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Impact of *Cucurbita maxima* Seed Extract on Hepatorenal Profile of Severely Diabetic Rats

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Abstract

Aims: Antidiabetic potential of *Cucurbita maxima* seeds extract (CMSE) has already been published by our research group. So, the present study deals with the impact of CMSE on hepatic and renal profile of diabetic rats to validate our previous findings. **Methods**: The long term study of four weeks with the most effective dose of 200 mg kg^{-1} of CMSE was carried out to assess its hepatoprotective and renal protective impact on streptozotocin (STZ) induced severely diabetic rats. The enzymes taken into consideration were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALKP) and creatinine (CRTN) in addition to hemoglobin (Hb) and total protein (TPR). **Results**: The maximum fall shown by CMSE in levels of AST, ALT, ALKP, CRTN was 45.9, 47.7, 40.4, 49.5% respectively. Whereas, in case of positive control, treated with 2.5 mg kg^{-1} of glipizide, the maximum fall was of 35.2, 40.9, 38.7, 30.0% in case of above enzymes respectively. Moreover, CMSE showed a maximum rise of 39.6 and 59.1% in Hb and TPR levels respectively. Whereas, glipizide treated rats showed much less improvement in Hb and TPR levels with a rise of only 7.3 and 20.3% respectively. The untreated diabetic rats continued to show enhanced levels of AST, ALT, ALKP and CRTN in addition to slight percentage fall in Hb and TPR levels. **Conclusion**: Thus, the present study not only reveals the impact of CMSE on hepatic and renal profiles of severely diabetic rats but also validates the previous reports of hypoglycemic and antidiabetic effects of CMSE.

Keywords

Cucurbita maxima, Hepatoprotective, Renal protective, Seeds, Streptozotocin.

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INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia associated with either deficiency in insulin secretion or insulin action or both. Constant hyperglycemia leads magnification of many complications which can affect multiple vital organs [1]. Liver and kidney are important organs of the body as they play key role in managing postprandial hyperglycemia and regulating the water fluid levels [2]. The biological markers of liver and kidney function viz., AST, ALT, ALKP and CRTN were taken in to consideration for evaluating the impact of CMSE

on hepatorenal profile of severely diabetic rats. Enhanced level of AST, ALT, ALKP and CRTN as hepatorenal functioning markers, is a clear indication of hyperglycemia [3]. Antidiabetic phytomedicines can counteract the harmful toxic side effects of synthesized drugs by providing by enhancing hepatorenal protective efficacy at low cost. Indeed, a number of *in vivo* studies have shown that plants can be used as chemopreventive agents against hepatic and renal damage [4, 5]. The current modern approach to treat diabetes, including insulin and various oral antidiabetic synthetic drugs, has demonstrated its limits, with unsatisfactory results and many side effects [6]. *C. maxima* Duch. (Family: Cucurbitaceae) commonly known as pumpkin in English and Kaddu in Hindi is an annual herb. Its fruits are the most valuable part with high nutritional value [7]. Its seeds have been identified as an effective antimicrobial agent [8]. Its seeds have also been explored by our research group for their antidiabetic [9, 10], antihyperlipidemic [11] and antioxidant potential [12]. Thus, the present study was taken into consideration for evaluating the hepatorenal protective effect of CMSE on STZ induced severely diabetic rats.

MATERIAL AND METHODS

Plant material and 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^{0}C for further use during experimental study.

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 Diseases (NICD), New Delhi, India were housed in polypropylene cages at an ambient temperature of 25-30^{0}C and 45-55% relative humidity with a 12 h each of dark and light cycles. 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 bya single intraperitonial injection of freshly prepared STZ of dose 50 mg kg^{-1} bw in 0.1 M citrate buffer (pH 4.5) [13]. After 3 days of STZ administration, rats with FBG > 250 mg dL^{-1} and PPG > 350 mg dL^{-1} were categorised into severely diabetic rats and selected for the study [14].

Estimations

STZ was purchased from Sigma-Aldrich, New Delhi, India. Serum levels of Aspartate Amino Transferase (AST), Alanine amino Transferase (ALT), Alkaline phosphatase (ALKP), Creatinine (CRTN) and Total protein (TPR) were estimated by using standard kit of Erba Diagnostics, Mumbai, India [15-18]. Hb in blood was also detected by Hemocor-D reagent [19]. All the parameters were measured initially before as well as after the treatment.

