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Serum lipid profile and *In-vivo* antidiabetic activity of medicinal plant *Costus spicatus* in diabetic albino wistar rats

Senthilkumar S[1], Azhagu Madhavan S[1], Mariappan P[2], Rajakumar R[1], Ganesan S[1*]

[1] PG & Research Department of Zoology, A Veeriya Vandayar Memorial Sri Pushpam College, (An Autonomous Institution Affiliated to Bharathidasan University) Poondi, Thanjavur, Tamil Nadu, India

[2] PG & Research Department of Zoology, Rajah Serfoji Government College, (Autonomous), Thanjavur, Tamil Nadu, India.

Abstract

The medication is solution properties arsenic harming, tonic, diuretic, uterotonic and sterility in ladies and leaves is shown especially in diabetes. Diabetes mellitus is an endocrine, metabolic problem wherein the homeo-balance of sugar and lipid digestion is inappropriately controlled by the pancreatic chemical, insulin, eventually bringing about expanded blood glucose. In our examination, diabetes was initiated in rodents by single intraperito-neal infusion of Streptozotocin (STZ) at a portion of 45mg/kgb.w. The hepatoprotective adequacy of ethanolic concentrate of *Costus spicatus* (ACS) at a portion of 500 mg/kg b.w. was examined. Diabetes mellitus is related with reformist metabolic insanity, deteriorating glycemic control, and morphological changes in the liver, pancreas and different organs. Compared to the lipid profile in control and experimental rats 167.3±7.* maximum values and minimum values103.6±6.7***.**

Keywords: albino rats, *Costus spicatus*, STZ, Insulin, HDL, LDL

Introduction

Histopathology

Histological procedures are used for the assessment of tissue changes in either induced or spontaneous diseases. Principally, it compares the experimentally altered or diseased tissues with matching samples from control or healthy counterparts (Slaoui and Fiette, 2011). In the streptozotocin model, the pancreatic β – cells can be damaged or destroyed with the help of streptozotocin [1]. Histopathological studies after administration of streptozotocin have altered the morphological features of the pancreas and kidney. After the administration of an antidiabetic drug, the islets of Langerhans containing beta cells were restored to nearly normal in streptozotocin-induced diabetes in rats. The observations like basal vacuolization, hypertrophy in glomeruli, glycogen accumulation, degeneration of tubular epithelium, glomerulosclerosis, increased mesangial matrix, hyalinization, thickening of membrane, etc. may be noted in the histopathology of the kidney [2]. The slight improvement in the pancreas, kidney, liver, lungs and brain tissue may be due to antioxidant properties of the antidiabetic drug and its capability to scavenge the free radicals generated from streptozotocin, apart from its role in enhancing the functional capabilities of the immune system [3]. In the kidney, hypertrophy is observed in both the glomerular basement membrane and capillaries that may contribute to ending stage renal damage [4].

Drug or chemical induced diabetes

Many drugs can impair insulin secretion. These medicine might not cause polygenic disorder by themselves, but they may precipitate diabetes in individuals with insulin resistance. Certain toxins like Vacor (a rat poison) and blood vessel pentamidine will for good destroy duct gland β-cells. Such drug reactions, fortunately, are rare. Some drugs and hormones can impair insulin action. Examples include nicotinic acid and glucocorticoids. Patients reception α-interferon has reported near develop diabetes connected through islet cell antibodies and, in certain instances, severe insulin deficiency [5].

Infections

Certain viruses have been associated with β-cell destruction. Diabetes happens in patients with inborn German measles, although most of these patients have HLA and immune markers characteristic of type 1 diabetes. Also, coxsackievirus B, cytomegalovirus, adenovirus, and mumps have been implicated in inducing certain cases of the disease or diabetes [6].

