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PHYTOCHEMICALS ANALYSIS OF ANTI-DIABETIC EFFECT OF *Costus spicatus* IN STREPTOZOTOCIN-INDUCED DIABETIC ALBINO WISTAR MALE RATS

Azhagu Madhavan S*, Ganesan S,
PG & Research Deparment of Zoology & Biotechnology,
A Veeriya Vandayar Memorial Sri Pushpam College, (Autonomous) Poondi,
Thanjavur, 613503, Tamil Nadu, India.
Email: mathavan062@gmail.com
Corresponding author ---Azhagu Madhavan S[a]*,

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Abstract:
The medicine is prescription properties arsenic hurting, tonic, diuretic, uterotonic and sterility in women and leaves is shown particularly in diabetes. Phytochemical screening of different concentrates of *Costus spicatus* exposed the presence and non-attendance of various phytochemicals are available in ethyl acetate remove, further examinations were done with rhizome of *Costus spicatus* ethyl acetate separate. *Costus spicatus* (ECS) in streptozotocin (STZ) 45mg/kg body weight induced diabetic rats was studied. Oral administration of effect of ECS to diabetic induced rats at a dose of 500 mg/kg body weight resulted in significant reduction of elevated blood glucose and hepatic transaminase enzyme levels, at different treatment period (0th day, 28st day and 45th day) which also showed the structural changes in cytoarchitecture of STZ induced diabetic rats. (SGOT), (SGPT) and (ALP) levels. Further of histopathology results of ECS treated rats also confirmed the significant recovery of pancrease damage to normalcy ($p<0.001$), with simultaneous increase in body weight. Confirmed the effect of ECS in antihyperglycemic effect in STZ-induced diabetic rats.

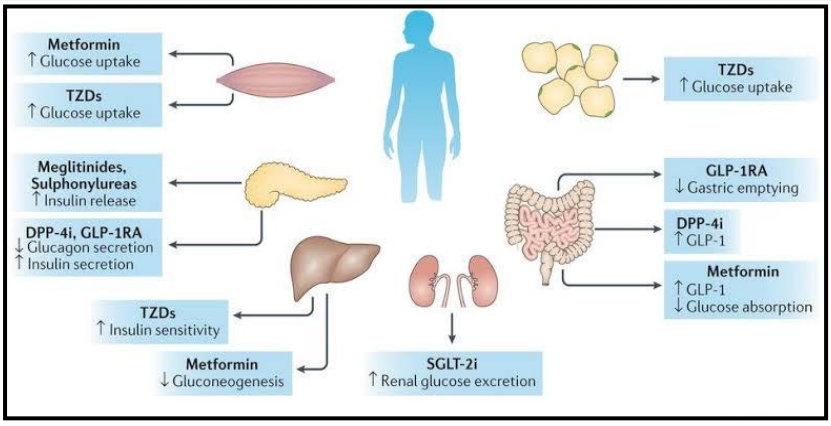
Keywords: Anti-hypolipidemic, diabetes mellitus, peripheral insulin resistance, pancreatic, STZ.

1.INTRODUCTION

Diabetes derives its source from a Greek word 'diabaino' which means to go through and "Mellitus" means sweet or sugar. Diabetes mellitus (DM) is one of the mainly important and major epidemic public health challenges of the twenty-first century worldwide [15,7]. Diabetes mellitus, more basically called diabetes, is a chronic condition in the occurs are raised levels of glucose in the blood because. The body cannot produce any or sufficient of the hormone insulin or make use of insulin effectively. It is an upset of multiple etiologists distinguished by a failure of aldohexose equilibrium with disturbances of macromolecule, fat and super molecule metabolism as a result of defects in insulin secretion and/or insulin action [6].

2.MECHANISM OF ACTION OF DIABETIC DRUGS

The mechanism of various drugs. Metformin would possibly target the liver to scale back gluconeogenesis and skeletal muscles to reinforce peripheral aldohexose utilization, with a possible role in the gut to increase levels of glucagon-like peptide 1 (GLP-1). Sulfonylurea's and meglitinides increase insulin secretion within the pancreas. Thiazolidinediones (TZDs) act as hormone sensitizers in striated muscle, adipose tissue and the liver. GLP-1 receptor (GLP-1R) agonists (GLP-1RA) target the duct gland to extend hormone secretion and cut back hormone production, as well as act in the gut to reduce gastric emptying [3,16].



