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Original Article

Evidence based study of antidiabetic potential of *C. maxima* seeds – *In vivo*

Devesh Kumar Kushawaha, Manjulika Yadav, Sanjukta Chatterji, Amrita Kumari Srivastava, Geeta Watal*

Alternative Therapeutics Unit, Drug Development Division, Medicinal Research Laboratory, Department of Chemistry, University of Allahabad, Allahabad, 211002, Uttar Pradesh, India

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ABSTRACT

Objective: *In vitro* antidiabetic efficacy of *Cucurbita maxima* seed extract (CMSE) has already been studied in our previous findings. Thus, in order to validate these findings in biological system, *in vivo* antidiabetic activity of aqueous extract was investigated in normal as well as diabetic experimental models.
Methods: Variable doses of extract were administered orally to normal and STZ induced mild diabetic rats during fasting blood glucose (FBG) and glucose tolerance test (GTT) studies. In order to determine the extract's antidiabetic potential long-term FBG and post prandial glucose (PPG) studies were also carried out.
Results: Most effective dose of 200 mg kg^{-1} of CMSE decreases the blood glucose level (BGL) in normal rats by 29.02% at 6 h during FBG studies and 23.23% at 3 h during GTT. However, the maximum reduction observed in BGL of mild diabetic rats during GTT the same interval of time was 26.15%. Moreover, in case of severely diabetic rats a significant reduction of 39.33% was observed in FBG levels whereas, in case of positive control, rats treated with 2.5 mg kg^{-1} of glipizide, a fall of 42.9% in FBG levels was observed after 28 days. Results of PPG level also showed a fall of 33.20% in severely diabetic rats as compared to the positive control showing a fall of 44.2% at the end of the 28 days.
Conclusion: Thus, the present study validate the hypoglycemic and antidiabetic effect of CMSE and hence this extract could be explored further for developing as a novel antidiabetic agent.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by high blood glucose level either due to insulin deficiency or insulin resistance. It is mostly associated with dyslipidemia and oxidative stress affecting nearly every organ in the systems. Any factor which delays intestinal carbohydrate absorption would help to reduce plasma glucose concentration and hence diabetic complications as well. Life expectancy for diabetic persons is estimated to be up to 10 years lesser than the non-diabetic individuals. It has been reported that the number of people who have diabetes will increase to 360–380 million in 2025–2030.[1] Thus, diabetes being global burden has to be treated dealt firmly.

Recently, herbal medicines are gaining importance due to their high margin of safety. There are number of medicinal plants well known for their medicinal usage for treating diabetes mellitus in traditional system of medicine. However, some of them have been studied systematically and scientifically for their antidiabetic efficacy[2–6] and *Cucurbita maxima* is one of them. Its fruits are used as vegetable, and its seed have been recommended for the treatment of several diseases due to their high medicinal value. Thus, the present study was conducted on aqueous CMSE in normal, mild and severely diabetic rats to elucidate its hypoglycemic and anti-hyperglycemic profile.

C. maxima Duch. (family: Cucurbitaceae) commonly known as pumpkin in English and Kaddu in Hindi is an annual herb. It is used as a vegetable and also in the traditional system of medicine.[7] Its fruits are the most valuable part with high nutritional value.[8] Its seeds have been identified as an effective antimicrobial agent.[9] Its seeds have also been explored by our research group for the first time for their antidiabetic effect *in vitro* by assessing their role

* Corresponding author.
E-mail address: geetawatal@gmail.com (G. Watal).
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involved in α-amylase and α-glucosidase inhibitory activities and was found to be of high impact.[10] Preliminary phytochemical screening of the *C. maxima* seeds reveals the presence of alkaloids, tannins, saponins, proteins, carbohydrates and glycosides in the same extract.[9]

Moreover, the present study which describes *in vivo* antidiabetic efficacy of *C. maxima* seeds in normal as well as diabetic models is also the first reporting of its type and was taken into consideration for validation of above mentioned *in vitro* findings of the extract's involvement in carbohydrate metabolism. However, the results of the present study clearly reveals that the seeds of *C. maxima* could be developed as antidiabetic agent having significant impact on lowering of enhanced BGL.

2. Materials and methods

2.1. Materials

Streptozotocin was purchased from Sigma–Aldrich, New Delhi, India. BGL for FBG, GTT and PPG studies was assessed by SD code-free blood glucose meter purchased from SD Biosensor Healthcare Pvt. Ltd., Gurgaon, India.

2.2. Preparation of CMSE

The seeds of *C. maxima* plant were procured from the local market of Allahabad, India and authenticated by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been submitted to the University herbarium (No. MRL/CM/01). The seeds were washed well with water and dried in shade. The shade dried seeds were powdered and extracted with hot distilled water. Extract obtained was filtered, concentrated and lyophilized till constant weight. The dry powder so obtained of CMSE was stored at −40 °C for further use during experimental study.