Acute toxicity

The wide market and growing demand for natural medicinal plants in the forms of food or health supplement, is increasing nowadays. The different processes employed in the development of such products resulted in qualitative or quantitative changes in the chemical profiles of the plant material causing the alteration of the biological profile. Most of the researches mainly focus on optimizing and advancing the extraction methods to extract maximum biological active material. However, assessing the toxicity profile of medicinal plants is of vital importance. It becomes particularly essential since most people have a general belief that all-natural products are safe and free to access and consume since most of these products are marketed as supplements. Hence it is important to have proper chemical, toxicological, and safety data for the usage of plants with traditional claims on health benefits [7]. Acute toxicity testing is usually carried out to determine the effect of a single dose on animal species like rodents and non-rodents. In acute toxicological testing, the investigational product is administered at different dose levels, and the effect is observed for two weeks [8]. Acute toxicity testing permits the five hundred doses (LD^{50}) of the investigational product to be determined. The LD^{50} was used as AN indicator of acute toxicity antecedently. The determination of the LD^{50} involves large numbers of animals, and the mortality ratio was high. Because of these limitations, modified methods were developed.

Material Methods

Collection, Identification and Authentication of plant species

Map 1: Study area

The plant, *Costus spicatus* were collected from the Saliyamangalam and Thanjavur district, Tamilnadu, India. It was taxonomically identified and authenticated by Dr. S.S. Aravindar, Sither Ashram, Thanjavur. A voucher specimen number is SSS01 of the plant was deposited in the Department archive.

Preparation of extracts

The powdered plant samples of flower (100mg) were used for successive solvent extraction (500ml) with increasing order of polarities like chloroform, ethyl acetate and petroleum ether. At to direct it is reserved during an orbital shaker at 190-220rpm for 48 hours. The supernatant was gathered, separated through Whatman No.1filter paper and the concentrate were concentrated by a Rotary jar evaporator at a particular temperature was utilized dependent on the dissolvable framework. Each time past to extricate through the following dissolvable the remaining parts was dried completely to eliminate the dissolvable utilized. The gained dried concentrate was then decisively measured, set aside in little vials at – 20 °C and used for the going with assessments [9].

Animal

Albino Wistar male rats; 10- weeks old through a bodyweight ranged connecting 180-250 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. Animals were handled according to Good Laboratory Practice. Ethical clearance was obtained from the Committee for the Purpose of Control and Supervision of experiments on Animal (CPCSEA). Institutional Animal Ethics Committee (IAEC) RegNo:685/PO/Re/S/2002/) KMCRET/Ph.D/22/2018-19).

Chemicals

Streptozotocin (STZ), Ethylene Diamine Tetra Acetic Acid (EDTA), Glibenclamide (Prudence Pharma Chem, India), Chloroform. In india.

Induction of Diabetes in Rats

After fasting, diabetes was induced by intra peritoneal (*ip*) injection of Streptozotocin dissolved in 0.1 M cold sodium citrate buffer, pH 4.5,at a dose of 55 mg/kg [10]. The control rats received the vehicle alone. The animals are allowed to drink 5% glucose solution over night to overcome the drug induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

Experimental Design

The animals were divided into six groups of six animals each as follows. Each animal was marked for identification and regularly monitoring. The animals of normal control (Group I) were injected with citrate buffer alone. Group II served as diabetagenic rats (Control). Group III and IV rats treated with *Costus spicatus* rhizome at a dose of 300 and 500mg/kg were orally given once a day for 4 weeks. Group IV rats treated with Glibenclamide at dose of 5mg/kg and served as reference stranded treatment continued for 4 weeks.

Collection of sample

After the termination of the experiment all the animals were anesthetized using ketamine chloride (24mg/kg bw) and sacrificed by cervical dislocation after an overnight fast. Blood was collected with and without EDTA. Plasma and serum were separated after centrifugation and used for various biochemical estimations.

Biochemical estimations

Serum glucose was estimated by the oxidase method [11]. The total cholesterol was estimated by the method. Triglyceride was estimated by the method of HDL cholesterol was separated by adding phosphotungsti magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by the methods. Concentration of LDL cholesterol was calculated by using the Fried wald formula and VLDL cholesterol was calculated by dividing the triglycerides value (in mg/dl) by 5. Heamoglobin estimated by the method. Insulin was assayed by the solid phase system amplified sensitivity immunoassay.

