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In-vivo assay methods to study the Anti-diabetic activity of ethanolic extract of flower of *Costus spicatus* in diabetic albino Wistar rats

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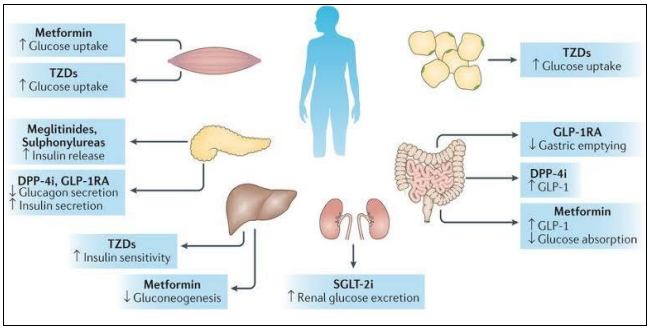
Abstract
The characteristic phytochemical constituents are steroids, tri-terpenoid, Anthraquinone glycosides, proteins are established in the concentrates of *Costus spicatus* flower. Established gentle to direct movement and better anthelmintic action after contrasted with ethanolic separate. Oral administration of effect of ECS to diabetic induced rats at dosage of 500 mg/kg body weight resulted during significant decrease of elevated blood glucose and hepatic transaminase enzyme levels, at different treatment period (0^{th} day, 28^{th} day and 45^{th} day) which also showed the structural changes in cytoarchitecture of STZ induced diabetic rats. (SGOT), (SGPT) and (ALP) levels. Confirmed the effect diabetic medicinal plants in restore in pancreas of ECS in anti-hyperglycaemic activity.

Keywords: *costus spicatus*, *diabetes mellitus*, streptozotocin, pancrease, insulin

Introduction
Diabetes mellitus (DM) is one of the oldest known human diseases and one of the biggest health problems of researchers, especially after the spread among children largely due to unhealthy nutrition and unhealthy lifestyle with less physical activity and the riskiness of this is on the health of individuals and society [1-3]. Persians, Indians, and ancient Egyptians described the symptoms of the disease, but the proper understanding of the situation has developed over the last 100 years. Diabetes has 3 major types: Diabetes mellitus type 1: the result of the destruction of insulin-producing cells [4]. These cells are called beta-cells (β-cells), which are treated with insulin, and diabetes mellitus type 2: which does not depend on insulin and depends on diet and medicines; beta cells secrete insulin, but the inability is to identify and benefit from insulin by the cells and is treated with drugs, including Sulfonylurea in addition to diet and physical activity. In addition, Diabetes may appear during pregnancy and is called Gestational Diabetes [5]. Diabetic quickly turns into a global health problem due to its complications, especially with the high population rate, aging, urbanization, urbanization, increased physical inactivity, and obesity [6-7]. *Punica granatum*, also called (Pomegranate), has been described as a treatment for diabetes in traditional Greek medicine Unani system of medicine.

Mechanism of action of diabetic drugs
The system of different medications metformin would perhaps focus on the liver to downsize gluconeogenesis and skeletal muscles to build up fringe aldohexose use, with a potential job in the gut to expand levels of glucagon-like peptide 1 (GLP-1). Sulfonylurea's and meglitinides increment insulin discharge inside the pancreas. Thiazolidinediones (TZDs) go about as chemical sensitizers in striated muscle, fat tissue and the liver. GLP-1 receptor (GLP-1R) agonists (GLP-1RA) focus on the pipe organ to broaden chemical emission and cut back chemical creation, just as act in the gut to lessen gastric purging [3, 16].



Target organs and action mechanism of and diabetic drugs.

Fig 1: Mechanism of action of diabetic drugs

Nanotechnology can be characterized as the science and designing associated with the combination, plan, portrayal, observing, fixing, development and control of the human organic framework at the atomic level. Nanomedicine is the combination of nanotechnology in medication for better human medical care.
Nano-materials have special physicochemical properties, like high surface to mass proportion, minuscule size, and high reactivity.
These properties can be utilized to conquer the constraints of conventional DM medicines and finding.. The kidney has a vital role in the normal physiology of humans. Its disorders have considered a major cause of disability and in worst circumstances lead to death.

Material Methods

Collection, Identification and Authentication of plant species

Fig 2: 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.

