood glucose level range above 250mg/dl) were considered as diabetic and used for plant (herbal) treatment. The macerated plant bulbs and standard pellet (guinea feed) were administered at a concentration of 6.6g/kg (6600mg/kg) body weight/rats/day for 7, 14 and 21 days.

Experimental animal /Study design

The animals were divided into five groups of five (5) rats each and treated as follows:

Group I (NC): Normal control rats were administered standard pellets and water *ad libitum* for 21 days.

Group II (DC): Diabetic control rats injected with 150mg/kg of STZ solution, standard pellets and water *ad-libitum* for 21 days.

Group III: Diabetic rats were given macerated preparation of *Costus spicatus* (rhizomes extract) at a dose of 6.6g/kg and standard pellets for 7 days.

Group IV: Diabetic rats were given macerated preparation of *Costus spicatus* (rhizomes extract) at a dose of 6.6g/kg and standard pellets for 14 days.

Group V: Diabetic rats were administered macerated preparation of *Costus spicatus* (rhizomes extract) at a dose of 6.6g/kg and standard pellets for 21 days.

The fasting blood glucose levels (BGL) of all rats were recorded at the regular intervals during the experimental period. For acute study, the BGL was monitored after 72 hours of administration of a single dose of macerated preparation of *Costus spicatus* and standard pellet (Guinea feed) and the end of 7, 14 and 21days for prolonged treatments.

The Blood Glucose Levels (BGL) in the blood of diabetic rats by tail tipping method. The blood was dropped in the dextrostix reagent pad, which was inserted into microprocessor digital blood glucometer and the readings were noted.

Sample collection for Histopathological analysis

At the end of the stipulated 21 days feeds were withdrawn, the rats were subjected to a 12 hours fast but had access to water. Sacrificed using chloroform vapour. Whole blood was collected by cardiac puncture (under light anaesthesia). The blood was transferred to plain sample bottles and allowed to clot for about 3 (three) hours. The clotted blood was then centrifuged at 3000 revolution per minute for 30 minutes to recover serum from clotted cells. Serum was separated using sterile syringes and stored under refrigerated condition before biochemical analysis were carried out.

The harvested kidney was carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. The tissue was infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney. The sections were designated "vertical sections". Serial sections of 5 μm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

Gross morphometrical analysis

The weights of body of the rats were measured daily using the weighing balance. The values of all the morphometric analysis were compared statistically using SPSS 17 Software

Photomicrography

The Histological and histochemical results was obtained from the by photomicrography using digital photomicrographic microscope.

Results

Plate 1: Normal control (NC) of Kidney tissue showing normal cellular architecture.

Plate 2: Diabetic control (DC) of Kidney tissue induced with 150mg/kg of STZ showed cellular abnormalities with area of vascular degeneration, tubular necrosis, glomerular inflammation, epithelial lining degeneration and desquamation as compared with normal control group.

Plate 3: Kidney tissue treated with macerated preparation of *Costus spicatus* (rhizomes extract) at a dose of 6.6g/kg and standard pellets for 7 days showed cellular regeneration with prominent nuclear rearrangement as compared with diabetic and non diabetic control group.

Plate 4: Kidney tissues treated with macerated preparation of *Costus spicatus* (rhizomes extract) at a dose of 6.6g/kg and standard pellets for 14 days

technique showed cellular regeneration with prominent nuclear rearrangement as compared with diabetic and non diabetic control group.

Plate 5: Kidney tissue treated with macerated preparation of *Costus spicatus* (rhizomes extract) at a dose of 6.6g/kg and standard pellets for 21 days showed increase in cellular regeneration with prominent nuclear rearrangement as compared with diabetic and non diabetic control group.

Finally, histopahological profile from the group treated with macerated *Costus spicatus* rhizomes extract) at a dose of 6.6g/kg at various days 7, 14 and 21 displayed tremendous recovering and restorative effect of the cellular components thereby signifying protective and anti-diabetic and nephroprotective role of *Costus spicatus* (rhizomes extract) on the kidney, however the cytoarchitectural alteration effect were completely restored.

