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Research article

Evaluation of hypoglycemic activity of *Costus igneus* extract (whole plant) on alloxan induced diabetic rats

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Abstract

Our present study was to evaluate hypoglycemic activity of *Costus igneus* (whole plant) ethanolic extract on alloxan induced diabetic albino wister strain rats. The ethanolic extract from whole plant of *Costus igneus* (ECI) was prepared and subjected to preliminary photochemical analyses to know the presence of alkaloids, flavanoid, phenolic compounds and steroids.Normal as well as diabetic rats was divided into groups (n=6) receiving different treatments i.e vehicle(diabetic control), standard drug, ethanolic extract of *Costus igneus*. Blood samples was collected and analyzed for fasting blood glucose levels (BGL) and lipid profile on days 0,7, and 14. Diabetic rats treated with Glibenclamide (10mg/kg) decreased blood glucose level from 245.66 ± 2.40 mg/dl to 95.00 ± 1.34 mg/dl (55.33%). Diabetic rats treated with ethanolic extract of *Costus igneous* (ECI) 250mg/kg showed decreased blood glucose levels from 243.83 ± 3.4mg/kg to 90.17 ± 5.04 mg/kg (50.46 %) at the end of 14th day of treatment indicating good hypoglycemic activity. *Costus igneous* ethanolic extract treated animals also showed decrease in serum cholesterol & serum triglyceride, LDL along with increase in HDL as compared to diabetic control group.

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Key Words
Antidiabetic activity, *Costus igneus*, Glibenclamide, ethanolic extract, Alloxan induced diabetic rats

INTRODUCTION

Diabetes mellitus (DM) is an endocrinal disorder associated with deficiency of insulin secretion due to damaged pancreatic β-cells which altered carbohydrate, lipid and protein metabolism and additionally increased risk of complications of various vascular diseases etc. It has been estimated that Indian people are more genetically susceptible to diabetes accounting about 30 to 33 million and would go up to 74 million by 2025[1]. Even though the oral hypoglycemic agents and bio-technically engineered insulin preparations are currently available for the treatment of diabetes mellitus, but are not free from undesirable side effects[2]. The management of diabetes mellitus thus is global challenge that demands for alternative therapy. Report of ethnobotany suggested that about 800 medicinal plants possess antidiabetic potential[3].

Traditionally people take 2-3 leaves of this plant twice a day for the management of diabetes and it is also known to be Insulin plant (*Costus igneus*) which is native of Southeast Asia, especially on the Greater Sunda Islands in Indonesia. It is a relatively new entrant to Kerala in India. The long red flower spikes of Costus pulverulentus are unique to the family. Costus igneus plant grows very quickly; propagation of this plant is by stem cutting. It needs sunshine but it also grows in slightly shady areas. In Ayurvedic treatment, diabetes patients are advised to chew down this plant leaves for a month. The patient has to take two leaves per day in the morning and evening for one week[4]. The leaves must be chewed well before swallowing. After one week the patient should take one leaf each in the morning and evening. This dosage should be continued for 30 days. Allopathic doctors too recommend it and it is found to be effective in bringing blood sugar levels completely under control. There is also dried and grounded powder of the leaves now available in the market. In Traditional Medicine it is also used to promote longevity, Treats rash, Reduces fever, asthma, bronchitis and eliminates intestinal worms. The diverse activity of *Costus igneus* inspired us to investigate its antidiabetic activity.

MATERIALS AND METHOD

Chemicals

Glibenclamide drug was obtained from Micro lab Hosur, Alloxan received from Himedia laboratory Mumbai; 90% Ethanol were purchased from SD Fine

Chem Ltd, Mumbai; all other chemicals used for the study were of analytical grade.

Collection of plant material and its authentication

The whole plant of Costus igneus (L.) was collected from ABS botanical garden, karripatti village, Salem, TamilNadu, India. The leaves were identified and authenticated by the botanist Prof.P.Jayaraman.Ph D, director national institute of herbal science, Chennai, India.