Phytochemical Studies

Secondary metabolites present in the studies were plant sample revealed the presence of medicinally active constituents. Beneficial drugs and to improve the patient health. Phytochemical studies was carried out by using standard procedure [8-11].

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.

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) Reg No: 685/PO/Re/S/2002/) KMCRET/Ph.D/22/2018-19).

Diabetes induction using Streptozotocin

Animals fasted overnight and diabetes was induced by single intra-peritoneal injection of STZ (45mg/kg body weight) prepared in 0.1 M Citrate buffer at pH 4.5. To overcome drug-induced hypoglycemia, animals were allowed to drink a 5% glucose solution overnight. Citrate buffer in place of Streptozotocin was injected to control rats. After 72 hours of STZ injection, (taken as 0^{th} day) fasting blood glucose levels of each animal was analyzed. Animals with the fasting blood glucose levels > 200 mg/dl were considered diabetic and considered used for studies.

Anti-diabetic treatment of animals

The rats were separated into 5 groups and each group consisted of 6 rats and the duration of treatment was 45 days. Group I: Animals fed among the distilled water (negative control). Group II: Diabetic animals fed among the distilled water (positive control). Group III: Diabetic animals fed among the Glibenclamide (5mg/kg/b.w./day). Group IV: Diabetic animals fed among the ECS (300 mg/ kgb.w./day). Group V: Diabetic animals fed among the ECS (500mg/kg/b.w./day). Before (0^{th}), during (28^{st}) and at the end of treatment (45^{th}), body weight, fasting plasma glucose levels, SGOT,SGPT and ALP levels were measured. Plasma glucose levels were determined by Ortho Toluidine reagent method. SGOT, SGPT and ALP levels were measured from serum separated from the blood which was collected from the retro-orbital plexuses of the rats of all groups under light ether anaesthesia using a semiautomatic biochemical analyzer with commercially available biochemical kits.

Collection of tissue samples and histological analysis

After 45 days of treatment, animals were sacrificed following the guidelines of the animal ethical committee. The Pancreas tissues were excised and fixed in 10% neutral buffered formalin (NBF). Thus fixed Pancreas tissues were sectioned with Leica rotary microtome to produce serial sections of 5µm thickness. Pancreas sections were stained with Hematoxylin and Eosin (H&E) stains. The stained specimens were then analyzed and photomicrography with APCAM-5 USB 2digital camera attached to a computer monitor (ADELTAVISION OPTEC India microscope Ltd).

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

Plants have contained in utilized for a long time for human wellbeing. There are as yet numerous plants which have different restorative qualities yet not investigated and utilized. Plants contain frequent novel mixes with therapeutic qualities which require logical investigation. Several chemicals which are derived from plants acts as a drug is currently used in more countries in the world [12].

Preliminary phytochemical screening

India is most likely the greatest creator of restorative flavors on the planet. These days allopathic utilization was

antagonistic responses, so now daily's natural medications use was expanded because of less results and tolerance acknowledgment in these manner home grown medications use was expanded. In the current examination, the endeavour is made to the phytochemical examination of the oil ether and ethyl acetic acid derivation concentrates of *Ipomoea sepiaria* leaves and performed antibacterial, antifungal and anthelmintic exercises [1, 13].

Table 1: Qualitative analysis of Phytochemicals analysis *Costus spicatus* flower extract

S. No	Analysed Phytochemicals factor	Ethanol	Methanol
1.	Tannin	++	+
2.	Phlobatannins	-	+
3.	Saponin	-	+
4.	Flavonoids	+++	++
5.	Steroids	++	-
6.	Terpenoids	+	+
7.	Triterpenoids	+	+
8.	Alkaloids	+++	++
9.	Carbohydrate	+	+
10.	Protein	++	-
11.	Anthraquinone	-	-
12.	Polyphenol	++	+
13.	Glycoside	+	-

Indications: "+" means positive activity, "-" means negative activity

Each constituent assumes a significant part and insufficiency of any one constituent may prompt strange improvements in the body [14-17]. Diabetes mellitus is an endocrine, metabolic problem wherein the homeostasis of carb 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 a solitary intra-peritoneal infusion of STZ at a portion of 45mg/kg b. w. what's more, the histological premise investigation of in hepatoprotective action of ECS 500 mg/kg b. w. is resolved. Diabetes mellitus is related with reformist metabolic insanity, deteriorating glycemic control, and morphological changes in the liver, pancreas and different organs [18]. Liver compounds SGOT, SGPT, ALP are available in high focus in the ordinary hepatocytes of the liver and these catalysts are spilled into the dissemination because of harm to the cell film of hepatocytes. Liver creations of significant job in the observing and settling glucose level so she could stay considered glucostat screen [5, 19].