Experimental design

CMSE was already assessed by our research group for their hypoglycemic and antidiabetic effects in normal as well as mild diabetic rats, respectively. The dose of 200 mg kg^{-1} of CMSE which was identified as the most effective dose during the studies of mild diabetic rats was administered to severely diabetic rats once daily for 28 days. Their fasting blood glucose (FBG) and post prandial glucose (PPG) studies showed noticeable hypoglycemic as well as antidiabetic effects. CMSE also possess significant antihyperlipidemic potential. These results encouraged us to assess the extract for its hepatic and renal profile in severely diabetic rats during long term treatment of 28 days. Other additional parameters viz. Hb and TPR were also taken into consideration for validating the results.

Assessment of hepatic, renal and other related bioactivities in severely diabetic rats

Three groups of six rats each of severely diabetic rats were used in the experiment. Group I was of normal rats served as control received vehicle (distilled water) only, Group II served as diabetic control received vehicle (distilled water) only whereas Groups III was treated daily with a single oral administration of 200 mg kg^{-1} identified as the most effective dose of CMSE and Group IV received glipizide (2.5 mg kg^{-1}) once a day, as a reference drug, for 28 days.

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 CMSE. Thereafter, rats were observed for gross behavioural, neurologic, autonomic and toxic effects continuously. Food consumption, faeces and urine were also examined at 2 h and then at 6 h intervals for 24 h.

Statistical analysis

The entire group of data was statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe's test using the SPSS computer software, version 7.5. The values were considered significant when P<0.001. Experiments were done in triplicate and the mean value was reported as mean ± S.D.

RESULTS

AST, ALT and ALKP studies of Severely diabetic rats

Table 1 demonstrates the hepatoprotective effect of CMSE on hepatic enzymes viz. AST, ALT and ALKP in blood serum of severely diabetic rats. The results reveal that CMSE significantly reduced hepatic enzyme levels in treated rats as compared to diabetic control rats. The most effective dose of CMSE produced a fall of 45.9, 47.7, 40.4% in AST, ALT and ALKP enzyme levels respectively after 28 days of treatment. Whereas, the fall observed in case of Glipizide treated animals, was of only 35.2, 40.9 and 38.7% in AST, ALT and ALKP levels.

CRTN, Hb and TPR studies of severely diabetic rats

Table 2 shows the effect of CMSE on renal enzymatic profile i.e. on CRTN level in addition to Hb and TPR. Diabetic rats treated with the extract showed reduced level of CRTN as compared to untreated diabetic rats. The most effective dose of CMSE showed the fall of 49.5% in CRTN level. A significant rise of 59.1% was observed in TPR level treated with CMSE. A noteworthy improvement of 39.6% in Hb level was observed in treated rats. Whereas, Glipizide improved CRTN level by a fall of 30.0% only. Glipizide treated rats also showed little improvement in Hb and TPR levels by a rise of 7.3 and 20.3% respectively.

LD_{50} studies

LD_{50} experiment was carried out on normal healthy rats. The behaviour of the treated rats was found to appear normal. No toxic effect was reported at doses up to 10 and 15 times of the effective dose of CMSE and there was no death reported in any of these groups.

Table 1: Effect of *C. maxima* on Hepatic profile of Severely diabetic rats

Groups	Doses	0 Days	7 Days	14 Days	21 Days	28 Days
AST/SGPT (U/L)						
Normal Control	DW	22.3±2.4	22.1±2.6	22.6±2.8	22.0±2.1	21.9±2.5
SD Control	DW	42.7±3.4	43.1±3.2	45.3±3.2	49.8±3.9	55.9±4.3
SD Treated	200mg/kg	34.8±4.5	30.2±2.3	26.0±3.9	20.7±3.2	18.8±2.6**
(*C. maxima*)						**Fall 45.9%**
SD Treated (Glipizide)	2.5mg/kg	37.2±3.3	34.2±2.8	30.1±2.1	28.5±3.1	24.1±2.9
						Fall 35.2%
ALT/SGOT (U/L)						
Normal Control	DW	19.7±3.9	19.1±3.1	20.1±4.2	20.7±2.5	19.9±2.9
SD Control	DW	33.9±3.7	36.9±4.1	38.5±4.2	40.3±3.8	42.7±3.1
SD Treated	200mg/kg	32.1±3.6	28.7±2.3	22.5±2.2	19.9±2.7	16.8±3.0**
(*C. maxima*)						**Fall 47.7%**
SD Treated (Glipizide)	2.5mg/kg	35.4±2.3	30.7±2.2	26.2±2.4	23.5±2.5	20.9±2.3
						Fall 40.9%
ALKP(U/L)						
Normal Control	DW	112.4±4.5	112.2±2.6	113.5±3.9	112.1±3.6	112.7±4.3
SD Control	DW	145.7±5.5	146.6±4.6	148.7±4.9	150.1±5.9	152.6±6.2
SD Treated	200mg/kg	167.8±4.6	158.0±4.5	145.0±4.9	131.1±2.8	99.9±3.3**
(*C. maxima*)						**Fall 40.4%**
SD Treated (Glipizide)	2.5mg/kg	156.4±2.9	138.7±3.1	115.2±3.7	98.7±3.5	80.2±2.9
						Fall 38.7%