Histological Assay

On the 28thday, pancreatic tissues were taken from animals which were fasted overnight under ether anesthesia. The whole pancreas from each animal was removed after killing the animals, was placed in 10% formulation solution and immediately processed by the paraffin technique section of 5 µm thickness were cut and strained by haematoxylin and Eosin (H and E) for histological examination. The photomicrographs of histological studies are taken.

Statistical analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were expressed as Mean ± Standard Deviation for p values<0.05 were considered significant for analysis of percent inhibition of cell growth.

Result and Discussion

Estimation of Body Weight

The body weight of diabetic rats significantly decreased when compared with the control group [12]. Supplementation of ethanolic extracts of *Costus spicatus* showed a significant improvement in the bodyweight of diabetic rats. There were no significant changes observed between control-treated group animals.

Estimation of Blood glucose, Plasma insulin and Hemoglobulin

Shows the blood glucose plasma insulin and total hemoglobulin levels of normal and experimental rats. There was a significantly increased level of blood glucose and plasma insulin was observed in diabetic animals compared to the corresponding control group [13]. *Costus spicatus* ethanolic flower extract with blood glucose Plasma insulin, and Hemoglobulin restored.

Estimation of Serum Lipid Profile

The lipids profile to *Costus spicatus* connective with Triglycerides, Total cholesterol, VLDL, LDL increased and HDL levels were significantly decreased in STZ treated rats. Oral administration of C. Rhizomes ethanolic extract in 300 and 500mg/kg of body weight restored the altered parameters, which was compared to that of the glibenclamide group. However, no significant changes were observed, control-treated groups.

Table 1: Effect of *C. spicatus* ethanolic flower extract on the changes of body weight (BW) of control and treated rats

Groups	Change in Bodyweight (gm)	
	0^{th} day	21^{th} day
Group I	197.21± 19.10	228.21 ± 19.01
Group II	186.13 ± 17.11*	148.11 ± 11.4*
Group III	201 ± 10.21 **	224 ±12.35 **
Group IV	196.06 ± 19.43*	227.01±11.39**
Group V	181.04 ± 12.2$^{\#}$	180.11±12.17*

Results are expressed as mean ±SEM; n=6; *=p<0.05, **=p<0.01 and#=NS

Table 2: Effect of *C. spicatus* ethanolic flower extract on the levels of blood glucose, plasma insulin and hemoglobin in control and experimental rats

	Blood glucose (mg/dL)	Plasma insulin (µg/mL)	Total Hemoglobin (g/dL)
Group I	83.41 ± 8.81*	7.24 ± 1.53	13.43 ± 1.37*
Group II	278.11± 19.33$^{\#}$	4.24 ±0.15$^{\#}$	8.97 ± 0.48$^{\#}$
Group III	82.27 ±6.23$^{\#}$	15.12±2.47 **	13.88±3.12 **
Group IV	106.17±4.57*	11.08±1.21*	11.87±0.47*
Group V	88.01±3.19*	16.27±1.08*	12.99±1.57**

Results are expressed as mean ±SEM;n=6; *=p<0.05, **=p<0.01 and#=NS

Table 3: Effect of *C. spicatus* ethanolic flower extract on lipid profile in control and experimental rats

Treatment	TGL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	Total Cholesterol (mg/dl)
Group I	75.15±5.03	38.6±1.83	15.03±1.06	41.9±4.37	96.16±6.41
Group II	124.32±6.7$^{\#}$	42.56±5.52$^{\#}$	32.14±1.7$^{\#}$	96.47±3.2$^{\#}$	215.2±7.4$^{\#}$
Group III	82.04±6.7*	25.38±4.75*	16.92±1.34*	33.9±2.66*	96.2±4.8*
Group IV	97.52±4.67*	38.27±2.56$^{\#}$	30.19±2.8*	88.41±2.34$^{\#}$	167.3±7.*
Group V	91.49±2.45**	33.46±1.36*	25.52±3.4*	81.27±5.28*	103.6±6.7*