Target organs and action mechanism of and diabetic drugs.

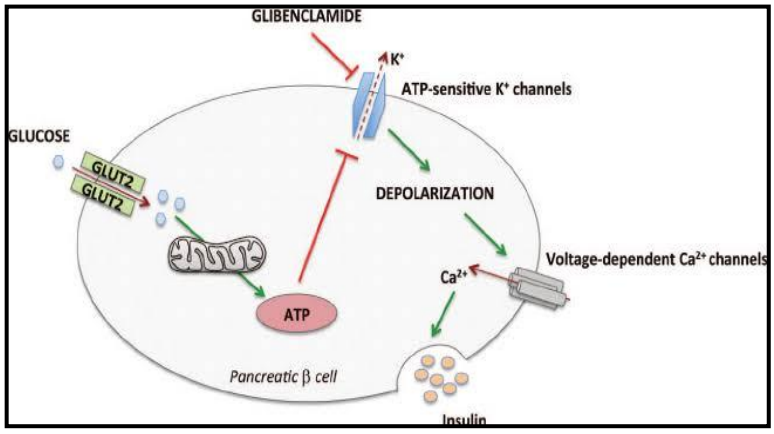
Fig 1: Mechanism of action of diabetic drugs

3.DIABETES INDUCED BY STREPTOZOTOCIN

Diabetes is regularly inspired by streptozotocin (STZ), glucosamine–nitrosourea compound got from actinomycete chromogens that territory unit utilized clinically as a chemotherapeutical specialist inside the therapy of conduit organ β cell malignant growth. STZ harms pancreatic β cells, diminishing the insulin level which further prompts hyperglycemia [9,17]. The enlistment of diabetics by STZ is relied upon the dosages. The beta cells selectivity is related with particular collection of the synthetic substances after section through the GLUT2 glucose carrier receptor: compound underlying closeness with glucose permits STZ to tie to this receptor [18]. High portions are normally given separately; STZ targets β cells by its alkylating property relating to that of cytotoxic nitrosourea mixes. Low dosages are by and large given in various openings, STZ evokes a safe and fiery response, probably identified with the arrival of glutamic corrosive decarboxylase autoantigens which thusly brings about the pulverization of β cells and acceptance of the hyperglycemic state. This is typically connected with fiery penetrates remembering lymphocytes for the pancreatic islets. The antagonistic results of STZ incorporate hepatotoxicity and nephrotoxicity [9,11].

4.GLIBENCLAMIDE

Physiologically, glucose deluge into pancreatic cells incites ATP blend through glycolysis and mitochondrial breath with ensuing K+ channel conclusion. In sort 2 diabetes mellitus patients, glibenclamide straightforwardly closes ATP-touchy K+ channels, diminishing layer potential and instigating calcium flood, which thus animates insulin emission. The kinds of harmfulness tests that region unit much of the time performed by drug producers inside the examination of a spic and span drug include intense, sub-intense and ongoing poisonousness. Intense harmfulness is engaged with the assessment of LD50 the portion which has end up being deadly (prompting demise) to half of a tried gathering of creatures. The motivation behind intense oral harmfulness is normally a unique screening step in the appraisal and assessment of the poisonous qualities, everything being equal [3]. Oral glucose resistance test (OGTT) is a generally utilized methodology in the ID of diabetes and middle of the road phases of hyperglycemia. In the diabetic condition, OGTT sets up the malabsorption of glucose related with insulin emission and insulin obstruction [6].



Glibenclamide-dependent secretion of insulin in pancreatic β-cells.

