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Original Article

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

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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<sup>-1</sup> 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<sup>-1</sup> 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.

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## 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.<sup>1</sup> 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<sup>2–6</sup> 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.<sup>7</sup> Its fruits are the most valuable part with high nutritional value.<sup>8</sup> Its seeds have been identified as an effective antimicrobial agent.<sup>9</sup> Its seeds have also been explored by our research group for the first time for their antidiabetic effect *in vitro* by assessing their role

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involved in  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitory activities and was found to be of high impact.<sup>10</sup> Preliminary phytochemical screening of the *C. maxima* seeds reveals the presence of alkaloids, tannins, saponins, proteins, carbohydrates and glycosides in the same extract.<sup>9</sup>

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  $-40^{\circ}\text{C}$  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  $25\text{--}30^{\circ}\text{C}$  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 intraperitoneal injection of freshly prepared STZ  $50\text{ mg kg}^{-1}\text{ bw}$  in 0.1 M citrate buffer ( $\text{pH} = 4.5$ ).<sup>11</sup> After 3 days of STZ administration, rats with marked hyperglycemia were selected for the study.<sup>12</sup> The rats with hyperglycemia were divided into two groups of 36 rats each: mild diabetic animals with FBG  $150\text{--}200\text{ mg dL}^{-1}$  and PPG  $> 250\text{ mg dL}^{-1}$  and severely diabetic rats with FBG  $> 250\text{ mg dL}^{-1}$  and PPG  $> 350\text{ mg dL}^{-1}$ .

### 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.<sup>13</sup> 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.<sup>14</sup>

### 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  $250\text{ mg kg}^{-1}$ , 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  $2\text{ g kg}^{-1}$  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  $250\text{ mg kg}^{-1}$  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  $2\text{ g kg}^{-1}$  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  $2.5\text{ mg kg}^{-1}$  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  $200\text{ mg kg}^{-1}$  and group III served as positive control received glipizide at a dose of  $2.5\text{ mg kg}^{-1}$  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<sub>50</sub> experiment

The toxic effect of the CMSE was also studied by a LD<sub>50</sub> 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  $200\text{ mg kg}^{-1}$ , respectively, after 6 h during FBG studies. However, a fall of only 16.73% was observed with an increased dose of  $250\text{ mg kg}^{-1}$  after the same interval of time.

**Table 1**  
Effect of CMSE on BGL of normal healthy rats during FBG studies (mean  $\pm$  SD).

Groups	Extract (mg kg <sup>-1</sup> bw)	Blood glucose levels (mg dL <sup>-1</sup> )			
		Pre-treatment FBG	Post-treatment (h)		
			2	4	6
I	Control	98 $\pm$ 2.51	96 $\pm$ 4.16	95 $\pm$ 3.51	94 $\pm$ 3.05
II	50	90 $\pm$ 2.08	81 $\pm$ 1.52	73 $\pm$ 4.04	66 $\pm$ 3.51
III	100	88 $\pm$ 1.25	85 $\pm$ 2.01	68 $\pm$ 4.58	62 $\pm$ 2.64
IV	150	86 $\pm$ 3.05	81 $\pm$ 4.58	63 $\pm$ 3.05**	58 $\pm$ 2.08**
V	200	86 $\pm$ 2.64	78 $\pm$ 4.50	60 $\pm$ 2.51**	49 $\pm$ 1.15***
VI	250	83 $\pm$ 3.51	79 $\pm$ 3.78	63 $\pm$ 3.51	55 $\pm$ 2.30

\*\*p < 0.01, \*\*\*p < 0.001 as compared with control.

For GTT studies the similar set of doses of CMSE was given orally to overnight fasted rats and FBG was checked after 2 h considered as 0 h value before giving 4 g kg<sup>-1</sup> of glucose. The fall was observed up to 3 h after glucose administration at 1 h intervals and the results reveal that the percentage fall in BGLs was regular up to the dose of 200 mg kg<sup>-1</sup> and reached its maximum at 23.23%. Moreover, the fall of 16.50, 17.46, 18.42 and 15.53% was observed with the dose of 50, 100, 150 and 250 mg kg<sup>-1</sup> at the same interval of time.

### 3.2. GTT studies of mild diabetic rats

Fig. 1 illustrates the antidiabetic effect of variable doses of CMSE in mild diabetic rats. Fall of 4.61, 15.76, 19.23, 26.15 and 23.07% in BGLs of mild diabetic animals was observed after 3 h of glucose administration with doses of 50, 100, 150, 200 and 250 mg kg<sup>-1</sup>, respectively. However, the dose of 2.5 mg kg<sup>-1</sup> of standard drug, glibenclamide reduced BGL only by 25% which was lesser than the maximum fall observed with the dose of 200 mg kg<sup>-1</sup> at the same interval of time.