****p < 0.01 as compared with control**

Table 2: Effect of *C. maxima* seeds on CRTN, Hb and TPR profile of Severely diabetic rats

Groups	Doses	0 Days	7 Days	14 Days	21 Days	28 Days
CRTN (U/L)						
Normal Control	DW	0.8±0.2	0.8±0.4	0.7±0.3	0.9±0.3	0.8±0.2
SD Control	DW	2.7±0.5	2.6±0.6	2.4±0.4	2.6±0.4	2.9±0.2
SD Treated (*C. maxima*)	200mg/kg	2.1±0.4	1.8±0.5	1.6±0.9	1.4±0.5	1.0±1.0*** **Fall 49.5%**
SD Treated (Glipizide)	2.5mg/kg	2.0±0.4	1.9±0.3	1.7±0.4	1.6±0.3	1.4±0.2 **Fall 30.0%**
Hb (g/dl)						
Normal Control	DW	12.3±2.4	12.0±3.1	12.2±2.1	12.3±2.2	12.5±2.2
SD Control	DW	9.5±3.7	9.9±2.6	8.4±2.4	8.2±2.5	7.3±2.7
SD Treated (*C. maxima*)	200mg/kg	8.7±0.8	9.7±0.4	11.9±1.6	12.8±0.6	14.5±1.3*** **Rise 39.6%**
SD Treated (Glipizide)	2.5mg/kg	9.5±0.5	9.7±0.4	9.9±0.7	9.9±0.6	10.2±0.5 **Rise 7.3%**
TPR (mg/dl)						
Normal Control	DW	8.9±1.8	8.7±1.2	8.5±1.4	8.8±2.1	8.9±2.2
SD Control	DW	5.6±2.6	5.1±2.5	5.3±1.7	5.7±2.3	5.6±1.5
SD Treated (*C. maxima*)	200mg/kg	4.7±0.9	7.1±0.5	8.8±1.1	9.8±1.5	11.5±1.0*** **Rise 59.1%**
SD Treated (Glipizide)	2.5mg/kg	5.4±0.5	5.9±0.4	5.9±0.3	6.3±0.4	6.5±0.3 **Rise 20.3%**

***p < 0.001 as compared with control**

DISCUSSION

The challenge in treatment of diabetes is to treat its related complication as well. The chief diabetic complications are hepatic and renal injury. The ability of a hepatoprotective drug is to preserve the activities of liver marker enzymes SGOT, SGPT and ALKP. The level of these enzymes rises dramatically in acute liver damage due to diabetes. The increased level of AST, ALT and ALKP therefore, increases the incidence of liver failure. In the present study, all treated groups with *C. maxima* seeds were found to reduce the enhanced activities of AST, ALT and ALKP effectively in severely diabetic rats, thereby suggesting that *C. maxima* seeds may prevent hepatic injury associated with diabetes.

Creatinine is chiefly filtered out of the blood by the kidneys and high level of it indicates towards renal dysfunction due to diabetes. Oral administration of *C. maxima* seeds ameliorated the impaired renal function as evidenced from the declined levels of CRTN in treated groups. It was however, observed that *C. maxima* seeds rendered not only greater renal protection by lowering the CRTN level significantly but also improved the levels of Hb and TPR in comparison to the standard drug, Glipizide.

Other important parameters such as Hb and TP which are generally lower than their normal values in chronic diabetic cases, were also controlled by the long term treatment of *C. maxima* seeds which was another additional benefit of the treatment. Moreover, the percentage rise in TP and Hb levels was found to be more in case of *C. maxima* seeds treated rats as compared to the rats treated with the standard drug, Glipizide. Since, high LD_{50} value indicates high margin of safety therefore the extract can be effectively used to control diabetes and its related complications.

CONCLUSION

These results conclude that our choice of selecting this plant and specially seeds as its part was the right choice for rational drug development for diabetes. This study also provided a new therapeutic avenue against human diabetes and diabetes related complications.

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