The outcomes showed that infusion of alloxan actuates hepatocellular harm, which remains demonstrated by a huge expansion in AST, ALT, and ALP in the diabetic gathering when contrasted with control bunches [20]. Raised levels showed that AST, ALT, and ALP hindered liver capacity [15, 22]. STZ-initiated diabetes is portrayed by an in unembellished loss of body weight.

Glibenclamide is routinely utilized as a standard enemy of diabetic medication in STZ-instigated moderate diabetes to be contrasted and an assortment of hypoglycemic mixtures and its proficiency is perceived [23]. The rise of liver biomarker catalysts, for example, AST, ALT, and ALP in diabetic control rodents shows that diabetes may actuate hepatic dysfunction [21].

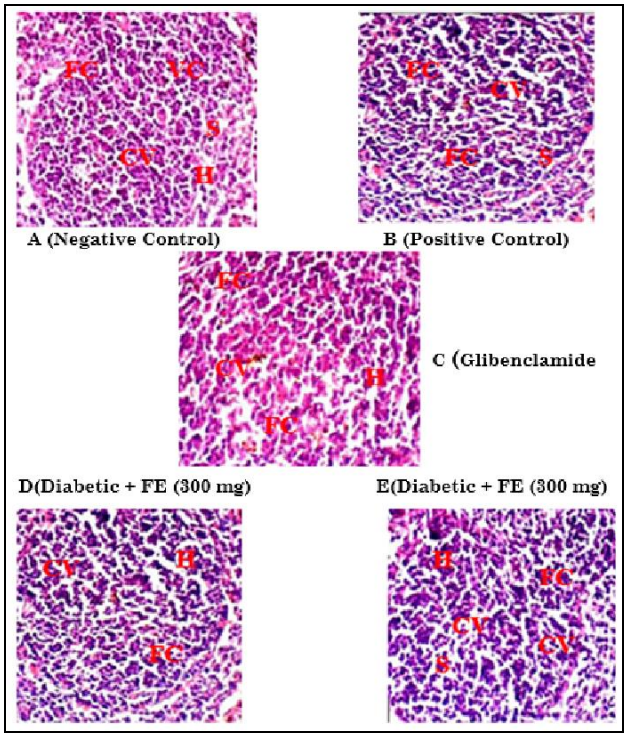


Fig 3: Cv-Central Vein; Vc-Vacuolation; Fc-Fatty Changes; H-Hepatocyte; S- Sinusoids

Figure 1A: Photomicrograph of Pancreas of normal control rats show clear central vein, well-arranged hepatocytes and sinusoids. (H & E magnification X100)

Figure 1B: Photomicrograph of Pancreas of STZ induced diabetic rat shows congested central vein, fatty degeneration and cytoplasmic vacuolation. (H & E magnification X100)

Figure 1C: Photomicrograph of Pancreas of diabetic rat treated with Glibenclamide (5 mg/kg b.w) shows restoration of hepatocytes structure, clear sinusoids and reduction in fatty degeneration. (H & E magnification X100).

Figure 1D: Photomicrograph of Pancreas of diabetic rats treated with ECS (300 mg/kg b.w) shows well-arranged hepatocytes in between sinusoids, with a clear central vein. (H & E magnification X100)

Figure 1E: Photomicrograph of Pancreas of diabetic rat treated with ECS (500 mg/kg b.w.) shows restoration of hepatocytes structure to near normal, still little congestion of central vein seen. (H & E magnification X100)

Table 2: Effect of ECS on body weight in normal & STZ induced diabetic rats

Groups	Change in Bodyweight (gm)		
	0st day	28th day	45thday
Group I	161±2.58	84.66±2.41	194.16±2.98
Group II	180.66±2.13**	160.83±1.47**	124.33±1.96**
Group III	171.33±2.15#	166.33±2.44**	187.16±1.97**
Group V	192±2.78#	180.66±2.21**	182.50±1.45**
Group IV	173.16±1.60	183.50±2.14**	176.66±1.60**