Fig 2: Glibenclamide

5.MEDICINAL PLANTS AND THEIR THERAPEUTIC VALUES

Therapeutic plants are found to fix different illnesses. Ayurveda was considered as a non-genuine treatment strategy, generally controlled by undeveloped doctors. Nonetheless, the investigation of phytochemicals (liable for restorative qualities in therapeutic plants) in some conventional Indian Ayurveda details has set up the viability of spices in the treatment of different physical and mental infirmities [5]. About 80% of the total populace rely upon conventional solutions for their medical care needs. Universally, around 70,000 to 80,000 plant species are utilized for restorative or sweet-smelling purposes. India is maybe the most extravagant country with a huge home grown restorative abundance in light of its environmental, topographical and climatic varieties [21]. Diabetes is a sickness brought about by a lopsidedness between glucose retention and insulin discharge. Postprandial indication assumes a crucial job in the advancement of the polygenic sickness. Directing plasma glucose level is essential for postponing or forestalling diabetes. [14,17]. Helpful methodologies for diminishing postprandial hyperglycemia [22]. The utilization of sugar processing compound inhibitors assumes an essential job in controlling hyperglycemia by lessening the intestinal assimilation of glucose [16]. A powerful method for bringing down the degrees of postprandial hyperglycemia has been offered by - amylase and - glucosidase inhibitors [19]. The treatment objective of diabetic patients is to keep up close typical degrees of glycemic the board, in each quick and post-prandial condition. Numerous normal sources are examined with respect to the concealment of aldohexose creation from the starches inside the gut or aldohexose assimilation from the gut [18]. In past reports, therapeutic plants are having acceptable organic movement against numerous infections. Accordingly, in this examination, we chose the restorative plant of *Costus spicatus*. To realize their phytochemical mixes, free extremist searching and hostile to diabetic movement by utilizing in vitro models.

6.MATERIALS AND METHODS

6.1Collection, Identification and Authentication of plant species:

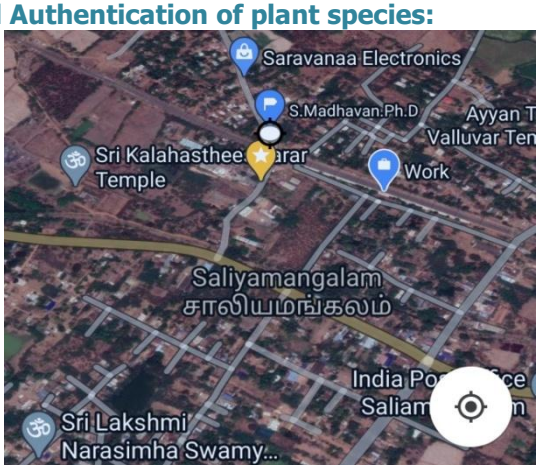


Fig: 3. Map 1: Study area

The plant, *Costus spicatus* were collected from the Saliyamangalam and Thanjavur district, Tamilnadu, India. It was taxonomically identified and authenticated by Rev Dr S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamilnadu, India. The voucher specimens are deposited at the Rapinat herbarium and the voucher number is SAM 001.

1.3. Phytochemical Studies

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

6.2Preparation Of Extracts

The powdered plant samples of rhizome (100 g) 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.

6.3Animal

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 theCommittee for the Purpose of Control and Supervision of experiments on Animal (CPCSEA). Institutional Animal EthicsCommittee (IAEC) RegNo:685/PO/Re/S/2002/) KMCRET/Ph.D/22/2018-19).

6.4Diabetes induction using streptozotocin

Animals fasted overnight and diabetes was induced by single intraperitoneal 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 among the fasting blood glucose levels > 200 mg/dl were considered diabetic and considered used for studies.

6.5ANTI-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.

6.6COLLECTION 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 photomicrographed with APCAM-5 USB 2digital camera attached to a computer monitor (ADELTAVISION OPTEC India microscope Ltd).

7.STATISTICAL ANALYSIS

The obtained data were analyzed using the SPSS program, version 24. Data were figured as mean ± SE (for data of the biological study n=10, where for data of the *in vitro* study 3 replicates were used). ANOVA test was used to compare results among groups and $P < 0.05$ was significant.

8.RESULT

Plants and their items have been 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 numerous novel mixes with therapeutic qualities which need logical investigation. Several chemicals which are derived from plants acts as a drug is currently used in more countries in the world.

