HISTOPATHOLOGICAL STUDIES OF METHANOL EXTRACT OF CORIANDRUM SATIVUM LINN FRUIT ON STREPTOZOTOCINE INDUCED DIABETIC RAT

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ABSTRACT
The present study was undertaken to evaluate the histopathological changes by 75% methanol extract of Coriandrum sativum Linn in streptozotocine induced diabetic rats which has been claimed to possess antidiabetic properties in Traditional System of Medicine. The streptozotocine induced albino rats were orally treated with vehicle[diabetic control], Glibenclamide (0.5 mg /kg; standard control) and 75% methanol extract [100 mg/kg and 200 mg/kg as CS1 and CS2] respectively for 14 days. Histopathological changes in the diabetic rat organ viz., heart, Kidney, liver and spleen were observed after 14 days of treatment. The 75% methanol extract showed significant decrease in blood glucose level at a dose of 100 mg/kg and 200 mg/kg. Histopathological changes are restored near to normal after treatment with CS1 and CS2. The results demonstrate that the fruit of C. sativum Linn possess significant antidiabetic activity in diabetic rats and possesses favourable effect on vital organ such as heart, Kidney, liver and spleen, thereby justifying the use of the plant as antidiabetic agent.

KEYWORDS: Coriandrum sativum, Diabetes mellitus, Streptozotocine, Histopathology.

INTRODUCTION
Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The total number of people with diabetes is projected to increase from 171 million in 2000 to 366 million in 2030.[1] The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonyl ureas and biguanides.[2] Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic drugs has been successful in diabetes management and controlling long term microvascualr and macrovascular complications.[3, 4] The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity and treatment.[5] Streptozotocin induced diabetes mellitus in many animal species has been reported to resemble human hyperglycemic non-ketonic diabetes mellitus.[6] This effect has been extensively studied and appears to be mediated through a lowering of beta cell nicotinamide adenine dinucleotide (NAD+) and results in histopathological alteration of pancreatic islet beta cells.[7] Kidney hypertrophy is observed in both the glomerular basement membrane and capillaries of diabetics and may contribute to end stage renal damage.[8] Alternative medicines, particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability.[9] Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity.[10-11] Furthermore, World Health Organization (WHO) has also recommended the evaluation of traditional plant treatment for diabetes.[12] Patients are therefore using herbal medicines which have fewer side effects and have the potential to impart therapeutic effect in complicated disorders like diabetes and its complication.[13] One such plant is Coriandrum sativum which has been used in traditional system of Indian medicine for treating diabetes.

Coriander [Coriandrum sativum Linn.] an annual of the Apiaceae family is one of the valuable medicinal and seasoning plant. This species comes from the Mediterranean region and it is grown all over the world. The coriander fruit and essential oil isolated from it are used for medicinal purpose.[14,15] C. sativum is widely used in traditional medicine to treat anxiety, dizziness, headache, edema, fever, digestive disorders, respiratory diseases, allergies, and burns.[16,17] The fruits are used as astrignent, anthelmintic, emollient, stomachic, antibilious, digestive, appetizer, constipating, diuretic, antipyretic, refrigerant, tonic, expectorant, anodyne, antidiabetic and dyspepsia. The phytochemical screening of Coriandrum sativum showed that it contained essential oil, tannins, terpenoids, reducing sugars, alkaloids, phenolics, flavonoids, fatty acids, sterols and...
glycosides. It also contained high nutritional values including proteins, oils, carbohydrates, fibers and wide range of minerals, trace elements and vitamins. The previous pharmacological studies revealed that it possessed anxiolytic, antidepressant, sedative-hypnotic, anticonvulsant, memory enhancement, improvement of orofacial dyskinesia, neuroprotective, antibacterial, antifungal, anthelmintic, insecticidal, antioxidant, cardiovascular, hypolipidemic, anti-inflammatory, analgesic, antidiabetic, mutagenic, antimutagenic, anticancer, gastrointestinal, deodorizing, dermatological, diuretic, reproductive, hepatoprotective, detoxification and many other pharmacological effects.  

