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Hepatoprotective and antidiabetic effect of aqueous extract of *Costus spicatus* jacq. Rhizome extract in streptozotocin induced diabetic Rats –histological study

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ABSTRACT

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 single intraperitoneal injection of Streptozotocin (STZ) at a dose of 45mg/kgb.w. and the hepatoprotective efficacy of aqueous extract of *Costus spicatus* (ACS) at a dose of 500 mg/kg b.w. was studied. Diabetes mellitus is associated with progressive metabolic derangement, worsening glycemic control, and morphological changes in the liver, pancreas and other organs. Diabetes is characterized by high blood plasma glucose levels and high concentration of liver enzymes like SGOT, SGPT and ALP in hepatocytes of the liver leaks into the circulation as a result of damage to cell membrane of hepatocytes. In the present study, administration of ACS to STZ induced diabetic animals restored the blood plasma glucose and liver enzymes to normal level which is comparable to the anti-diabetic efficacy of Glibenclamide a synthetic drug. Though both showed its anti-diabetic efficacy, ACS exerted its anti-diabetic activity without damaging the liver in comparison with Glibenclamide which damaged the liver.

Keywords: Hepatoprotective, Insulin, Diabetes mellitus, Pancreatic hormone, STZ.

INTRODUCTION

Diabetes mellitus (DM) has shown an exponential rise of causing serious economic, social and health repercussions. Diabetic retinopathy (DR), the most common and serious complication of DM, are characterized by vascular alterations including retinal blood flows changes, endothelial cells dysfunction, breakdown of the blood-retinal barrier, ischemia and neovascularisation. DR has no or mild symptoms at early stages; however, if not

properly treated, DR may progress to the advanced stage, during which severe pathologies often lead to irreversible blindness (Cheung *et al*., 2010; Antonetti *et al*., 2012; RaskpMadsen *et al*., 2013).

Diabetes is becoming the third killer disease of mankind, after cancer and cardiovascular disease, because of its high prevalence, morbidity and mortality (Li *et al*., 2004). Diabetes is the fastest growing chronic disease in the world; the number of diabetic patients is increasing, almost half of all deaths before the age of 70 have been attributed to high blood glucose levels associated with diabetes (WHO, 2016). Diabetes is being treated using synthetic drugs to achieve euglycemia and eliminating or minimizing the chronic complications as well. But the usage of synthetic anti-diabetic drugs is associated with lot of side effects (Holman 1991).

Herbal medicines have received more attention than synthetic drugs for the treatment of various human diseases including diabetes due to their less or nil side effects and cost effectiveness (Wachtel-Galor 2011). Nowadays, treating diabetes using medicnal herbs gaining its ground at a fast pace. However, the mechanism of action of anti-diabetic activity of medicinal herbs has not yet been clearly established. It has been proposed that the hypoglycemic effect of these medicinal herbs is due to their ability to restore or stimulate the function of pancreatic tissues or β-cells thereby causing an increase in insulin level or inhibition of intestinal absorption of glucose.

Costus igneus is also known as fiery *costus* or spiral flag or insulin plant belongs to the *costaceae* family, contains a range of phytochemicals which include flavonoids, alkaloids, terpenoids and it is traditionally being used in India to control diabetes (Devi *et al*., 2008; Saraswathi *et al*., 2010; Bhat *et al*., 2010; Shetty *et al*., 2010 a,b; Krishnan *et al*., 2011; Pazhanichamy *et al*., 2011). The present study aimed to elucidate the anti-diabetic activity of *Costus igneus* and the mechanism by which it exerts its anti-diabetic activity in Streptozotocin induced diabetes rat models.

MATERIALS AND METHODS

Animal

Albino wistar male rats; 10- weeks old with a body weight ranged between 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 Institutional Animal Ethics Committee and all experiments were conducted according to the Indian National Science Academy guidelines for the use and care of the experimental animals.