9.PRELIMINARY PHYTOCHEMICAL SCREENING

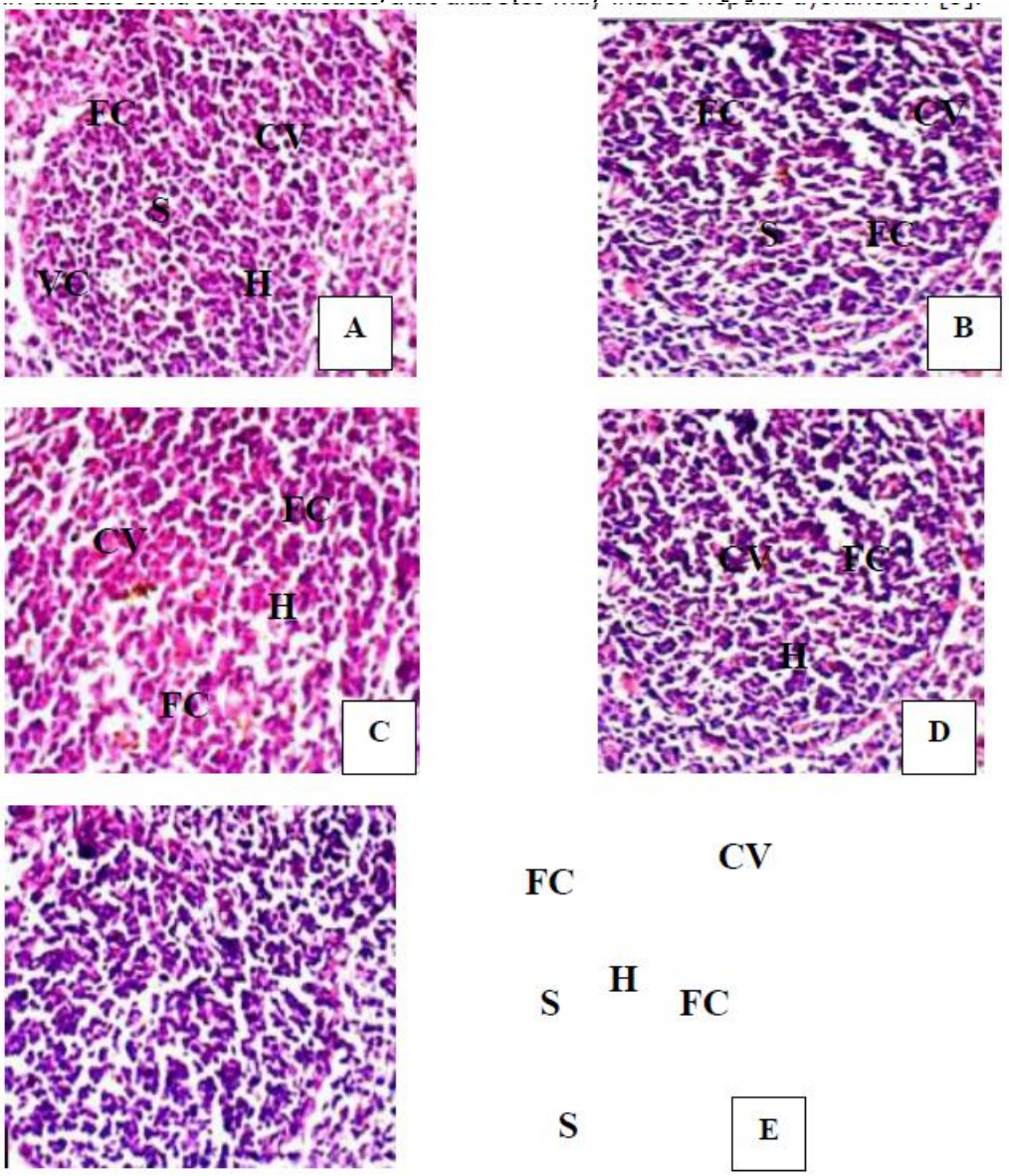
India is most likely the greatest creator of restorative flavors on the planet. These days allopathic framework utilization was diminished because of results, antagonistic responses, so now daily's natural medications use was expanded because of less results and tolerance acknowledgment in these manner home grown medications use was expanded. In the current examination, the endeavor 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]. The characteristic phytochemical constituents are steroids, triterpenoid, Anthraquinone glycosides, proteins are establish in the concentrates of *costus spicatus* rhizome. Demonstrated gentle to direct movement and better anthelmintic action when contrasted with ethyl acetate separate.

