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EVALUATION OF ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACT OF *CARDIOSPERMUM HALICACABUM* LINN STEM ON STREPTOZOTOCIN INDUCED DIABETIC RATS

Sheik Nasar I *[1]& K L Senthilkumar [2]

[1] Sun Rise University, Bagdad Rajput, Teh. Ramgarh. Dist. Alwar 301030, Rajasthan, India.
[2] Sri Vijay Vidyalaya College of Pharmacy, Nallampalli, Dharmapuri, Tamilnadu -636807, India.

ABSTRACT

Purpose: To investigate antidiabetic and antioxidant activity of the ethanolic extract of *Cardiospermum halicacabum Linn* on Streptozotocin induced diabetic rats modeldiabetic rats.*Methods:* Streptozotocin (45 mg/kg b.w) was administered to wistar albino rats via the intraperitoneal route. The diabeticrats were then placed in 5 groups, following stabilization of hyperglycemia. The first group was untreated, thenext receives Streptozotocin (45 mg/kg b.w) the next two groups received, each day 200 and 400 mg/kg body weight of the ethanol extract *Cardiospermum halicacabum* and the fifth group received a reference standard, glibenclamide (0.5 mg/kg). Treatment was via the oral route for 28 days and fasting blood sugar level was monitored over this period. Acute toxicity (oral)studies on the extract was carried out, as well as phytochemical screening of the extract. *Results:* All doses of the extract (200 and 400 mg/kg) significantly ($p < 0.05$, $p < 0.0001$, $p < 0.05$,respectively) lowered fasting blood glucose level, notably at the 7th , 14th 21th and 28th day. Glibenclamide (0.5 mg /kg) also significantly lowered fasting blood glucose ($p < 0.0001$). The results on acute toxicity revealed that for the oral route is high safety status of the plant. Phytochemical screening revealed the presence of saponins, tannins, alkaloids and flavonoids. *Conclusion:* This study supports the use of *Cardiospermum halicacabum* in traditional medicine as well as highlights the need to further explore the potentials of the plant extract as a antihyperglycemic agent.

Key words: Diabetes mellitus, *Cardiospermum halicacabum,* Antidiabetic, Hypoglycemia, Blood glucose.

Corresponding Author **Sheik Nasar I** Email: sheiknasarpharmacist@gmail.com

INTRODUCTION

Diabetes mellitus

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels(Richard kahn *et al*.,1997).

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (Genuths *et al*.,2003).

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EXPERIMENTAL

Plant material

Cardiospermum halicacabum is a member of the Sapindaceae. It is an herbaceous vine about 3 m long, stem slender, grooved. The leaves are stalked, alternate, bipinnate, pinnae divided into three pinnules, pinnuleslanceolate and long-pointed. The flowers are white in colour with a pair of tendrils at the base of the clusters, in axillary racemes. The fruit a capsule in shape, three-celled, winged at the angles. The seeds are globose, smooth and black in colour (Manandhar *et al*., 2002).

Chemical Constituents

(+)-pinitol; β-Sitosterol; β-sitosterol-β-D-galactoside; apigenin-7-O-glucuronide; arachidic acid; chrysoeriol-7-O-glucuronide; lineleic acid; luteolin-7-O-glucuronide;stearic acid(Chungkisung *et al*., 1998).

Extract preparation

The fresh stem of *Cardiospermum halicacabum Linn* was collected and washed with running water. It was shad dried at room temperature the dried stem was made in to coarse powder. The powdered stem were extracted with ethanol in Soxhlet extractor.

Drugs and chemicals

Streptozotocin , glibenclamide and 95 % ethanol (Sigma Chemicals Ltd, Mumbai) were used.

Experimental Animals:

Adult male Wistar rats of weighing 250-300 gms were used for this study. The inbred animals were procured from the animal house of Padmavathi college of Pharmacy, Dharmapuri. They were housed six per cage under standard laboratory conditions at a room temperature at 22 ± 2^0 C with 12 hr light/dark cycle. The animals were provided with pellet chow and water and labium. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Phytochemical screening

The ethanolic extract of stem of *Cardiospermum halicacabum* (EECH) showed presence of various phytochemical constituents such as Alkaloids, carbohydrates, flavonoids, gums and mucilage's, tannins, phenols, saponins and terpenes, proteins, steroids and glycosides.

ACUTE TOXICITY STUDIES (Ecobichan *et al*., 1997; Leelavinothan pari *et al*., 2006).