Preparation of extraction

The plant material first washed with water thoroughly to remove dirt and soil deposits and dried under shade until complete removal of moisture content, such dried plants were powdered by mechanically and passed through sieve no 80. 500gm of powdered extract with 300 ml of 90% ethanol solvent heated to 60-70ºC by using soxhlet apparatus for 48 hours. The extract thus obtained was concentrated with the help of rotator vacuum evaporator and stored in –2ºC [5]. and was subjected to preliminary photochemical analysis to know the nature of various phyto-constituents.

Animals used

Albino Wister strain rats (150-200gm) of either sex were used for this study and these animals were kept at Padmavathi College of pharmacy animal house.

Throughout the experiment, the animals were housed, four animal per cage, maintained at ambient temperature of (25ºC ±2); 30-60% humidity, under 10 h light-dark cycle. They were fed with standard pellet diet and water *ad libitum*. The animals were habituated to laboratory conditions for 36 h prior to the experimental protocol to minimize any non specific stress. All the experimental protocols were approved by Institutional Animal Ethical Committee (IAEC) of Padmavathi College of Pharmacy, Dharmapuri, India. All animal studies were performed as per rules and regulations in accordance to guideline of CPCSEA. CPCSEA Reg No: 1143/ac/07/CPCSEA. Acute toxicity study was carried out in animals by using OECD guidelines before proceedings treatments.

Experimental Design

Four groups of rats, six in each received the following treatment schedule:

Group I: Normal control (Sodium CMC)

Group II: Diabetic control - Diabetic rats receiving vehicle (0.05% Sodium CMC)

Group III: Diabetic rats receiving Glibenclamide (10 mg/kg)

Group IV: Treatment group - Diabetic rats receiving *Costus igneous* (250 mg/kg)

Induction of Diabetes

Diabetes was induced by single dose i.p. of alloxan monohydrate (120 mg/kg)[6]. Except Non diabetic control rats received only saline solution, After 72 h, the blood glucose levels were checked. Diabetic rats were considered with the blood glucose levels200 - 260 mg/dl.

Evaluation of hypoglycemic activity

Blood samples were collected on 0th, 7th and 14th day. Fasting blood glucose levels were estimated. The blood glucose levels were estimated on the 0th day, 7th day and 14th day of the treatment. After the treatment period on the 15th day, blood samples were collected from the overnight fasted animals from the retro orbital plexus under mild ether anesthesia for estimation of various biochemical parameters like Urea[7], AST , ALT[8], Cholesterol[9], Triglyceride, HDL, LDL[10] and Creatinine[11] were also estimated.

Histopathology studies

At the end of the study, all the animals were sacrificed under light ether anaesthesia, by Decapitation. The relevant organs pancreas, liver and kidneys were dissected out and collected in 10% formalin solution and immediately processed by the paraffin technique. Sections of 5µ thickness were cut and stained haematoxylin and eosin (H&E) for histo-pathological examination.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests to determine level of significance. A value of $P<0.01$ was considered significant results are expressed as mean SEM.

RESULTS AND DISCUSSION

Acute toxicology study showed no toxic effects or mortality up to 14 days in animals with ethanolic extract of *Costus igneous*, dose was selected 250mg/kg body weight based on the LD50 cut off value for the assessment of hypoglycemic activity.

The results of the phytochemical screening of ECI showed the presence of alkaloids phytosterols, saponins, glycosides, carbohydrates, tannins & phenolic compounds flavanoid, terpenoids and lignin and absence of proteins, amino acids, gums & mucilage and fixed oils & fats in ethanolic extract.

Tab 1. Fasting blood glucose levels of normal control, induced control, drug and & extract treated at the dose 250mg/kg body wt in rats.