Plant collection and extraction

Costus spicatus were collected from Saliyamangalam, Thanjavur District, Tamil Nadu, India. Rhizome were cut into small pieces and shade dried at room temperature. The dried rhizome was subjected to size reduction to a coarse powder by using dry grinder and sieved. About 100 g was continuously extracted with ethanol (95%) using Soxhlet extractor up to 48 h. The extract was filtered through Whatmann filter paper and concentrated using rotary evaporator at 40-60ºC under reduced pressure to prepare final crude extract.

Diabetes induction using Streptozotocin

Animals were fasted overnight and diabetes was induced by single intra peritoneal injection of Streptozotocin (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 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 fasting blood glucose levels > 200 mg/dl were considered as diabetic and considered for the study.

Anti-diabetic treatment of animals

The rats were randomly divided into 5 groups and each group consisted of 6 rats and the duration of treatment was 45 days. Group I: Animals fed with distilled water (Negative control). Group II: Diabetic animals fed with distilled water (positive control). Group III: Diabetic animals fed with Glibenclamide (5mg/kg/b.w./day). Group IV: Non-diabetic animals fed with ACS (500 mg/kgb.w./day). Group V: Diabetic animals fed with ACS (500mg/kg/b.w./day). Before (0^{th}), during (21^{st}) and at the end of treatment (45^{th}), body weight, fasting plasma glucose levels and serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) levels were measured. Plasma glucose levels were determined by Ortho Toludine reagent method. SGOT, SGPT and ALP levels were measured from serum separated from 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 animal ethical committee. The liver tissues were excised and fixed in 10% neutral buffered formalin (NBF). Thus fixed liver tissues were sectioned with Leica rotary microtome to produce serial sections of 5µ thickness. Liver 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).

Statistical Analysis

One-way analysis of variance (ANOVA) was performed using the software "Graphpad Instat". Results were expressed as mean ± SEM. $p<0.05$ was considered as statistically significant.

RESULTS

The administration of STZ resulted in a significant increase in plasma glucose level, SGOT, SGPT and ALP along with a reduction in body weight (Table 1, 2 & 3). After treatment of animals with 500 mg/kg/ b.w of ACS, the plasma glucose levels significantly reduced and returned to normalcy ($p<0.001$), with simultaneous increase in body weight (Table 1 & 2).

Table 1: Effect of ACS on body weight in normal & STZ induced diabetic rats

Groups	Change in Body weight (gm)		
	0 day	21st day	45 st day
Group I	167±2.58	86.66±2.41	197.16±2.98
Group II	185.66±2.13**	164.83±1.47**	127.33±1.96**
Group III	174.33±2.15#	170.33±2.44**	189.16±1.97**
Group IV	178.16±1.60	186.50±2.14**	180.66±1.60**
Group V	195±2.78#	183.66±2.21**	185.50±1.45**

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

Table 2: Effect of ACS on plasma glucose values in normal & STZ induced diabetic rats

Groups	Change in Body weight (gm)		
	0 day	21st day	45 st day
Group I	99.16±2.12	97.33±1.76	96.5±2.12
Group II	279.33±8.80**	339±11.07**	379.83±11.85**
Group III	266.66±8.53#	197±7.10**	123.5±2.95**
Group IV	89.70±0.76	89.5±1.47**	89.16±0.70**
Group V	267.50±7.02#	199.5±10.67**	127.5±2.39**

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

Table 3: Effect of ACS on SGOT, SGPT and ALP levels in normal & STZ induced diabetic 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	65.01±3.40	62.58±1.43	77.33±0.66	77±1.34	77±1.24	79.33±0.82
Group II	154.45±2.64**	225±3.50*	249.87±2.05**	147.57±6.67**	144.68±1.57**	209±1.17**
Group III	142.37±1.67#	103±1.28**	97.86±1.07**	145.33±1.89#	146.33±1.78#	95.4±1.53
Group IV	46.25±0.65	61.33±0.63**	67.89±55.3**	78.85±0.08	78.87±0.70	79.3±0.99**
Group V	141.37±3.77#	177±1.94#	76±2.17**	145.66±2.37#	147.60±2.34#	81.17±0.85**