Procedure:

Adult male wistar rats weighed 250- 300 gms were used for the study. The starting dose level of ethanolic stem extract of *Cardiospermum halicacabum Linn* was 2000mg/kg body weight p.o. as most of the crude extracts possess. LD_{50} value more than 2000 mg/kg, p.o. so starting dose used was 2000mg/g p.o. Dose volume administered was 1ml/100 gm body weight to fasted rats with 1%w/v CMC. Food was with held for a further 3-4 hrs after administration (p.o) of drugs and observed for the signs of toxicity. Body weight of rats before and after determination were noted and any changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behaviour pattern were observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

Induction of diabetes mellitus in experimental animals (Benny Kwong Huat Tana *et al*., 2005)

Experiments were performed in adult Wistar rats (n=6) of sex, aged 6–8 weeks and weighing 220–250g. The animals were housed under standard environmental conditions (23±1°C, with 55±5% humidity and a 12 h light/12 h dark cycle) and the rats were fed with normal Diet .After 2 weeks the animals were administered with Streptozotocin (45 mg/kg, in 0.1 M citrate buffer, pH 4.5) intraperitoneally to induce diabetes.After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. The development of diabetes was confirmed after 72 hours of the Streptozotocin injection.

Antidiabetic treatment

The animals were dived into 5 groups .Group I consists of normoglycaemic rats. The remaining 4 groups consisted of 6 STZ induced diabetic rats. The test sample was administered orally by using oral feeding needle.

- Group I-Normal control animals received 1 % CMC.
- Group II - Animal injected with Streptozotocin (45 mg/kg b.w) and treated with 1% CMC
- Group III- Animal injected with Streptozotocin (45 mg/kg b.w) and treated with low dose of EECH 200mg/kg b.w/ p.o
- Group IV- Animal injected with Streptozotocin (45 mg/kg b.w) and treated with high dose of EECH 400 mg/kg b.w/ p.o
- Group V - Animal injected with Streptozotocin (45 mg/kg b.w) and treated with Glibenclamide 0.5mg/kg b.w/p.o

The above mentioned treatment schedule was followed for the respective group of animals for 28 days.

Measurement of blood glucose levels

Blood samples were collected from tail vein in overnight fasted animals on 0th, 7th, 14th 21st and 28th day to estimate blood glucose levels using a commercial

glucometer and glucose-oxidase strips. (One touch glucometer).

Statistical analysis

The data were expressed as mean ± standard error (S.E.M). The significance of differences among the groups was assessed using one way analysis of variance (ANOVA). The test followed by Dunnet's test p values lees than 0.05 were considered as significance.

Biochemical Studies:

At the end of the study, all the animals were sacrificed under light ether anaesthesia. The rats were sacrificed by decapitation and blood was collected by bleeding of retro-orbitol plexus using micro capillary technique from all the groups of overnight fasted rats and serum was separated to study the biochemical parameters.

Histopathology:

The relevant organs like liver and pancreas were removed dissected out and washed with ice-cold saline. The pancreatic tissues and liver were preserved in 10% formalin solution for Histopathological study.

RESULTS

Phytochemical analysis

The presence of various phytochemical constituents such as Alkaloids, carbohydrates, flavonoids, gums and mucilage's, tannins, phenols, saponins and terpenes, proteins, steroids and glycosides.

Acute toxicity

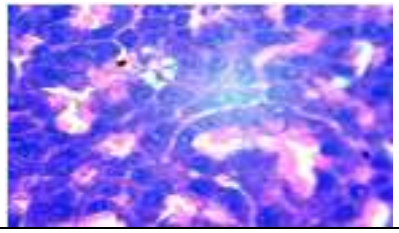
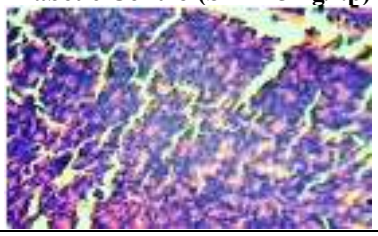
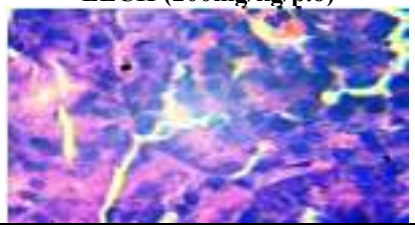
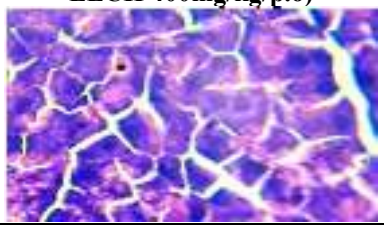
The Acute Oral Toxicity Study was done according to the OECD guidelines 423 (Acute toxic class method). A single administration of starting dose of 200mg/kg b.w /p.o EECH was administrated to three rats and observed for 3 days. There was no considerable change in body weight before and after treatment and no sign of toxicity was observed. When the experiment was repeated again with same dose level, 2000 mg/kg b.w /p.o of the EECH for three more days and observed for 14 days. No change was observed from first set of experiment**.**