Groups	Blood glucose levels (mg/dl)			
	0 Day	7th Day	14th Day	% Activity
Normal	84.83 ± 0.70	83.80 ± 0.25	84.83 ± 0.70	-
Diabetic Control	252.67 ± 3.11	268.67 ± 5.15	283.00 ± 1.52	-
Standard GBC	245.66 ± 2.40	143.50 ± 2.19	95.00 ± 1.34	55.33 %
ECI 250mg/kg	243.83 ± 3.4	181.17 ± 4.97	90.17 ± 5.04	50.46 %

Values are expressed as mean **±** SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

Tab 2.Depicts the effect on Liver markers on treated and untreated rats

Groups	**Parameters (U/L)**	
	AST	**ALT**
Normal	58.65 ± 0.12	45.2 ± 0.19
Diabetic control	151.52 ± 0.14	197.92 ± 0.24
Diabetic standard	85.38 ± 0.12	87.93 ± 0.22
ECI (whole plant) 250mg	92.92 ± 0.11	80.25 ± 0.21

Values are expressed as mean ± SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

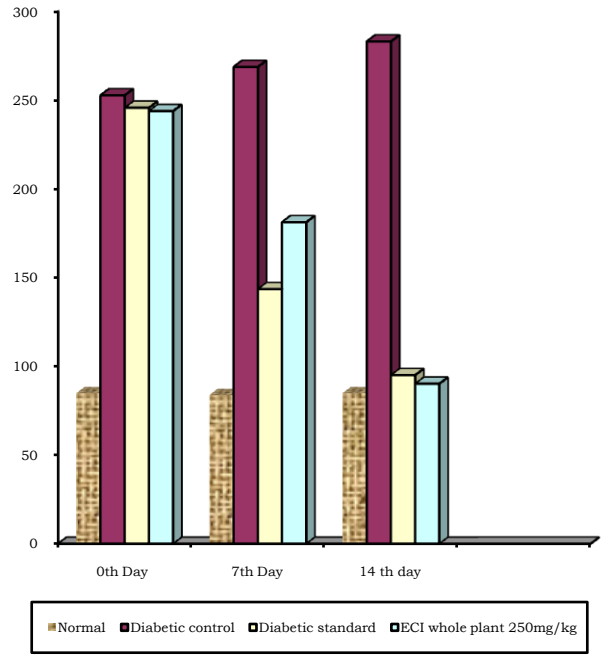

Fig 1. Shows the glucose level in different groups

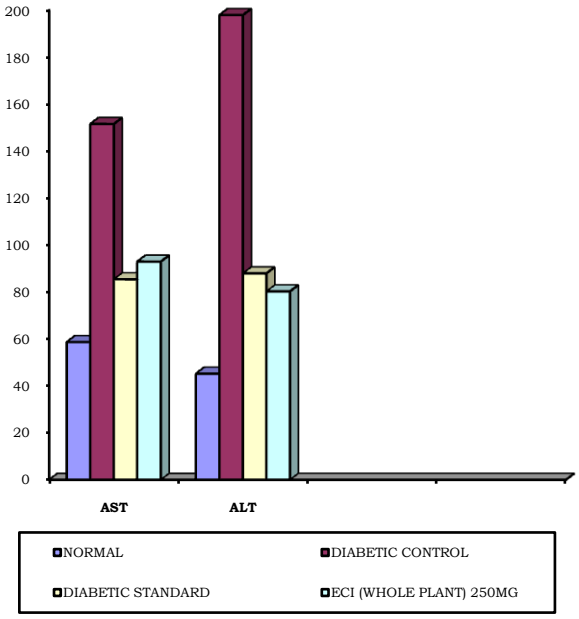

Fig 2. Depicts the effect on Liver markers on ECI treated and untreated rats

Non diabetic control rats receiving saline were considered as normal & healthy, diabetic control rats showed what could have happened without treatment. Diabetic rats treated with Glibenclamide (10mg/kg) decreased BSL 245.66 ± 2.40 mg/dl to 95.00 ± 1.34 mg/dl (55.33%). Diabetic rats treated with *Costus igneous* ethanolic extract (Whole plant) 250mg/kg showed decreased blood glucose levels from 243.83 ± 3.4mg/kg to 90.17 ± 5.04 mg/kg at the end of 14th day of treatment indicating good hypoglycemic activity (50.46 %)(Tab 1& Fig 1). *Costus igneous* ethanolic extract 250mg/kg also showed decrease in Serum Cholesterol & Serum Triglyceride, LDL along with increase in HDL as compared to diabetic control group (Tab 4). Rats treated with *Costus igneous* ethanolic extract (Whole plant) 250mg/kg also showed statistically significant decrease ($p<0.01$) in AST and ALT levels (Tab & Fig 2) as compared to Diabetic control, that's indicating hepatoprotective effect. Rats treated with *Costus igneous* ethanolic extract (Whole plant) 250mg/kg also showed statistically significant decrease ($p<0.01$) in Urea and Creatinine levels as compared to Diabetic control that indicating nephroprotective effect(Tab 3).

Tab 3. Shows the levels of Blood Urea and Serum Creatinine on the 14th day

Groups	**Parameters (U/L)**	
	Urea	**Creatinine**
Normal	15.94 ± 0.01	0.7 ± 0.094
Diabetic control	34.58 ± 0.15	1.60 ± 0.007
Diabetic standard	20.97 ± 0.12	0.60 ± 0.006
ECI 250mg	22.64 ± 0.12	0.71± 0.008