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

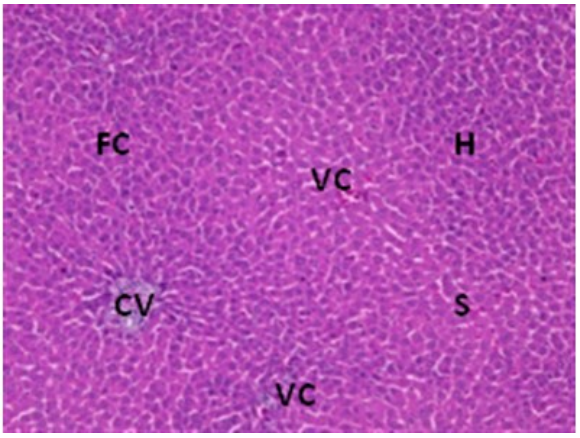
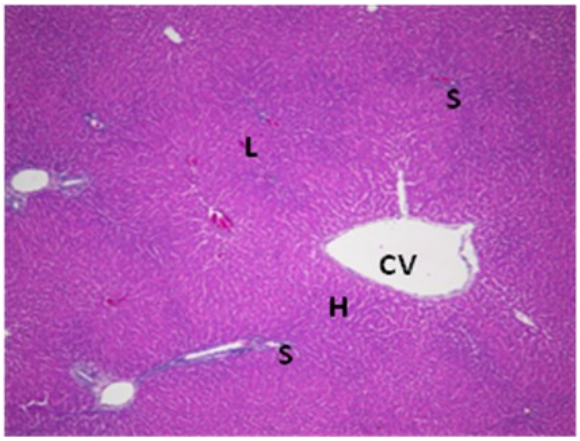


Figure (1A)



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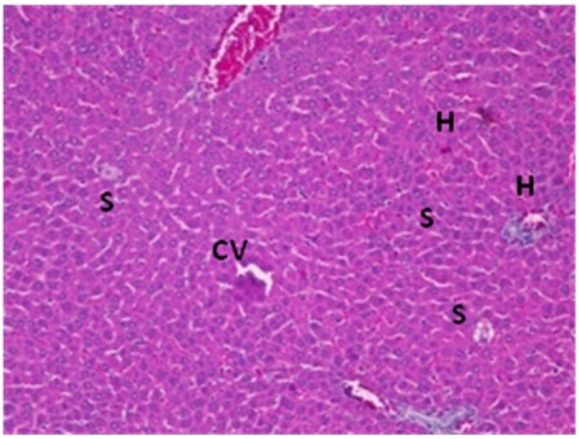

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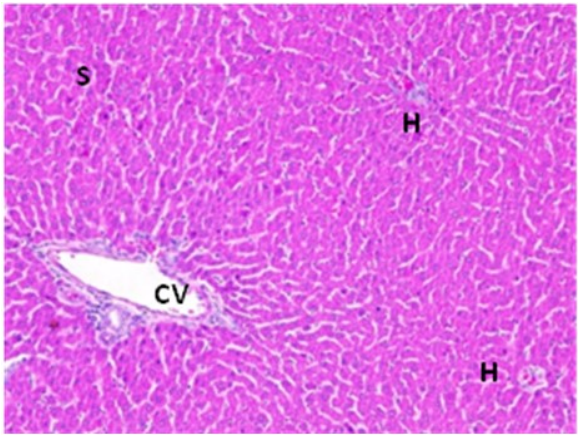

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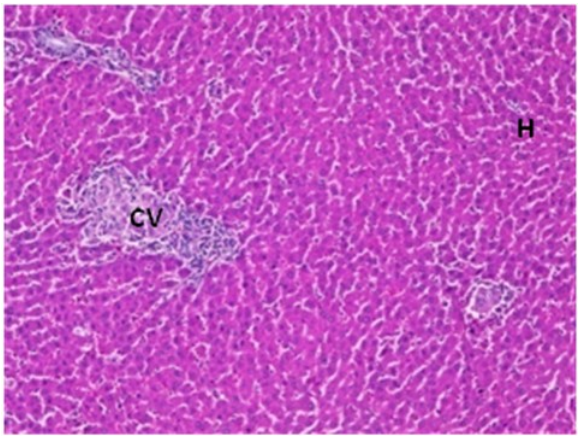

