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Research Article

ANTI-DIABETIC ACTIVITY OF METHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* SEEDS ON STZ INDUCED DIABETIC RATS

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ABSTRACT

Objective: The present study was designed to evaluate the anti-diabetic activity of methanolic extract of *Calotropis gigantea* seeds on streptozotocin induced diabetic rats.

Methods: The acute toxicity studies were performed on mice while adopting the OECD-420 Guidelines (fixed dose procedure). The anti-diabetic activity of plant seeds were investigated on the standard 21 days Streptozotocin induced diabetes model.

Results: Treatment with methanolic extract of *Calotropis gigantea* (MECG) seeds at the dose 200 mg/kg and 400 mg/kg body weight, significantly decrease the blood glucose levels from 277.33±5.207 and 294.5±12.26 to 141.5±8.807 and 120.5±9.68 respectively. The activities were also compared with the effects produced by the standard anti-diabetic drug, glimipride 10 mg/kg.

Conclusion: The present findings indicate that the seeds of *Calotropis gigantea* possess potent anti-diabetic effect on streptozotocin diabetic rats.

Keywords: Madar, *Calotropis gigantea*, OECD, MECG, Streptozotocin and glimipride.

INTRODUCTION

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and/or insulin action [1]. It is one of the common metabolic disorders with micro-and macrovascular complications that results insignificant morbidity and mortality. It is considered as one of the five leading causes of death in the world [2].There are an estimated 143 million people in the world with diabetes mellitus and this number will probably double by the year 2030. A medicinal plant, *Galega officinalis*, led to the discovery and synthesis of metformin [3]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times, there has been a renewed interest in the plant remedies [4-5].

Calotropis gigantea (Crown flower) is a species of *Calotropis*, commonly known as 'madar' in Hindi, belonging to the family Asclepiadaceae, is a milky shrub up to 1-3m in height found throughout India [6]. *C. gigantea* has been reported to contain proteases, 3'-methylbutanoates of amyrin, flavonol glycosides, calotropins, stigmasterol and sitosterols, cardenolides, and pregnanone. *Calotropis gigantea* is also used in ayurvedic system of medicine especially the powdered root used in asthma, bronchitis and dyspepsia, dried whole plant is a good tonic [7]. *Calotropis procera* has also been reported to possess anti-diabetic activity [8]. The plant selected for this present work is locally available in the Bareilly district and has been used for long a time in local folklore medicine for the treatment of diabetics.

MATERIALS AND METHODS

Plant Material and Preparation of Extracts

The plant material (seed) was collected in the forest area of Bareilly (Uttar Pradesh). The plant was authenticated and identified at raw material herbarium and museum by Dr. D.S Saini, Birbal Sahani Institute of Paleobotony, Lucknow and a voucher specimen (13391) was deposited in the department as per the standard method [9]. The plant material was shade dried at room temperature for 10 days, coarsely powdered, and the powder was passed through sieve No.60 and used for extraction [10-11]. The powdered *C. gigantea* seed material was extracted separately using methanol by Soxhlet extraction method. The extracts were concentrated using rotary vacuum evaporator. The dried extracts were stored in airtight container & placed in refrigerator.

Animals

Wistar albino rats, 9-12 weeks old with an average weight of 150-175 g and Swiss Albino mice were obtained from the animal colony of National Laboratory Animal Centre (NBRI), Lucknow. They were randomly distributed into various groups and housed in cages (5 animals per cage) and maintained under standard conditions i.e. 26 ± 2º C and relative humidity 44-56%. They were housed in polypropylene cages and fed with a standard chow diet and water *ad libitum*. The animals were exposed to an alternating 12 h and light cycle. Before each experiment, the animals were fasted for at least 18 h. The experimental protocols were approved by Institutional Animal Ethical Committee.

Toxicity evaluation in mice

Acute toxicity study was carried out as per the procedure given in OECD Guideline No. 420. Male albino mice weighing between 25-30gm were used in the study. After sighting study, the methanolic extract of *Calotropis gigantea* at the dose of 2 g/kg body weight (b.w.) was given to five animals. The animals were continuously observed for 14 days for mortality and general behavior. No death was observed till the end of the study. We have selected 200 and 400 mg/kg b.w. dose of MECG seeds to test the anti-diabetic effect [12].