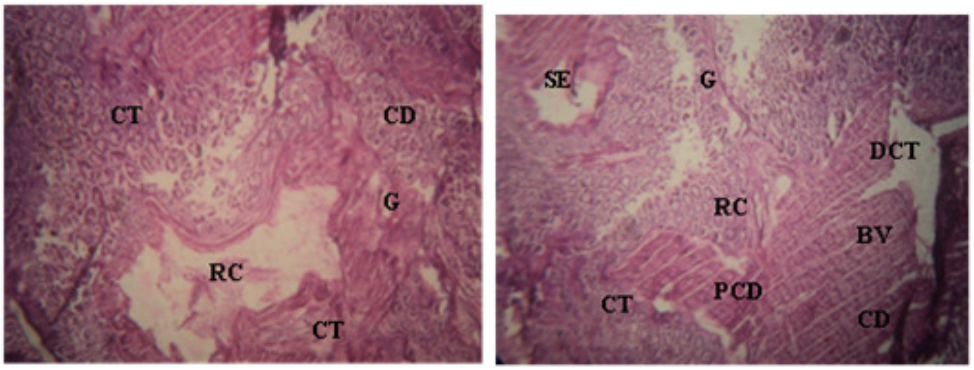


Plate :1- Normal control kidney tissues at Magnification a (α100) and B(α400) stained with H & E techniques showing normal cellular architecture note: G glomerulus, RC Renal corpuscle, DCT distal convoluted tubule, PCD- proximal convoluted tubule CD- collecting tubules, SE- Squamous Epithelial Lining and BV- Blood vessels.

Plate :2- Diabetic control of kidney tissues induced with 150kg/mg of STZ at Magnification C(α100) and D (α400) stained with H & E techniques, note: GI Glomerular Inflammation, RC Renal corpuscle, DCT distal convoluted tubule, PCT- proximal convoluted tubule, CD- collecting ducts, CT- collecting tubules, ELD- Epithelial Lining Degeneration, VC Vascular congestion and I- Focal area inflammation.

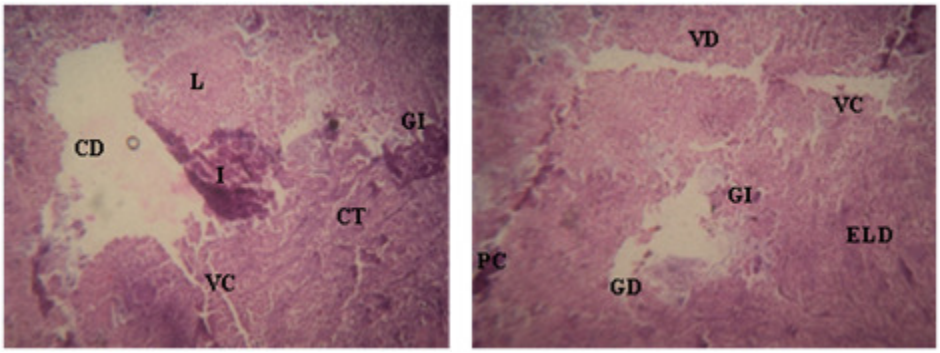


Plate :3- Kidney tissues treated with *Costus spicatus* rhizomes extract (7th Day) at Magnification a (α100) and B(α400) stained with H & E techniques note: G Glomerular Inflammation, RC Renal corpuscle , DCT distal convoluted tubule, PC- proximal convoluted tubule, CD- collecting ducts, CT- collecting tubules, SEL- Squamous Epithelial Lining and I- Focal area inflammation .