Results are expressed as mean ±SEM; n=6; **=p<0.001 and# =not significant

Table 3: Effect of ECS on plasma glucose values in normal & experimental rats

Groups	Change in Bodyweight (gm)		
	0st day	28th day	45th day
Group I	95.16±2.12	94.33±1.76	95.5±2.12
Group II	276.33±8.80**	335±11.07**	377.83±11.85**
Group III	262.66±8.53#	194±7.10**	120.5±2.95**
Group IV	267.70±0.76	101.5±1.47**	95.16±0.10**
Group V	263.50±7.02#	96.5±10.67**	91.5±2.39**

Results are expressed as mean ±SEM;n=6; **=p<0.001 and # =not significant

Table 4: Effect of ECS on SGOT, SGPT and ALP levels in normal & experimental rats

Groups	SGOT (IU/L)		SGPT (IU/L)		ALP (IU/L)	
	0 day	45th day	0 day	45th day	0 day	45th day
Group I	62.01±3.40	61.58±1.41	74.33±0.63	75±1.34	74±1.23	76.33±0.81
Group II	152.45±2.63**	222±3.50*	246.87±2.05**	144.57±6.67**	139.68±1.56**	205±1.16**
Group III	140.37±1.67#	101±1.28**	94.86±1.07**	142.33±1.88#	143.31±1.77#	91.4±1.52
Group IV	131.15±0.63	157.33±0.62**	124.89±54.3**	127.85±0.08	120.85±0.69	110.3±0.96**
Group V	108.37±3.73#	85±1.92#	106±2.17**	73.66±2.35#	124.59±2.33#	80.17±0.81**

Results are expressed as mean ± SEM; n=6; ** =p<0.001 and # = not significant

The fasting plasma glucose levels were significantly from increased in STZ induced diabetic rats, which was significantly (p<0.001) reduced by 45 days of treatment for ECS (Table 3). In STZ induced diabetic rats, ECS treatment significantly (p<0.001) increased body weight (Table 2) [16]. These results showed that decreased plasma glucose levels may be correlated with decreased gluconeogenic activity. Which may be the reason for an increase in body weight in ECS and glibenclamide treated diabetic rats. The raised degrees of SGOT, SGPT in serum are a sign of harmed liver tissue. Organization of ECS improves the liver capacity by diminishing the degrees of SGOT, SGPT in diabetic treated rodents, showing its hepatoprotective impact. Snow-capped mountain goes about as a marker for biliary capacity [8]. Decrease in ALP levels in ECS treated diabetic rodents further to approve its hepatoprotective impact [21, 23]. Treatment of ordinary rodents with ECS kept up the degrees of hepatic proteins consequently showing its non-harmful nature. Treatment for glibenclamide it's re-established the more ordinary design of liver tissue in STZ diabetic rodents, yet showed the presence of vascular clog of focal vein and few hepatocyte cores vocalizations [24-25]. These histopathological changes got in our investigation like Cassia auriculate. Expanded degrees of SGPT and SGOT were seen in the diabetic actuated rodents, the occurrence of heart and liver sickness. Since SGPT and SGOT levels are markers of pancreas capacity. There by the rebuilding of their levels demonstrate typical working as the pancreas. The blossom remove from treated rodents showed no huge change, contrasted and control rodents subsequently demonstrating the non-harmfulness of the plants.

Conclusion

The potential anti-diabetic activity of *C. spicatus*, flower may be due to the phytochemicals flavonoids, terpenoids, etc. present in *C. spicatus*, flower. Hence, it would facilitate in preventing diabetic complications and is an honest adjuvant within the gift assemblage of anti-diabetic medication. Diabetic therapeutic worth 300mg not needed and pancreas of diabetic rodent treated with ECS (500 mg/kg b.w.) shows reclamation of hepatocytes construction to approach typical, still little clog of focal vein seen. Huge decrease of raised blood glucose and hepatic transaminase compound levels, at various treatment period (0th day, 28st day and 45th day). The two ECS and Glibenclamide of STZ incited diabetic creatures reestablished the typical plasma glucose levels and SGOT, SGPT and ALP levels. In any case, ECS re-established the ordinary plasma glucose and SGOT, SGPT and ALP levels without harming the pancreas. The current investigation builds up the viability of low portion in diabetics and histopathological of studies.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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