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (18 h) normal rats. Rats were divided into three groups (n = 6). Group I served as normal control (glucose 2 g/kg b.w., per oral (p.o.)) and Group II and Group III received methanol extract of *Calotropis gigantea* (MECG) seeds orally at the doses of 200 and 400 mg/kg b.w. respectively, whereas Group IV received Glimipride (10mg/kg b.w.). Glucose (2 g/kg b.w.) was fed to Group II and Group III, 30 min prior to the administration of extracts. Blood was withdrawn from tail vein before administration of glucose and at 30, 60,120, 240 and 360 min after the oral glucose administration and glucose levels were measured using single touch glucometer (Accu Check Active) [13].

Drugs and chemicals

Streptozotocin was purchased from Sisco Research Laboratories, Pvt. Ltd, Mumbai, India. Glimipride was obtained as gift sample from Rani Life Sciences Pvt, Ltd, Rudrapur.

Preparation of extract dose, reference and STZ

The extracts were administered orally to rats, as a suspension in 1% Carboxy Methyl Cellulose (CMC). Glimipride (10mg/kg) was suspended with 1% w/v CMC and administered orally (Standard drug). STZ was dissolved in 0.1 M citrate buffer (pH 4.5) immediately before use [14].

Experimental design

Induction of diabetes

The STZ was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5) and was administered by *intra-peritoneal* route at the dose of 55 mg/kg body weight for each rat and their serum glucose levels were checked after 72 hr. Animals were considered diabetic when their blood glucose level was raised beyond 225 mg/dl [14].The rats' whose serum glucose levels was 250 mg/dl were considered as diabetic resembling chronic diabetic condition and hence they were selected and divided into five groups of six rat each [15-16].

Experimental procedure

In experiment, total 30 rats were used (24 diabetes surviving rats, 6 normal control rats) for the execution of the experiment [17,18]. The rats were divided as follows into five groups:

- Group I: Normal control rats (vehicle treated).
- Group II: Diabetic control (received 0.5ml of 1% CMC solution)
- Group III: MECG 200mg/kg body weight.
- Group IV: MECG 400mg/kg body weight.
- Group V: Glimipride 10mg/kg body weight.

The blood was withdrawn by tail vein puncturing method. The samples of blood were obtained just before drug administration on the first day and 1 h after drug administration on 0, 7, 15 and 21st day. Blood glucose levels were determined by using glucometer [19-20].

Statistical evaluation

All the data are presented as mean ±SEM, n= 6. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by the Newman-Keuls Multiple Comparison Test. $P<0.05$ was considered to be significant.

RESULTS

Acute toxicity studies

While performing preliminary test for pharmacological activity in rats, aqueous extract did not produce any significant changes in the behavioral or neurological responses upto 2500 mg/kg body weight acute toxicity studies revealed the non-toxic nature of the methanol and chloroform extracts of the seeds of *C. gigantea.*

Oral glucose tolerance test

Administration of glucose (2g/kg) produces significant change in SG level of normal rats. Treatment with MECGS 200mg/kg, 400mg/kg and GLB (10mg/kg) exhibited significant reduction ($p<0.05$, $p<0.01$ and $p<0.001$ respectively) in SG level over the period of 120 min as compared to normal control group as shown in fig 1. MECGS showed significant reduction in plasma glucose level at 2 hr in comparison with the control.

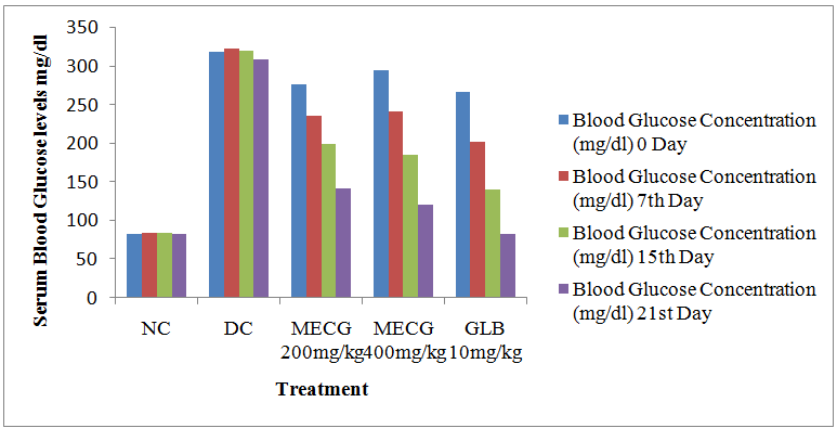

Fig. 1: Histogram showing the effect of MECG seeds on oral glucose tolerance in normal rats