Silver nanoparticles were synthesized using methanol and aqueous extract of fruit of *Coriandrum sativum* and its antioxidant activity were reported. We have reported better activity with 75 % methanol extract of fruit of *C.sativum* and HPTLC data also showed that 75 % methanol extract has more number of phytoconstituents than all other extract. In the light of the above information, the present investigation was undertaken to evaluate the histopathological changes in the streptozotocine(STZ) induced diabetic rat organs like Heart, Kidney, Liver and Spleen.

**MATERIALS AND METHODS**

**Plant material**
The *Coriandrum sativum* fruits were collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by Botanist, Natural Remedies Pvt Ltd., Bangalore. A voucher specimen was deposited in The Oxford College of Pharmacy, Bangalore. The fruits were dried in shade and powdered coarsely, passed through sieve no. 40 and stored in air tight container for further use.

**Preparation of fruit extract**
Coarsely powdered fruits of *C.sativum* 200 g was extracted with 75 % methanol [1500 ml] in soxhlet apparatus till the complete exhaustion, filtered. The methanol extract was concentrated by rotary vacuum evaporator and evaporated to dryness.

**Chemicals used**
The streptozotocine was procured from Hi-Media and all other chemicals and solvents used were of analytical grade.

**Animals**
Mature Albino rats obtained from the Animal house of The Oxford College of Pharmacy, Bangalore were used for the studies. Rats were maintained under standard conditions (27 ± 2°C; relative humidity 60 ± 5 %, light dark cycle of 12 hrs) and fed with standard pellet diet and water ad libitum. Prior to the experiment the animals were fasted for 12 h with water ad libitum given and weighed. All procedures described were reviewed and approved by Institutional Animal Ethics Committee.

**Acute Toxicity Study**
The toxicity study reveals the safety of *Coriandrum sativum* in Rat. There was no marked change in the general behavior up to 2000 mg/kg, body weight of *C.sativum*. No mortality was observed during the observation period as per literature study.

**Antidiabetic Activity**
Antidiabetic activity of *C.sativum* were determined by Streptozotocine induced diabetes mellitus model.

**Induction of Diabetes**
The animals were fasted for 16 hour prior to the induction of diabetes. STZ freshly prepared in citrate buffer (pH 4.5) was administered i.p. at a single dose of 50 mg/kg. Development of diabetes was confirmed by polydypisia, polyurea and by measuring blood glucose concentrations 72 hour after injection of STZ. Rats with blood glucose level of 250 mg/dl or higher were considered to be diabetic and selected for experiment.

**Experimental Design**
Animals were divided into five groups, each consisting of six rats. The extracts were administered for 14 days.

Group 1: Normal rats received only vehicle (Normal control)
Group 2: Streptozotocine induced rats received only vehicle (Diabetic control)
Group 3: Streptozotocine induced rats received Glibenclamide (0.5 mg /kg) daily for 14 days. Group 4: Streptozotocine induced rats received lower dose (100 mg /kg body wt. p.o) as CS1. Group 5: Streptozotocine induced rats received higher dose (200 mg/kg body wt .p.o) as CS2.

**Testing of fasting blood glucose level and biochemical parameters**
Fasting blood glucose levels were measured on 0, 7, and 14 days of treatment of all these groups. Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer. The results were expressed in terms of milligram per decilitre (dL) of blood. The body weight of each animal was noted. At the end of the experimental period, all the animals were sacrificed by decapitation and blood were collected and the serum was used for the estimation of various biochemical parameters and reported. [Communicated to EJPBS Journal].

**Histopathological studies**
At the end of 14th days, all the animals were fasted overnight. All animals from each group were euthanized by cervical dislocation under general anesthesia. Tissue samples were thereafter collected from the heart, kidney, liver and pancreas. These tissue samples were preserved in 10 % neutral buffered formalin for 48 hours, processed for paraffin embedment. Section were cut at 5 µm thickness and stained with hematoxylin and eosin dye (H & E) for histopathological evaluation under a photomicroscope (x 40). Histopathological studies
were carried out at The Oxford Dental College, Histopathology Lab, Bangalore.