Table: 1. Qualitative analysis of Phytochemicals analysis *Costus spicatus rhizome* extract

S. No	Analysed Phytochemicals factor	Ethyl acetate	Petroleum ether
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

Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body [12]. 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. In our study, diabetes was induced in rats by a single intraperitoneal injection of STZ at a dose of 45mg/kg b.w. and the histological basis study of in hepatoprotective activity of ECS 500 mg/kg b.w. be determined. Diabetes mellitus is associated with progressive metabolic derangement, worsening glycemic control, and morphological changes in the liver, pancreas and other organs [13]. Liver enzymes SGOT, SGPT, ALP are present in high concentration in the normal hepatocytes of the liver and these enzymes are leaked into the circulation as a result of damage to the cell membrane of hepatocytes. Liver productions of important role in the monitoring and stabilizing glucose level so she could remain considered glucostat monitor [5,19]. The results showed that injection of alloxan induces hepatocellular damage, which remains indicated by a significant increase in AST, ALT, and ALP in the diabetic group as compared to control groups. Elevated levels showed that AST, ALT, and ALP impaired liver function [15,22]. STZ-induced diabetes is characterized by an inaunembellished loss of body weight. Glibenclamide is regularly used as a standard antidiabetic drug in STZ-induced moderate diabetes to be compared with a variety of hypoglycemic compounds and its efficiency is recognized [23] The elevation of liver biomarker enzymes, such as AST, ALT, and ALP in diabetic control rats indicates that diabetes may induce hepatic dysfunction [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)**		
	0 day	28st day	45 stday
Group I	161±2.58	85.66±2.41	195.16±2.98
Group II	181.66±2.13**	160.83±1.47**	125.33±1.96**
Group III	171.33±2.15#	167.33±2.44**	188.16±1.97**
Group V	191±2.78#	180.66±2.21**	183.50±1.45**
Group IV	174.16±1.60	184.50±2.14**	177.66±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)**		
	0 day	28st day	45 day
Group I	96.16±2.12	95.33±1.76	95.5±2.12
Group II	277.33±8.80**	336±11.07**	378.83±11.85**
Group III	263.66±8.53#	195±7.10**	121.5±2.95**
Group IV	268.70±0.76	101.5±1.47**	96.16±0.10**
Group V	264.50±7.02#	97.5±10.67**	92.5±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	**45 th day**	**0 day**	**45 th day**	**0 day**	**45 th day**
Group I	63.01±3.40	61.58±1.43	75.33±0.66	76±1.34	75±1.24	77.33±0.82
Group II	153.45±2.64**	223±3.50*	247.87±2.05**	145.57±6.67**	140.68±1.57**	206±1.17**
Group III	141.37±1.67#	101±1.28**	95.86±1.07**	143.33±1.89#	144.31±1.78#	92.4±1.53
Group IV	132.15±0.65	158.33±0.63**	125.89±55.3**	128.85±0.08	120.85±0.70	110.3±0.97**
Group V	109.37±3.77#	86±1.94#	107±2.17**	74.66±2.37#	125.59±2.34#	80.17±0.82**

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 elevated levels of SGOT, SGPT in serum are an indication of damaged liver tissue, Administration of ECS improves the liver function by decreasing the levels of SGOT, SGPT in diabetic treated rats, indicating its hepatoprotective effect. ALP acts as a marker for biliary function [8]. Reduction in ALP levels in ECS treated diabetic rats further to validate its hepatoprotective effect [21,23]. Treatment of normal rats with ECS maintained the levels of hepatic enzymes thereby showing its non-toxic nature. Treatment for glibenclamide it's restored the more normal architecture of liver tissue in STZ diabetic rats, but showed the presence of vascular congestion of central vein and few hepatocyte nuclei vocalizations [23]. These histopathological changes obtained in our study similar to *Cassia auriculate*. Increased levels of SGPT and SGOT were observed in the diabetic

induced rats, the incidence of heart and liver disease. Since SGPT and SGOT levels are markers of liver function .there by the restoration of their levels indicate normal functioning as the liver. The rhizomes extract from treated rats showed no significant change, compared with control rats thereby proving the nontoxicity of the plants.

10.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 rhizomes to combat the diabetic condition in rats. Phytochemical examination of critical functions in ID, verification, and 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 anti-diabetic activity. The potential anti-diabetic activity of *C. spicatus*, rhizomes may be due to the phytochemicals flavonoids, terpenoids, etc. present in *C. spicatus*, rhizomes. Hence, it would facilitate in preventing diabetic complications and is an honest adjuvant within the gift assemblage of anti-diabetic medication. Both ECS and Glibenclamide of STZ induced diabetic animals restored the normal plasma glucose levels and SGOT, SGPT and ALP levels. But ECS restored the normal plasma glucose and SGOT, SGPT and ALP levels without damaging the pancreas. The present study establishes the efficacy of low dose rifabutin in diabetics and other associated cardiovascular symptoms ascertained and histochemical of studies. 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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