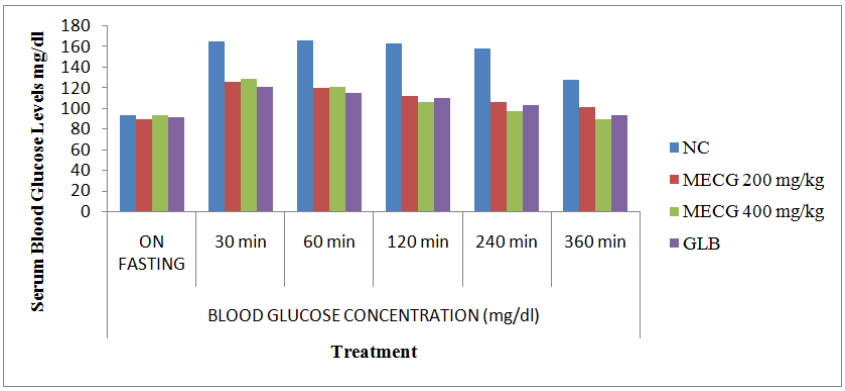

Fig. 2: Histogram showing the effect of MECG seeds on the blood glucose level in STZ induced diabetic rats

Evaluation of anti-diabetic activity

The effects of extracts on blood glucose levels in diabetic rats are reported in fig 2. Blood glucose levels of the STZ treated rats were significantly higher than those in normal rats. In STZ (50 mg/kg) induced rats, the blood glucose level significantly increased from 93.89±1.47 to 319.5±18.19 mg/dl. Methanol extracts were given up-to 21 days at a dose of 200 mg/kg b.w and 400 mg/kg b.w.

After extracts treatment, the blood glucose levels were decreased from 277.33±5.20 to 141.5±8.8 and 294.5±12.26 to 120.5±9.68 mg/dl,

respectively. Whereas in glimipride treated rats, blood glucose levels were decreased from 267.66±8.56 to 83.16±4.68 mg/dL.

Effect of MECG seeds on weight reduction in STZ diabetic rats

The change in the body weight of animals in different groups is compared at the end of study on 21st day. Normal control animals were showed increase in their body weight, but diabetic control rats showed significant reduction in the body weight, whereas MECG seeds 200mg/kg, 400mg/kg and Glimipride (10 mg/kg) treated group exhibited highly significant prevention of reduction in body weight (*P<0.01, P<0.001 and P* <0.001 respectively) when compared with initial body weight of same animals as shown in fig 3.

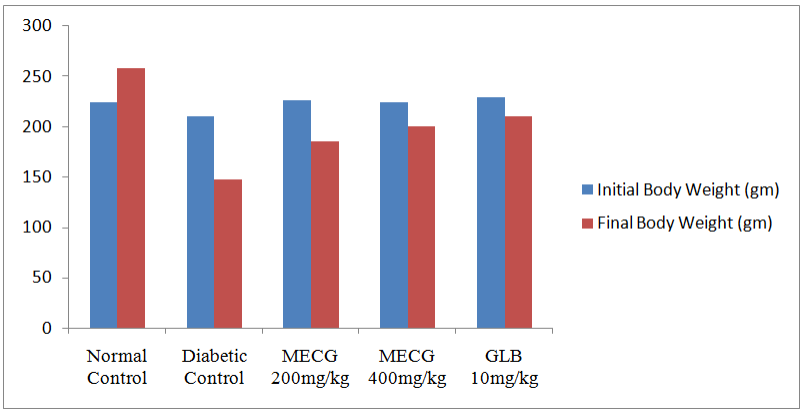

Fig. 3: Histogram showing effect of MECG seeds on weight reduction in STZ diabetic rats