Values are expressed as mean **±** SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

Tab 4.The levels of different constituents of lipid profile on the 14th day

Groups	**Parameters (mg/dl)**			
	Cholesterol	**Triglyceride**	**HDL**	**LDL**
Normal	69.16 ± 0.79	71.00 ± 0.85	51.00 ± 0.73	3.96 ± 0.45
Diabetic control	141.83 ± 1.07	124.33 ± 0.88	21.66 ± 0.71	95.30 ± 0.71
Diabetic std	80.167 ± 0.9	81.00 ± 0.85	42.83 ± 0.70	21.13 ± 0.33
ECI (whole plant)250 mg/kg	89.5 ± 0.88	93.00 ± 1.00	37.33 ± 0.66	32.53 ± 0.82

Values are expressed as mean **±** SEM, n = 6; n= Number animals per treatment;

** Significantly different from Control, p<0.01

Tab 5.Interpretation of histopathology studies

Figure no	Report
Pancreas	
Group I (Fig 5A)	In the pancreas of normal control rats showed normal pancreatic islets pattern and exocrine pancreatic architecture, many rounded and elongated islets were evenly distributed throughout the cytoplasm, with their nucleus lightly stained than the surrounding cells.
Group II (Fig 5B)	Histopathology of pancreas of Diabetic rats showed diffuse degeneration of islet of langerhans.
Group III (Fig 5C)	Only minor pathological features were identified in pancreas of the diabetic rats treated with Glibenclamide.
Group IV (Fig 5D)	Degenerative changes are very few in Costus igneus (whole plant) ethanolic extract treated rats
Liver	
Group I (Fig 6A)	Histopathalogical of liver showed normal hepatic structure with hepatic lobule.
Group II (Fig 6B)	The histopathalogical examination of diabetic rats showed hepatocellular injury pronounced in loss of the normal architecture of the liver. Histopathology showed that sinusoidal spaces are dilated in diabetic rats with multifocal fatty degeneration.
Group III (Fig 6C)	Degenerative changes are very few in this group.
Group IV (Fig 6D)	*Costus igneous* ethanolic extract250mg/kg treated rats showed minimal single cell necrosis and kuffer cell infiltration.
Kidney	
Group I (Fig 7A)	Examination of the kidney of the normal control rats revealed normal glomeruli with thin glomerular basement membranes, normal cellularity and patent capsular space were normal. No major detectable abnormalities were noted.
Group II (Fig 7B)	Histopathology showed that diffuse degeneration of tubular epithelium and multifocal area of haemorrhages in diabetic rats.
Group III (Fig 7C)	Minimal changes are seen in this group
Group IV (Fig 7D)	These changes are minimal in Costus igneus ethanolic extract (Whole plant) 250mg/kg treated rats.