2.3. Experimental animals

Albino Wistar rats of the same age group and body weight 150–200 g were selected for the experiments. Animals obtained from the National Institute of Communicable Disease (NICD), New Delhi, India were housed in polypropylene cages at an ambient temperature of 25–30 °C and 45–55% relative humidity with a 12 h each dark and light cycle. Animals were fed pellet diet (Paramount Techno Chem, Lanka, Varanasi, India) and water *ad libitum*. The study was approved by the Institutional Ethical Committee (Reg. No. 839/a/04/CPCSEA). Diabetes was induced to overnight fasted rats by a single intraperitonial injection of freshly prepared STZ 50 mg kg^{-1} bw in 0.1 M citrate buffer (pH = 4.5).[11] After 3 days of STZ administration, rats with marked hyperglycemia were selected for the study.[12] The rats with hyperglycemia were divided into two groups of 36 rats each: mild diabetic animals with FBG 150–200 mg $dL^{-1}$and PPG > 250 mg dL^{-1} and severely diabetic rats with FBG > 250 mg $dL^{-1}$and PPG > 350 mg dL^{-1}.

2.4. Experimental design

Initial screening of the CMSE for the hypoglycemic activity was done with a range of variable doses in normal healthy rats by conducting FBG and GTT studies. The antidiabetic effect was assessed in mild diabetic models with the same range of doses based on similar studies of FBG and GTT.[13] The most effective dose found during mild diabetic studies was administered to severely diabetic rats once daily for 28 days to determine its effect during long-term treatment.[14]

2.4.1. Hypoglycemic activity assays in normal healthy rats

Overnight fasted normal rats were used in the experiment for FBG and GTT studies. Group I served as control treated with distilled water only, whereas the animals of groups II, III, IV, V and VI were treated with lyophilized extract suspended in distilled water at doses 50, 100, 150, 200 and 250 mg kg^{-1}, respectively. FBG levels of all the groups were checked at 2, 4, and 6 h after treatment. For GTT studies the CMSE was given orally to different groups of overnight fasted normal healthy animals and the FBG was checked at 1.5 h and treated as 0 h value for GTT. The animals were then given 2 g kg^{-1} of glucose orally. The glucose tolerance was studied for next 3 h at regular intervals of 1 h each. Thus, the total period of blood collection was up to 5 h.

2.4.2. Antidiabetic activity assay in mild diabetic rats

The antidiabetic effect of CMSE in mild diabetic rats was also assessed by improvement in glucose tolerance. The rats were divided into six groups. Group I control, received vehicle (distilled water) only, whereas variable doses of 50, 100,150, 200 and 250 mg kg^{-1} of CMSE extract were given orally to groups II, III, IV, V and VI, respectively. BGLs were first checked after 90 min of FBG, considered as 0 h value, and then 2 g kg^{-1} glucose were given orally to all the groups. Blood glucose levels were further checked up to 3 h at regular intervals of 1 h each, considered as 1, 2, and 3 h values. The results were compared with group VII rats, which were treated with 2.5 mg kg^{-1} of glibenclamide (synthetic hypoglycemic agent).

2.4.3. Antidiabetic activity assay in severely diabetic rats

FBG and PPG based long-term study of severely diabetic rats: Three groups of six rats each were used in the experiment. Group I served as SD control received distilled water only, group II received extract at a dose of 200 mg kg^{-1} and group III served as positive control received glipizide at a dose of 2.5 mg kg^{-1} as a reference drug. All the groups were treated once a day up to 28 days. Blood samples were collected at the beginning and then weekly up to 28 days and levels of FBG and PPG were assessed.

2.5. LD_{50} experiment

The toxic effect of the CMSE was also studied by a LD_{50} experiment. Two groups of rats of both sexes (six animals per group, three females and three males), weighing about 180–200 g, were orally treated with a single dose of 2 and 3 g of the CMSE. Then, rats were observed for gross behavioral, neurologic, autonomic and toxic effects continuously. Food consumption, feces and urine were also examined at 2 h and then at 6 h intervals for 24 h.

2.6. Statistical analysis

Data were statistically evaluated using two-way ANOVA, followed by a post hoc Scheffe's test considered significant when $p < 0.001$.

3. Results

3.1. FBG and GTT studies of normal healthy rats

Table 1 and Table 2 depict the hypoglycemic effect of an oral treatment of variable doses of CMSE in normal healthy rats during FBG and GTT studies respectively. Treated rats showed a regular fall of 16.66, 17.54, 18.55 and 29.02% from the doses of 50, 100, 150 and 200 mg kg^{-1}, respectively, after 6 h during FBG studies. However, a fall of only 16.73% was observed with an increased dose of 250 mg kg^{-1} after the same interval of time.

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