Anti-diabetic effect

Effect of EECH on blood glucose level in STZ induced diabetic rats for 28 days The blood glucose level increased significantly in STZ treated group when compared to control group. The STZ induced rats were treated with EECH 200mg/kg/p.o and 400mg/kg/p.o for the duration of 28 days. Treatment with EECH at the dose of 200mg/kg/p.o did not show significant decrease in the blood glucose level after second week. Treatment with EECH at the dose of 400mg/kg/p.o showed significant decrease in the blood glucose level at first week ($p<0.001$), which further reduced in the second and third weeks ($p<0.001$), respectively. Treatment with glibenclamide (0.5mg/kg b.w/p.o) produced a significant ($p<0.001$) decrease in blood glucose level from first week to third week.

Effect of EECH on Body weight in STZ induced diabetic rats for 28 days: The Body weight level decreased significantly in STZ treated group when compared to control group. The STZ induced rats were treated with EECH 200mg/kg/p.o and 400mg/kg/p.o for the duration of 28 days. Treatment with EECH at the dose of 200mg/kg/p.o showed significant increase ($p<0.001$) in the body weight level after second week. Treatment with EECH at the dose of 400mg/kg/p.o showed significant increase in the body weight level at first week ($p<0.001$), which further reduced in the second and third weeks ($p<0.001$), respectively. Treatment with glibenclamide (0.5mg/kg b.w/p.o) produced a significant ($p<0.01$) increase in body weight level from first week to third week.

Fig: 1. Histopathology of Pancreas

Normal Control 	**Diabetic Control(STZ 45mg/i.p)** 
EECH (200mg/kg/p.o) 	**EECH 400mg/kg/p.o)** 