Results are expressed as mean ± SEM; n=6; *=p<0.05, **=p<0.01 and#=NS

Histological Assay

Multiple sections of the pancreas were taken and studied for histological changes in the plant extract-administered group and control group. Histological findings of the pancreas in the extract-administered group and control group were tentatively similar. STZ-induced diabetic rats showed extensive damage to the islets of langer hans cells. The orally administered flower extracts of *C. spicatus* extracts (500 mg/kg) and commercial drug, Glibenclamide (100 mg/kg) were showed restoration of normal cellular population and enlarged size of beta cells with hyperplasia found in islets of langer hans cells in the pancreas [14]. The pancreas present in the group of animals treated with the extracts of *C. spicatus* in plant rhizomes extracts (500 mg/kg) clearly showed that the partial restoration of normal cellular population and enlarged size of beta cells with hyperplasia on 30th day [15]. The islets were normal in size, shape, and number comparatively similar to that of the standard treated drug.

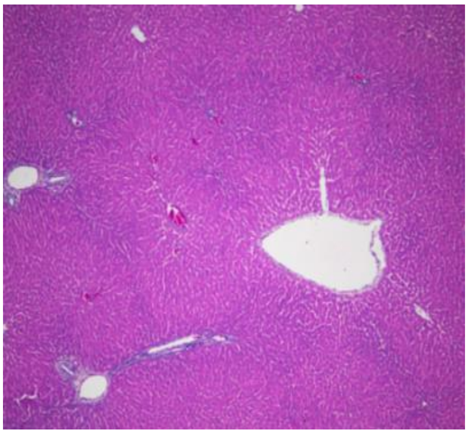

Fig A: Histopathology of islets of Langerhans Normal Rats

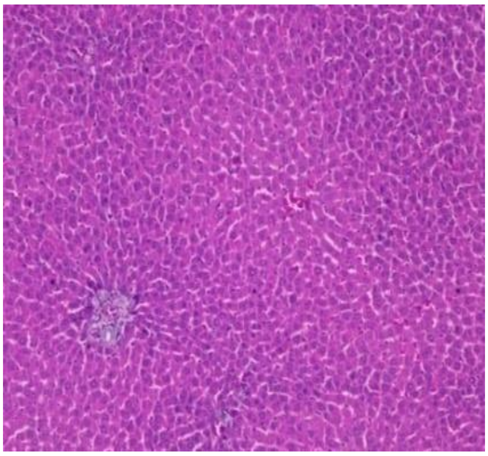

Fig B: Histopathology of islets of Langerhans diabetic Rats (STZ-Induced)

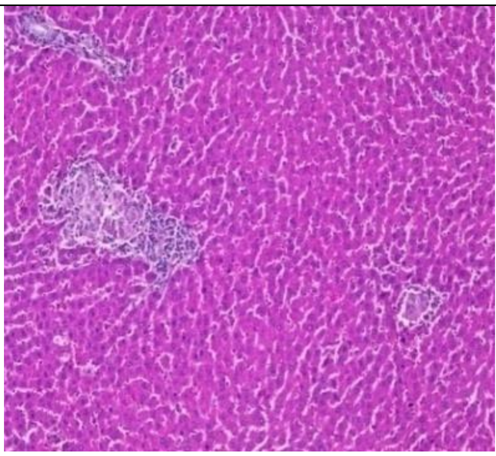

Fig C: Histopathology of islets of Langerhans diabetic Rats treated with Glibenclamide (5mg/kg)