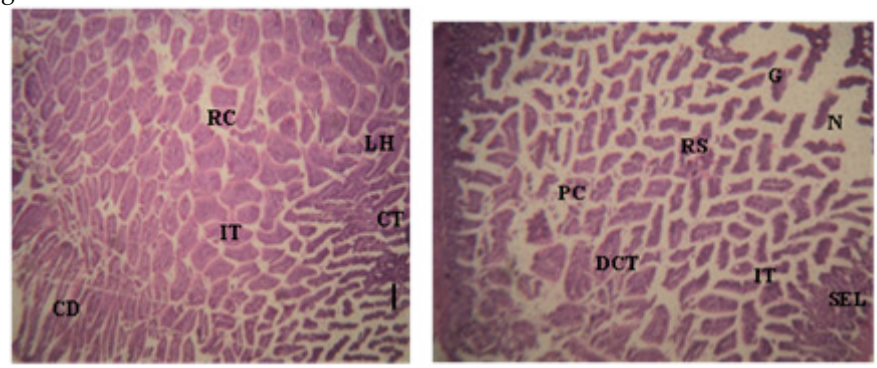


Plate :4- Kidney tissues treated with *Costus spicatus* rhizomes extract (14th Day) at Magnification a (α100) and B(α400) stained with H & E techniques note: G - Glomerular Inflammation, RC Renal corpuscle , DCT distal convoluted tubule, PCT- proximal convoluted tubule, CD- collecting ducts, CT- collecting tubules, SEL- Squamous Epithelial Lining and I- Focal area inflammation .

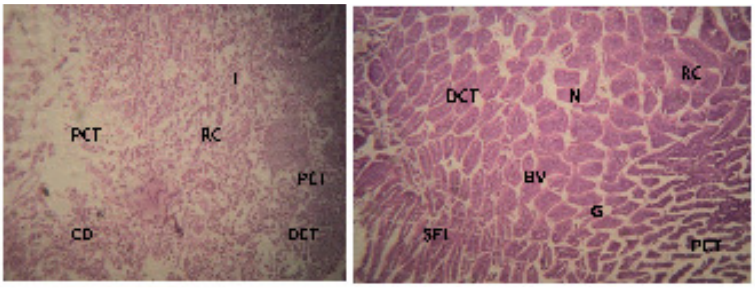
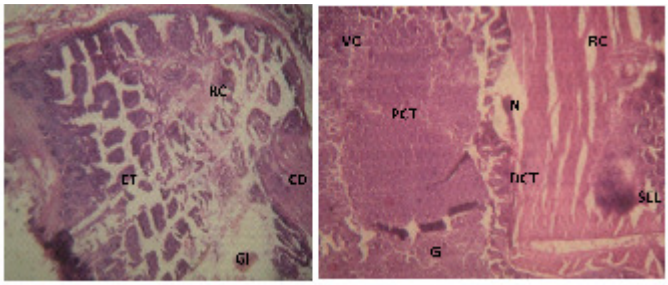


Plate :5- Kidney tissues treated with *Costus spicatus* rhizomes extract (21st Day) at Magnification a (α100) and B(α400) stained with H & E techniques note: GI Glomerular Inflammation, RC Renal corpuscle , DCT distal convoluted tubule, PCT- proximal convoluted tubule, CD- collecting ducts, CT- collecting tubules, SEL- Squamous Epithelial Lining, VC- Vascular congestion



Discussion

Diabetes mellitus is a serious chronic metabolic disorder. Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both types 1 and 2 diabetic patients [12–14]. This study was undertaken to assess the nephroprotective and possible reversible effect on cyto-architectural alterations observed following administration of STZ (150mg/kg) which was maintained over a given period of time. After the estab-

lishment of diabetics in the rats, they were treated daily for 3 weeks with the rhizomes extract for the restoration in kidney. Normal control (NC) diabetic rats kidney tissues were stable while diabetic control group showed high level cellular abnormalities including tubular necrosis, thickening of basement membrane, glomerular damages and edematous convulated tubules, atrophy and disarrangement of cytoartitectural component. It is also established, STZ induced diabetic administration to experimental rats selectively causes pancreatic β-cell membrane disruption and cyto-toxicity after its intracellular accumulation [15]. In India, *C. speciosus* and *C. igneus* (C. pictus) are the two commonly found species of Costus. *C. pictus* leaf extracts showed good inhibitory activity against a-glucosidase and a-amylase enzymes[16].

The administration of macerated preparation of *Costus spicatus* (rhizomes extract) to diabetic rats restored the changes in the architecture of kidney tissues to near normal level. These findings are suggestive of a possible nephroprotective role played by the macerated preparation of *Costus spicatus* (rhizomes extract).

Conclusion

The present study established the possible therapeutic value of *Costus spicatus* (rhizomes extract) in treating diabetes ascertained by histopathological studies. It is believed that this research will be helpful for future research like compound isolation and biological activity of the pure compound.

Conflict of Interest

The authors declare that they have no conflict of interest.

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