RESULTS AND DISCUSSION
The gross pathological evaluation of heart, Kidney, liver and spleen of the rats from the diabetic control and treatment groups revealed no abnormalities and remained similar to those of vehicle control animals [Fig 1 to 4]. The histopathological examination of Hematoxylin and Eosin stained sections of heart, Kidney, liver and spleen of all the rats from vehicle control group revealed normal histological pattern.

The heart of diabetic control rats administered with 50 mg STZ/ kg bw revealed more interstitial edema, leukocyte infiltration, and myonecrosis[Fig 1B]. The treated diabetic rats [Fig 1 C-E] exhibited reduction in edema with less fragmentation of fibres, which reflects the cardio protective effect of CS.

The normal kidney section shows the well arranged cells[Fig 2A]. The glomerular basement membrane is compact. Diabetic groups showed that the endocytic vacuoles as characteristically seen in the proximal tubules and the thickening of the glomerular basement with glomerulosclerosis[Fig 2B]. The damage was recovered with the treatment of CS1 and CS2 at the dose of 100 mg/ kg and 200 mg/kg[Fig 2 D-E].

The liver of diabetic control rats administered with 50 mg STZ/ kg bw revealed multifocal hepatocellular hypertrophy with intracytoplasmic microvesicular steatosis, bridging coagulative necrosis of centrilobular hepatocytes characterized by cytoplasmic eosinophilia, karyolysis and mononuclear cell infiltration in the Liver[Fig 3B]. The histopathological evaluation of liver from the diabetic rats treated with CS at 100mg/kg and 200mg/kg and glibenclamide revealed normal histological pattern[Fig 3C-E], respectively and remained similar to those of vehicle control group.

The spleen of diabetic control rats administered with 50 mg STZ/ kg bw showed that the white pulp was greatly diffused. Mature lymphocytes in peripheral sections of the spleen were also dramatically reduced[Fig 4 B]. The histopathological evaluation of spleen from the diabetic rats treated with CS at 100mg/kg and 200mg/kg and glibenclamide revealed normal histological pattern [Fig 4 C-E], respectively and remained similar to those of vehicle control group.

These findings suggest that the pathological progression of streptozotocin induced diabetes were found to be suppressed by the treatment of CS at the doses of 100 mg/kg bw and 200 mg/kg bw, respectively for 14 days and were comparable to those of glibenclamide treatment.

Histopathology of Heart myocardium

Fig: 1 Effect of CS1 and CS2 on Heart in different group of rats.
A: Normal group; B: STZ group; C: Glibenclamide group; D: CS1 group and E: CS2 group.

Histopathology of Kidney

Fig: 2 Effect of CS1 and CS2 on Kidney in different group of rats.
A: Normal group; B: STZ group; C: Glibenclamide group; D: CS1 group and E: CS2 group.

Histopathology of Liver

Fig: 3 Effect of CS1 and CS2 on Liver in different group of rats.
A: Normal group; B: STZ group; C: Glibenclamide group; D: CS1 group and E: CS2 group.
Histopathology of Spleen

Fig: 4 Effect of CS1 and CS2 on Spleen in different group of rats.
A: Normal group; B: STZ group; C: Glibenclamide group; D: CS1 group and E: CS2 group.

CONCLUSION
In conclusion, the results of the present study showed that the fruits of Coriandrum sativum are an alternative or complimentary medicine in the management of diabetes mellitus. The results of the present study indicate that the CS1 and CS2 were found to have antidiabetic activities in STZ induced diabetic rats. However, further investigations are needed to identify the lead molecule and to elucidate the exact mechanism of action for antidiabetic effect.

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Conflict of Interest
None to be declared.

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