### 3.3. FBG and PPG studies of severely diabetic rats

Table 3 shows the impact of the most effective dose identified as 200 mg kg<sup>-1</sup> on BGL of severely diabetic rate after long term treatment of 28 days.

A significant reduction of 39.33% and 33.20% was observed in their FBG and PPG levels at the end of the treatment. Whereas, in case of positive control, rats treated with 2.5 mg kg<sup>-1</sup> of glipizide, FBG level has shown fall of 42.9% and PPG level has show fall of 44.2% after 28 days. Moreover, BGL of normal control was maintain through out the treatment while BGL of SD control was continuously enhancing.

### 3.4. LD<sub>50</sub>

The experiment was carried out on normal healthy rats. The behavior of the treated rats appeared normal. No toxic effect was

reported at doses up to 10 times and 15 times. The effective dose of the CMSE, and there was no death in any of these groups.

## 4. Discussion

CMSE had been used in Indian traditional system of medicine since long but no scientific data has been reported so far for its glycemic profile *in vivo*. Hence, this study deals with complete screening of its glycemic attribute based on FBG and GTT studies in normal as well as STZ-induced diabetic animal models. The observed difference between initial and final BGLs of different groups of animals on treatment with variable doses of CMSE during these studies revealed a significant reduction in BGL of treated groups as compared with the control. Maximum hypoglycemic effect was observed at 6 h and maximum glucose tolerance was observed at 3 h in case of Normal rats. Maximum antidiabetic effect was also observed at 3 h in case of mild diabetic rats based on their GTT studies. It is interesting to note that the maximum hypoglycemic effect as well as maximum antidiabetic effect, both were associated with the dose of 200 mg kg<sup>-1</sup>. Moreover, this identified most effective dose of CMSE of 200 mg kg<sup>-1</sup> also showed the maximum reduction in BGL of severely diabetic rats on its long term treatment of 28 days. The antidiabetic effect of the dose of 200 mg kg<sup>-1</sup> of the CMSE was found to be even more effective than the dose of 2.5 mg kg<sup>-1</sup> of glibenclamide in case of mild diabetic rats. Though, the effectiveness of the extract in severely diabetic rats was almost at par as compared with the synthetic drug, glipizide during FBG studies. Moreover, during PPG studies the fall produced by CMSE was found little lesser as compared to glipizide. Since, glipizide has been used to treat diabetes, by stimulating insulin secretion from pancreatic beta cells, therefore results of CMSE which are comparable with those of glipizide suggest that the mechanism of action of this plant is somewhat similar to the reference drug. Thus, the plausible mechanism by which CMSE decrease blood sugar level may be by increasing the pancreatic secretion of insulin from beta cells of islets of langerhans.

It is generally accepted that the sulfonylureas, including glibenclamide, produce hypoglycemia in normal as well as diabetic animals by stimulating the pancreatic  $\beta$ -cells to release more insulin.<sup>15,16</sup> Hence, the significant reduction as shown in BGLs of diabetic rats treated with the CMSE as well as glibenclamide may be due to stimulation of the residual pancreatic mechanism, probably by increasing peripheral utilization of glucose.<sup>17</sup> This validates the efficacy of the extract to control elevated blood sugar levels.

Moreover, phytochemical analysis of the *C. maxima* seeds reveals the presence of number of phytoconstituents viz. alkaloids, tannins, saponins, proteins, carbohydrates and glycosides in the same extract<sup>9</sup> and hence it could be presumed that the synergistic effect of these may be responsible for bioactivities of CMSE as none of these was found active independently.

**Table 2**  
Effect of CMSE on BGL of Normal healthy rat during GTT studies (mean  $\pm$  SD).

Groups	Extract (mg kg <sup>-1</sup> bw)	Blood glucose levels (mg dL <sup>-1</sup> )				
		Pre-treatment FBG	Post-treatment (h)			
			0	1	2	3
I	Control	93 $\pm$ 1.15	91 $\pm$ 2.64	118 $\pm$ 1.73	109 $\pm$ 2.88	104 $\pm$ 1.15
II	50	92 $\pm$ 0.57	90 $\pm$ 2.88	98 $\pm$ 2.64	94 $\pm$ 3.05	91 $\pm$ 3.21
III	100	92 $\pm$ 1.73	91 $\pm$ 3.46	97 $\pm$ 02.08	93 $\pm$ 3.51	90 $\pm$ 0.57***
IV	150	91 $\pm$ 2.88	88 $\pm$ 1.73	96 $\pm$ 0.57	92 $\pm$ 0.57***	89 $\pm$ 2.64**
V	200	86 $\pm$ 2.30	80 $\pm$ 1.15	95 $\pm$ 4.50	88 $\pm$ 2.64**	84 $\pm$ 3.05
VI	250	90 $\pm$ 2.08	87 $\pm$ 2.08	100 $\pm$ 3.21	90 $\pm$ 3.21	92 $\pm$ 4.04

\*\*p < 0.01, \*\*\*p < 0.001 as compared with control.