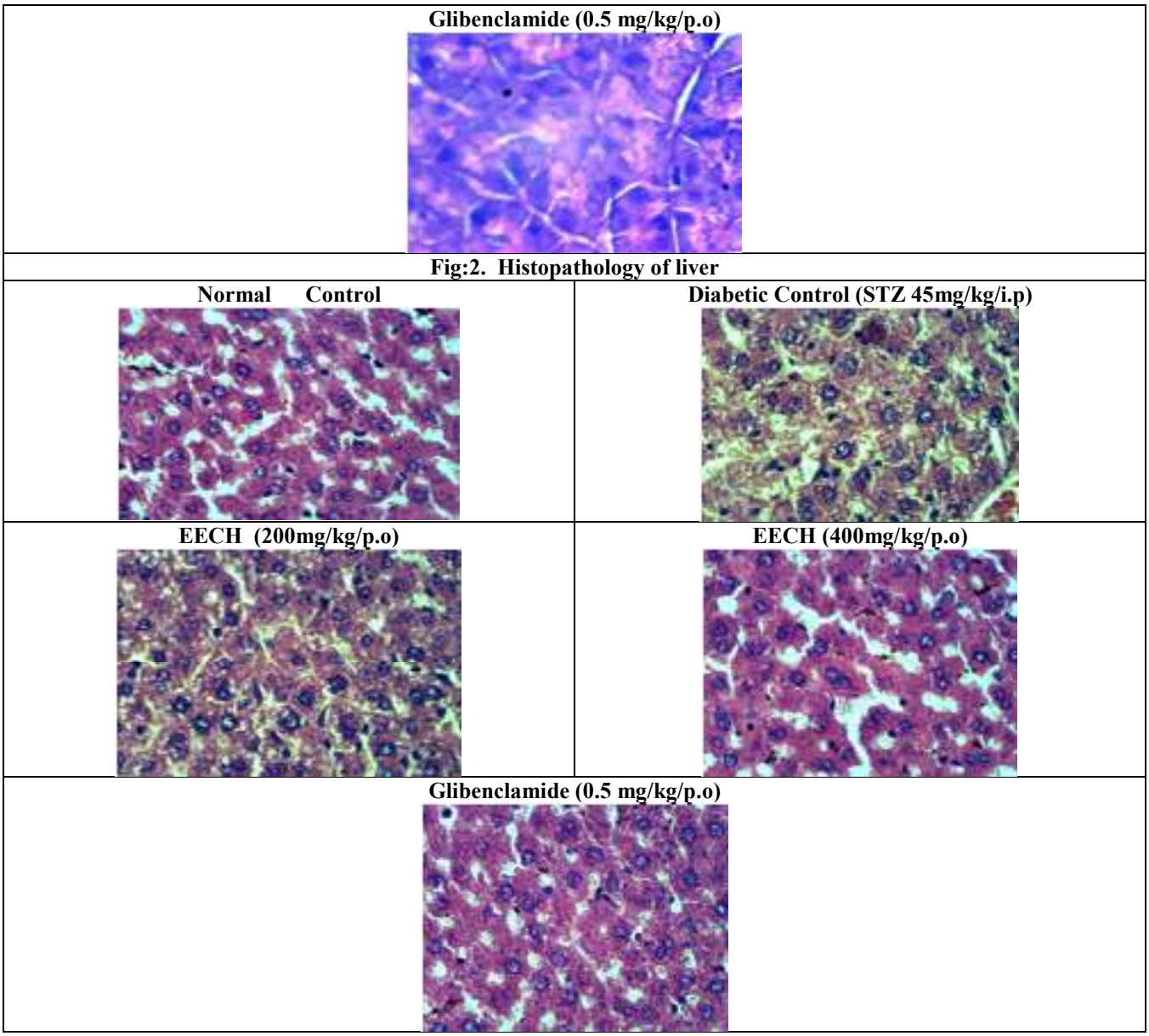


Fig:2. Histopathology of liver

DISCUSSION

Preliminary Phytochemical analysis of ethanolic extract of stem of *Cardiospermum halicacabum Linn* (EECH) showed the presence of Phytochemical such as alkaloids, carbohydrates, flavonoids, flavones, gums and mucilage's, tannins, phenols, saponins and terpenes, proteins, steroids and glycosides.

Acute oral toxicity study of EECH did not exhibit mortality or any profound toxic reactions at a dose of 2000mg/kg/p.o. The EECH at a dose 200mg/kg/p.o and 400mg/kg/p.o did not significantly suppress blood glucose level in over night fasted normal animals after 1st, 2ndand 3rd hours of oral administration when compared with normal animals (Cisse A *et al*.,2005).

Experimental induction of diabetes with low dose of STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting and due to loss of tissue protein. Reduced glucose transport or absorption from the gut. Extra pancreatic action probably by stimulation of glucose utilization in peripheral tissues. Increase in glycogenic or glycolytic enzyme activities in peripheral tissues. Decrease in the secretion of counter-regulatory hormones like glucagon, growth hormones (Didemdo *et al*., 2005).

Hyperlipidemia is pathological state observed in the DM, elevated serum total cholesterol, triglycerides, LDL- cholesterol, VLDL- cholesterol and reduced serum HDL level consequently increases the risk of diabetic complications and atherosclerosis. In the present study

treatment with EECH of 200mg/kg and 400 mg/kg significantly ($p<0.001$) reduced the total cholesterol, triglycerides, LDL- cholesterol, VLDL- cholesterol and significantly increased the HDL levels. This effect may not only due to better glycaemic control but also due to its action on the lipid metabolic pathway(Annie shirwaikark *et al*., 2005).

In the present study we have examined the oxidative stress pathway marker in STZ induced diabetes rats, SOD and catalase is the most important enzyme that scavenge the toxic free radicals and form the major anti oxidant system. Treatment with EECH at a dose 200 mg/kg and 400 mg/kg, glibenclamide significantly increased the enzyme activity. EECH at dose of 400 mg/kg exhibit pharmacological response related to glibenclamide.

In Histopathological examination of diabetic control animal, the sections revealed exocrine pancreatic tissue compound of acini with draining docutles, the endocrine component was found as scattered nodules within the substance of the exocrine pancreas relating to focal mild infiltration by mononuclear cell. In EECH (200mg/kg/p.o), the peripheral widening between acinar and islets cells were excited and more intracellular space and inflammatory cells were observed. Histology of pancreas in EECH (400 mg/kg/p.o) showed predominant exocrine pancreatic tissue compound of acini with draining ductules. In case of glibenclamide, neither hemorrhage nor massive infiltration of inflammatory cells was observed. So Histopathological studies prominent islet cell, hyperplasia and regeneration of islet cells show a proof for possible anti-hyperglycemic property of the EECH.

CONCLUSION

The ethanol stem extract of *Cardiospermum halicacabum* demonstrated antidiabetic activity in rats and acute toxicity studies also show it to be relatively safe. The findings from this work supports its use in traditional medicine. However, further studies are required to elucidate its mechanism of action.

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