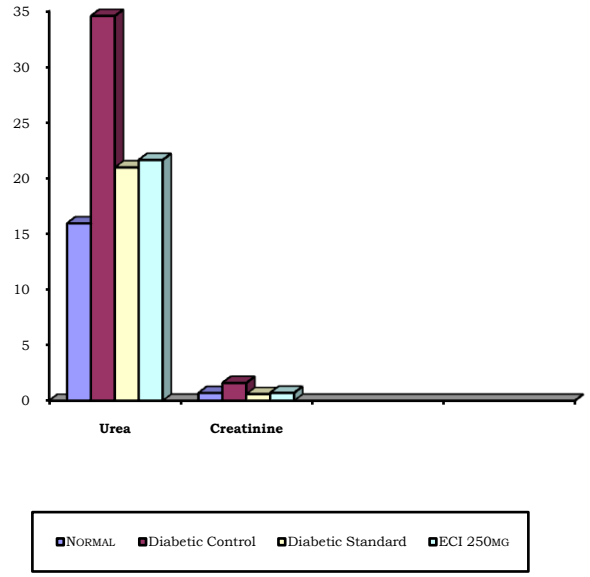

Fig 3.Shows the Effect of ECI on Blood Urea and Serum Creatinine in different groups

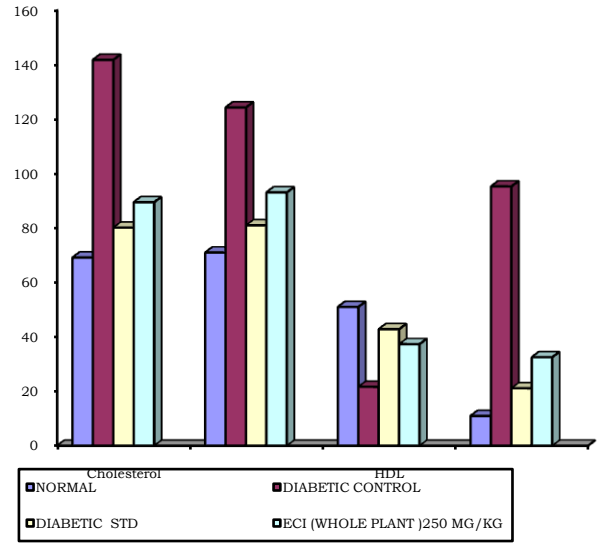

Fig 4. Shows the effect of ECI on lipid profile in different groups

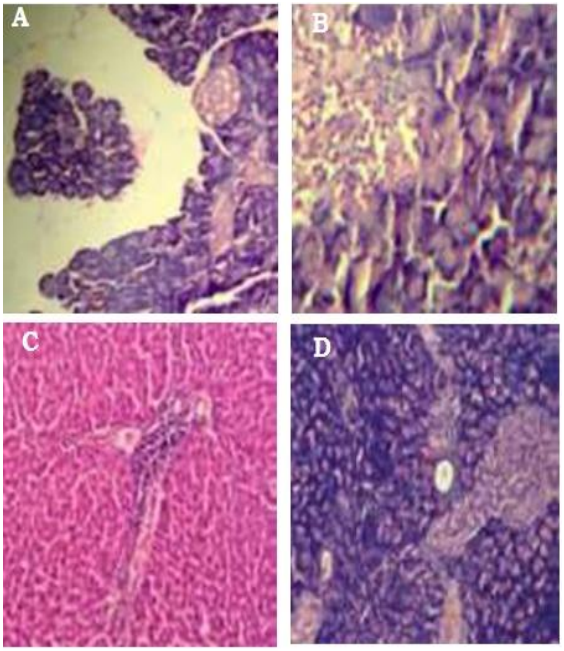

Fig 5. Histopathological slides of pancreas for A) Normal control; B) Diabetic control;C) Diabetic Standard; D) ECI250mg/kg