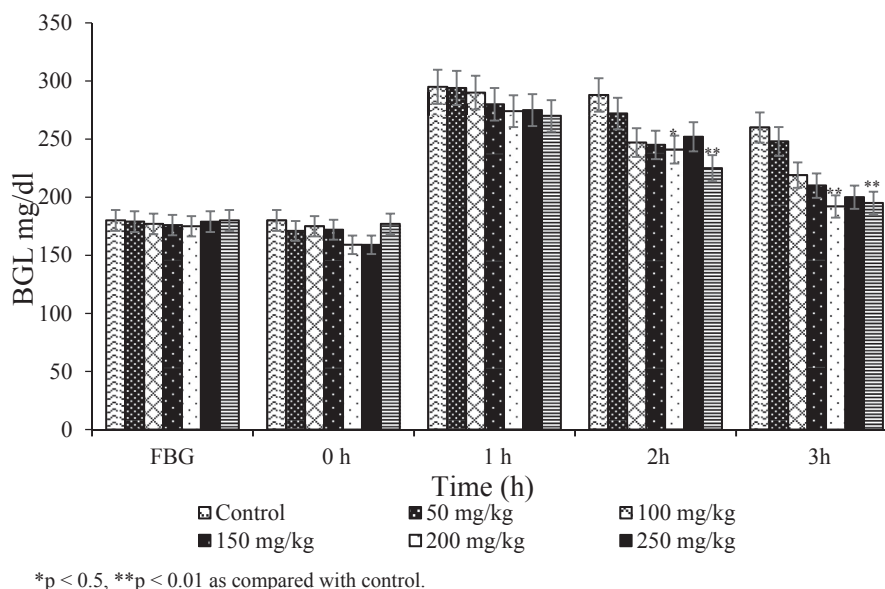


Fig. 1. Effect of CMSE on BGL of mild diabetic rats during GTT.

Table 3

Effect of long term treatment of CMSE on BGL of severely diabetic rats (mean  $\pm$  S.D.).

Experimental animals & Treatment	Doses	Pre-treatment levels	Post-treatment levels			
			7 days	14 days	21 days	28 days
<b>FBG (mg/dl)</b>						
Normal (control)	D W	80 $\pm$ 4.9	82 $\pm$ 3.2	78 $\pm$ 5.2	79 $\pm$ 5.3	80 $\pm$ 4.7
SD (control)	D W	452 $\pm$ 3.8	477 $\pm$ 5.1	512 $\pm$ 4.6	538 $\pm$ 4.2	548 $\pm$ 5.8
SD (treated with CMSE)	200 mg kg <sup>-1</sup>	300 $\pm$ 4.9	249 $\pm$ 3.8**	240 $\pm$ 4.1	235 $\pm$ 6.3	182 $\pm$ 5.5*
SD (treated with Glipizide)	2.5 mg kg <sup>-1</sup>	312.4 $\pm$ 6.7	301.2 $\pm$ 5.9*	288.4 $\pm$ 7.4	205 $\pm$ 7.3	178.4 $\pm$ 7.2
						<b>42.9% Fall</b>
<b>PPG (mg/dl)</b>						
Normal (control)	D W	172.5 $\pm$ 5.4	171.4 $\pm$ 4.6	171.6 $\pm$ 5.7	172.3 $\pm$ 4.8	170.2 $\pm$ 4.8
SD (control)	D W	480 $\pm$ 5.2	500 $\pm$ 4.9	525 $\pm$ 5.1	550 $\pm$ 5.2	550 $\pm$ 3.8
SD (treated with CMSE)	50 mg kg <sup>-1</sup>	509 $\pm$ 7.6	523 $\pm$ 5.2**	445 $\pm$ 6.6	395 $\pm$ 7.1	340 $\pm$ 7.3
SD (treated with Glipizide)	2.5 mg kg <sup>-1</sup>	412.6 $\pm$ 7.0	376.5 $\pm$ 8.3	309.2 $\pm$ 8.1	270.4 $\pm$ 7.6	230.31 $\pm$ 7.3
						<b>44.2% Fall</b>

\*p < 0.5, \*\*p < 0.01 as compared with control.

## 5. Conclusion

Conclusively it could be stated that the results of the present study are quite evident in support of the traditional use of *C. maxima* as a remedy for diabetes. *C. maxima* seeds have high hypoglycemic as well as antihyperglycemic profile, managing thereby BGL of normal, mild and severely diabetic models. Thus, the study could be further explored for developing CMSE as a herbal remedy for diabetic people.

## Declaration of interest

The authors declare no competing interests. The authors alone are responsible for the content and writing of the paper.

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