Figure (1E)

Cv-Central Vein ; **Vc**-Vacuolation ; **Fc-**Fatty Changes ; **H**-Hepatocyte ; **S-** Sinusoids

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

Figure 1B: Photomicrograph of liver of normal control rat shows clear central vein, well arranged hepatocytes and sinusoids. (H& E magnification X100)

Figure 1C: Photomicrograph of liver of normal rats treated with ACS(500mg/kg b.w) shows well arranged hepatocytes in between sinusoids, with clear central vein. (H& E magnification X100)

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

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

The elevated SGOT, SGPT, ALP levels in STZ induced diabetic animals were significantly reduced ($p<0.001$) in comparison with diabetic control (positive control) and Glibencl-amide treated groups (Table 3). Meanwhile non-diabetic animals (group IV) treated with ACS 500 mg/kg b.w. showed no disturbances in the levels of plasma glucose, SGOT, SGPT, ALP in comparison with negative controls.

Liver histopathology of treated animals

Examination of the stained sections of the liver of STZ induced diabetic rats revealed necrotic changes including nucleus and cytoplasmic vacuolation, hepato-cytes and sinusoids fragmentation, vascular congestion of the central vein and fatty degeneration (Fig.1.A). The negative controls (Fig.1.B) and non-diabetic ACS treated (group IV) animals (Fig.1.C) showed normal cyto-architecture of liver tissue with clearly defined hepatocytes around the central vein and well arranged sinusoids between the hepatic plate of cells. Diabetic rats treated with ACS (group V) and with Glibenclamide (group III), also showed the normal restoration of liver cyto-architecture (Fig.1 D & E) which was almost similar to control group of rats.

DISCUSSION

In our study, diabetes was induced in rats by single intraperitoneal injection of STZ at a dose of 45mg/kgb.w. and the anti-diabetic activity of *Costus spicatus* and its effect on liver histology and enzymes of liver function was studied. Diabetes mellitus is associated with progressive metabolic derangement, worsening glycemic control, and morphological changes of in the liver, pancreas and other organs (Cook *et al*., 2005; Cristina *et al*., 2008). In diebetic individuals liver enzymes such as SGOT, SGPT and ALP are present in higher concentrations in the normal hepatocytes of the liver and these enzymes are leaked into the circulation as a result of damage to cell membrane of hepatocytes (Ahsan *et al*., 2009).

The present study found that, ACS administration to STZ induced diabetic animals significantly reduced the abnormal fasting blood glucose level which was comparable to control. This decreased plasma glucose levels may be correlated with decreased gluconeogenic activity (Oliveira *et al*., 2005) which may be the reason for weight gain in ACS and Glibenclamide treated diabetic animals (Pandikumar *et al*., 2009). The elevated levels of SGOT, SGPT in serum are an indication of damaged liver tissue, administration of ACS 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 (Kumar *et al*., 2008). Reduction of ALP levels comparable with control in ACS treated diabetic animals further confirms its hepatoprotective effect.

In ACS treated non-diabetic animals the levels of hepatic enzymes were not disturbed which reveals the non-toxic nature of ACS (Godam *et al*., 2014). Though Glibenclamide treatment restored the normal levels of liver enzymes like ACS, but liver tissue showed the presence of vascular congestion of central vein and few hepatocyte nuclei vocalizations.

In conclusion, both ACS and Glibenclamide of STZ induced diabetic animals restored the normal plasma glucose levels and SGOT, SGPT and ALP levels. But ACS restored the normal plasma glucose and SGOT, SGPT and ALP levels without damaging the liver.

Conflicts of interest: The authors stated that no conflicts of interest.

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