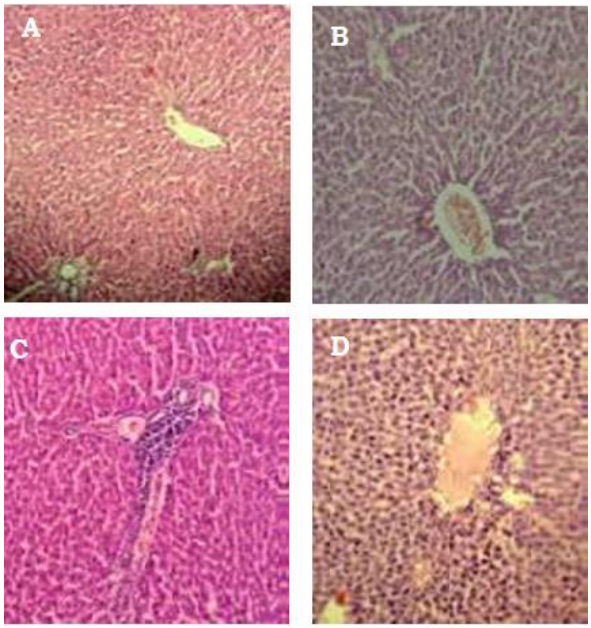

Fig 6. Histopathalogical slides of liver for A)Normal control; B) Diabetic control ; C) Diabetic Standard; D) ECI 250mg/kg

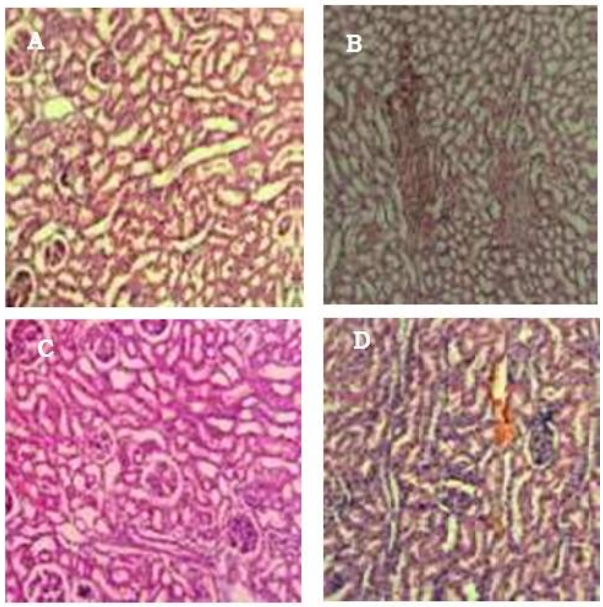

Fig 7. Histopathalogical slides of kidneys for A) Normal control) Diabetic control; C) Diabetic Standard; D) ECI 250mg/kg

Histopathalogical study on the pancreas confirmed the necrosis of β- cells in alloxan induced group of rats, whereas the glibenclamide-treated group showed the preserved cytology. At 250mg/kg b.w., ECI-treated rats showed small islet cells, i.e. regeneration of β-cells (Fig 5) and also in alloxan induced untreated rats observed heptotoxic and nephrotoxic effect (Figs 6 &7), ECI treated induced animal showed minimal single cell necrosis and kuffer cell infiltration in liver and minimal degeneration of tubular epithelium in kidneys when compared to untreated induced rats.

CONCLUSION

Ethanolic extract of CI (whole plant) at dose of 250 mg/kg showed significant hypoglycemic activity in alloxan induced hyperglycemic rats. Also shows hepatoprotective activity and significantly improvement in reducing nephrotoxcity. Thus ethanolic extract of *Costus Ignious* (Whole plant) is a multi targeted, cheaper, herbal drug which is a promising candidate for consideration for the treatment of diabetes mellitus. However, further study is needed to find out the exact mechanism and the phytoconstituents responsible for observed effects.

Abbreviations used: CI= *Costus igneus* (whole plant); ECI= Ethanolic extract of *Costus igneus;* OECD= Organization of Economic Co-Operation and Development; AST= Aspartate aminotransferase; ALT= Alanine transaminase, HDL= high density lipids, LDL= low density lipids.

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