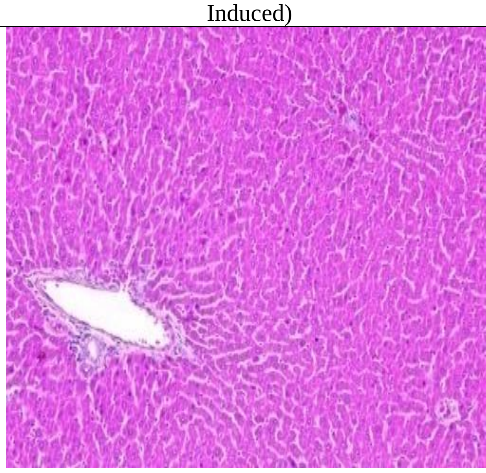

Fig D: Histopathology of islets of Langerhans of treated with *Costus spicatus* 300mg/kg diabetic Rats

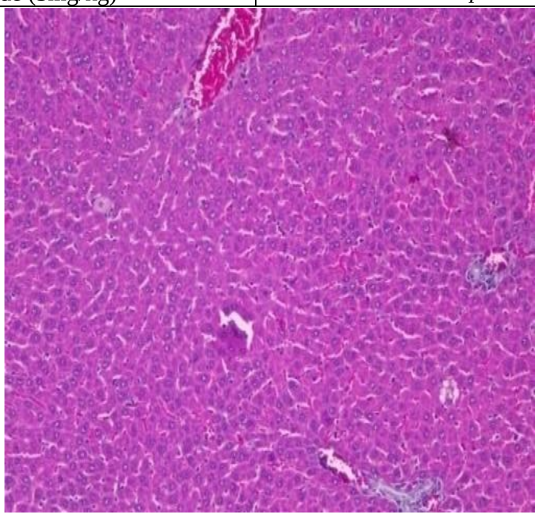

Fig E: Histopathology of islets of Langerhans of treated with *Costus spicatus* 500mg/kg diabetic Rats

Fig 1: Antidiabetic and hypolipidamic effects of flower extracts of *Costus spicatus.*

The present study investigates the anti-diabetic effect of leaf extract of *C. spicatus* on STZ diabetic rats. The fundamental mechanism underlying hyperglycemia in diabetes mellitus. Therefore, the total hemoglobin level is decreased in STZ diabetic rats [16]. So the total hemoglobin level is lowered in diabetic rats. Administration of *C. spicatus* reversed the total hemoglobin levels in STZ diabetic rats. Twenty-eight days administration of ethanolic extract of leaf of the *Costus spicatus* resulted in a significant reduction in the fasting blood glucose level compared to diabetic rats. The difference observed between the initial and final fasting levels of different groups revealed a significant elevation in blood glucose in the diabetic control group compared to normal [17-19]. It is evident from these investigations that the leaf extract is effectively maintaining the blood glucose levels in normal and STZ induced diabetic rats. Ethanolic extract of *C. spicatus* treated groups III, IV and aqueous solution of *C. spicatus* treated groups V rats showed a significant ($p < 0.00$ 1) increase insulin level when compared with group II as well as group V. STZ, aβ-cytotoxin, induces chemical diabetes in a wide variety of animal species by damaging the insulin-secreting β – cells of the pancreas.

Summary and Conclusion

The present study suggests that the *Costus spicatus* rhizomes extracts had cooperative symptom results disclosed by belittled body fluid lipoid levels, renovated hemoglobin and thus attribute to the therapeutic worth of the *C. spicatus* extracts of flower to combat the diabetic condition in rats [20-22]. Phytochemical examination of critical foundation of value boundaries of the species is additionally significant and drug organizations for the novel medications for the treatment of different sicknesses. Along these lines, improving the strategies for the subjective and quantitative assurance of restorative plants is significant for quality evaluation in the therapeutic plant industry. Among the two doses, 500mg/kg of *C. spicatus* rhizome extract to possess potential antidiabetic activity. Diabetes mellitus is an endocrine, metabolic disorder in which the homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin, ultimately resulting in increased blood glucose. The major side effect of the treatment is assumed to be hypoglycemia. However, it is believed that our research will be helpful for future research like compound isolation and biological activity of the pure compound.

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