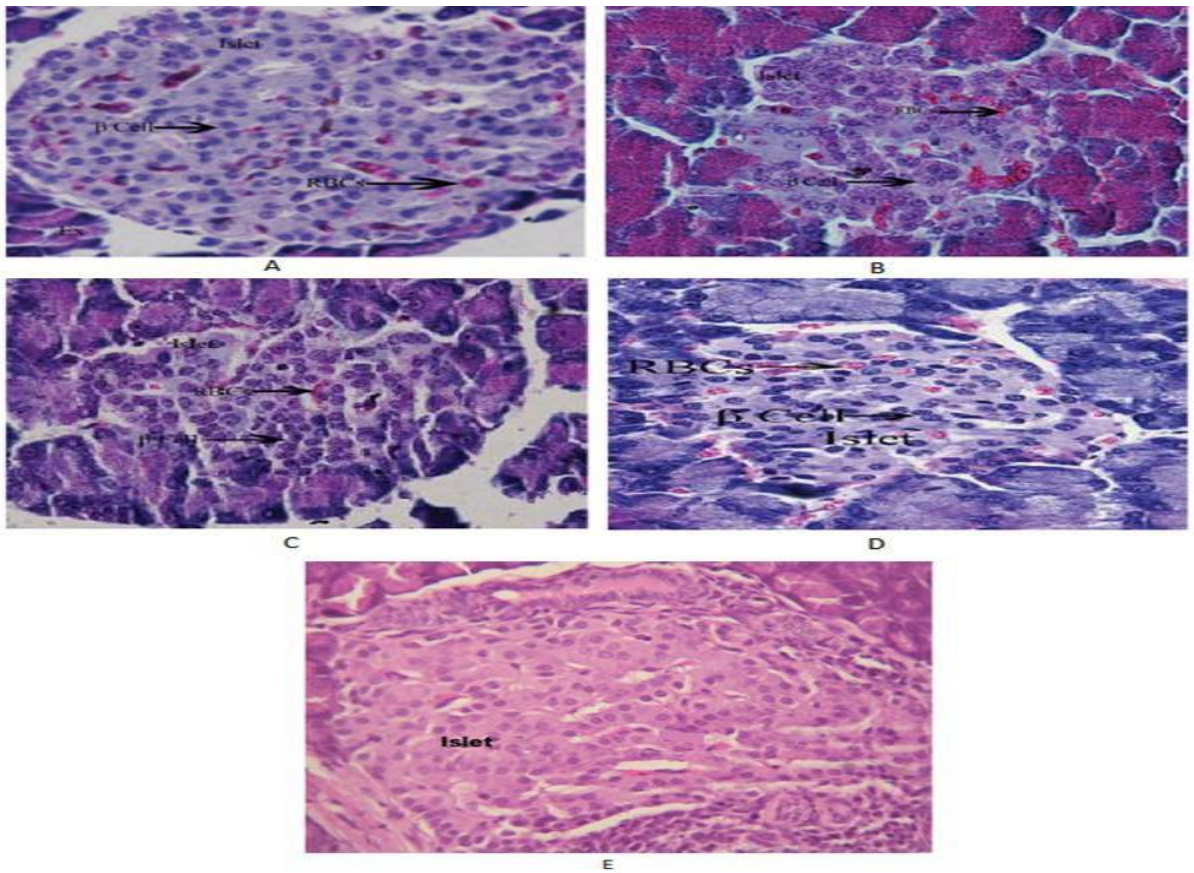

Fig. 4: A-Photomicrograph of a pancreatic section from the normal control group showing the exocrine region and islets of Langerhans, with scattered β cells and red blood cells visible in the vicinity.

B- Photomicrograph of STZ-induced diabetic pancreatic section showing the exocrine region and islets of Langerhans with damaged β cells due to necrosis and a decreased number of β cells.

C- Photomicrograph of STZ-induced diabetic pancreatic section treated with 200 mg/kg of methanolic extract of *C. gigantea* showing evenly distributed β cells and an increased number of β cells.

D- Photomicrograph of STZ-induced diabetic pancreatic section treated with 400 mg/kg of methanolic extract of *C. gigantea* showing evenly distributed β cells and an increased number of β cells.

Histopathological Examination

The cellular integrity and architecture were intact in the normal control group (fig 4 A). It showed normal acini and normal cellular population in the islets of langerhans in Pancreas whereas diabetic animal showed STZ induced severe necrotic changes of pancreatic islets, especially in the centre of islets and relative reduction in size and number of islets especially around the central vessel and severe reduction of beta cells was clearly seen (fig 4 B).

Both the higher doses of MECG seeds 200mg/kg (fig 4 C) and MECG seeds 400mg/kg (fig 4 D) and GLB (fig 4 E) showed increased size of islets relative increase of granulated and normal beta cells. Both the dose of extract improved the condition of pancreas compared with diabetic animal.

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