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Antidiabetic potential of a novel hypoglycemic active principle from bittergourd (*Momordica charantia*) seeds in alloxan-induced diabetic rats.

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Abstract-The present study evaluates antidiabetic potential of fractionated bittergourd (*Momordica charantia*) seed extracts in alloxan-induced diabetic rats. A novel hypoglycemic active principle (MCK3P8) obtained from a fraction of the ethanolic extract (MCK3) of bittergourd (*Momordica charantia*) seeds, given by intraperitoneal injection to alloxan-induced diabetic rats at a dose of 15 mg/kg b.wt., showed a significant hypoglycemic activity. Loss in hypoglycemic activity of the MCK3P8 upon proteinase-K treatment indicates the proteinaceous nature of the novel hypoglycemic active principle.

Keywords: Bittergourd (*Momordica charantia*), Antidiabetic potential, Hypoglycemic activity, alloxan-induced diabetic rats.

INTRODUCTION

1. Use in traditional medicine

Momordica charantia L. commonly known as bittergourd, is one of the most used plants for the treatment of diabetes and some of its late complex abnormalities including nephropathy, neuropathy and retinopathy etc. Bittergourd whole fruit, fruit pulp, seeds in diabetes mellitus¹⁻⁵, roots as an abortifacient⁶ and the leaves for hypoglycemic activity in diabetic animals⁷.

2. Previously isolated constituents

Sterols, charantin, momordicine⁸⁻¹⁰, cardenolides¹¹, and polypeptide-P.¹²

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MATERIAL AND METHODS

1. Plant Material

Momordica charantia L. (Cucurbitaceae), seeds purchased from sales counter of Indian Agriculture Research Institute (IARI), Pusa Road, New Delhi in August 2010, in large quantity to maintain the consistency of the stock for extract preparation and was authenticated by the Taxonomist of University Department of Botany, Patna University, Patna, Bihar, India. A voucher specimen is deposited in the Department of Biochemistry of the Patna University, Patna, Bihar, India.

2. Chemical

All the chemicals were of analytical grade and were procured from Sigma Aldrich Chemical Co., USA, or Boehringer - Mannheim, Germany, unless otherwise stated.

Protamine Zinc Insulin was procured from Boots Pharmaceuticals Ltd., India.

3. Animal

Random bred male wistar rats, 175-200 g (12-14 weeks), were housed in standard laboratory conditions, in the small animal facility of Department of Biochemistry of the Patna University, Patna, Bihar, India. The animals were provided with rat feed (Hindustan Liver Ltd, India) and water ad libitum.

4. Induction of Diabetes

The male wistar rats were made diabetic by using alloxan. Briefly, alloxan was administered i.e. after starving the animals for 36 hrs at a dose of 150 mg/kg b.wt. Animals were stabilized for 3 days by insulin administration, 1-2 units per day for 2 days. Only those animals having blood glucose level more than 300 mg/100 mL blood were selected for further analysis.

5. Tested Material

From decorticated bittergourd (*Momordica charantia*) seeds, fraction MCK3 was obtained from ice cold ethanol extract (75% C₂H₅OH, 1 mM PMSF, 0.2 N HCl), centrifuged and concentrated in speed vac at 4°C. The supernatant was neutralized with (NH₄)₂CO₃ to pH 7.2 and centrifuged with liquid ammonia. The supernatant, fraction MCK3 was further subjected to differential precipitation with (NH₄)₂SO₄ containing 0.25% TCA which resulted in precipitation of all protein.

The hypoglycemic MCK3P8 was obtained from the fraction MCK3 (14 ml containing 196 mg of proteins) by gel filtration CC with Sephacryl S100 eluting with 0.2 M NH₄HCO₃ (pH 7.2-7.4). Bioactivity of the fractions was measured at each step of purification.

6. Statistical Analysis

All the results were analyzed statistically using student's paired t-test for paired data of different levels of significance. All the results were expressed as mean S.E. P values less than 0.05 were considered significant. N represents number of experimental animals.

RESULTS & DISCUSSION

The present study reports purification of a hypoglycemic active principle from bittergourd (*Momordica charantia* seeds). Hypoglycemic activities studied in control, insulin, fraction MCK3, MCK3P8, Proteinase-K plus MCK3P8 treated alloxan - induced diabetic rat by measuring the blood glucose level enzymatically.¹³ by drawing blood from the tale vein during the study period. Results are reported in Table 1.

The hypoglycemic active principle (MCK3P8) was able to bring down the blood glucose level significantly by 3 hours after administration. The hypoglycemic activity brought about by the MCK3P8 was comparable to that observed with insulin treatment of the diabetic rats.

Table 1- Hypoglycemic activity of fraction MCK3, hypoglycemic active principle (MCK3P8) and Proteinase-K treated MCK3P8 of bittergourd (*Momordica charantia*) seeds

Treatment i.p.	Blood glucose level (mg/dL)			
	0 h		3 h	
Normal control + saline (0.5 ml)	90	10.0	85	9.1
Control Diabetic + saline (0.5 ml)	347	44.92	355.4	28.20
Diabetic + insulin (5 U/kg b.wt.)	351	18.0	205	16.0 □
Diabetic + fraction K3 (15 mg/kg b.wt.)	399.7	38.5	344.76	43.88
Diabetic + MCK3P8 (15 mg/kg b.wt.)	385.04	47.86	252.9	20.24 □
Diabetic + Proteinase-K treated MCK3P8	414.3	92.4	378.16	46.95

Values are mean S.E., N=5.

C^{**} P 0.001 (Student's t-test) vs control diabetic + saline (0.5 ml).

Based on the results of this study, we conclude that the novel hypoglycemic active principle (MCK3P8) of bittergourd (*Momordica charantia*) seed, given intraperitoneally at a dose 15mg/kg b.wt., possesses significant hypoglycemic activity in alloxan-induced diabetic rats, and it is proteinaceous in nature.

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