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Effect of a Novel Antihyperglycemic Active Principle from the Seeds of *Momordica Charantia* Linn. on Glucose Transporters 2 and 4 in Experimental Diabetes

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Abstract: ***Background: The current study evaluates changes in glucose transporters (GLUTs) mainly GLUT2 and GLUT4 observed with a novel antihyperglycemic active principle (MCK_3P_8) purified from a fraction of the ethanolic extract MCK_3 from the seeds of Momordica charantia Linn. in alloxan- induced experimental diabetic rats. Materials and Methods: MCK_3P_8 purified from a fraction of the acid-ethanolic fraction MCK_3 of the seed extract of bittermelon seeds (14 ml containing 196 mg of proteins) by gel fitration column chromatography. This antihyperglycemic active principle and the protamine zinc insulin were given by intraperitonial injection to alloxan-induced male wistar diabetic rats at a dose of 15 mg/kg body weight of experimental animals for 20 days. Hypoglycemic activities and changes in the levels of GLUT2 and GLUT4 were studied in control, insulin, fraction MCK_3, MCK_3P_8 treated alloxan-induced diabetic rats by measuring the blood glucose level enzymatically and levels of glucose transporters 2 and 4 were determined by Enzyme-Linked Immunosorbent Assay (ELISA) drawing blood from the tail vein during the study period. Results: Results of the present study unveiled that MCK_3P_8 - the antihyperglycemic active principle of bittermelon seeds when given intraperitoneally at a dose of 15 mg / kg b. wt. exhibited significant antihyperglycemic activity along with significant decrease in the levels of by 3 hours after administration in alloxan-induced diabetic rats. Loss of hyperglycemic activity upon proteinase –K treatment indicates that MCK_3P_8 is proteinaceous in nature. Present study also indicates that MCK_3P_8 - the antihyperglycemic active principle of bittermelon seeds have significantly influenced the serum levels of glucose transporters GLUT2 and GLUT4 levels in experimental diabetes. Conclusion: The novel antihyperglycemic active principle (MCK_3P_8) of bittermelon seeds exhibited significant antihyperglycemic activity and significantly reduced the glucose transporters 2 and 4 levels in alloxan- induced experimental diabetic rats.***

Keywords: Momordica charantia, Antihyperglycemic principle, Glucose transporters. Experimental diabetes

1. Introduction

Since ancient times *Momordica charantia* Linn. the fruit-vegetable commonly known as "Karella" in Hindi, Bittermelon / Bittergourd / Balsam pear in English has drawn considerable attention from scientists as a monoherbal medicine for the treatment of diabetes mellitus [1-5], roots as an abortifacient [6] and the leaves as vitamins, minerals and antioxidants [7]. Previously isolated constituents from *Momordica charantia* include charantin, sterols, alpha and beta-momorcharins [8-9], cardenolides [10], and polypeptide-P [11]. Earlier investigations indicate that the bioactive compounds in bitter melon, including triterpenoids and conjugated linoleic acid, have been shown to have their hypoglycemic and antioxidant effects that reduce oxidative stress [12 – 13].

The reduction in oxidative stress may improve GLUT2 and GLUT4 translocation and regulation, thereby enhancing glucose uptake and homeostasis [14].

However, there are inadequate information's about regarding the effect of antihyperglycemic active principle(s) from *Momordica charantia* on glucose transporters (GLUTs) as these are essential for regulating glucose metabolism, with GLUT2 and GLUT4 serving as the primary transporters in muscle cells. , current study has been carried out to evaluate the changes brought about by a novel antihyperglycemic active principle (MCK_3P_8) which is obtained from the seeds of *Momordica charantia*, on GLUT2 and GLUT4 levels, in alloxan induced diabetic rats which is at present unclear.

2. Material and Methods

2.1 Plant Material

Seeds of *Momordica charantia* Linn. (Cucurbitaceae family) purchased from Indian Agriculture Research Institute (IARI), New Delhi sales counter in a large quantity to maintain consistency of the stock for extract preparation and was authenticated by the taxonomist of the botany department, Patna University, Patna, Bihar, India. A voucher specimen is deposited in biochemistry department, Shyamlal Chandrasekhar Medical College (SLCMC), Khagaria, Bihar, India.

2.2 Chemical

All the chemicals were of analytical grade procured from Boehringer Mannhiem, Germany, or Sigma Aldrich Chemical Company., USA unless otherwise stated. From Boots Pharmaceuticals Limited India, the Protamine Zinc Insulin was procured. For the determinations of glucose transporters 2 and 4, sandwich ELISA Kit was procured from Kohrain Biotech, China.

2.3 Animal

Randomly bred male wistar rats, of 12 - 14 weeks (175 - 200 grams), were housed in hygienic cages under standard conditions, in the small animal facility of biochemistry department, SLCMC, Khagaria, Bihar, India. The animals were fed on rat feed (balanced pellets) made by Hindustan Liver Limited, India and water *ad libitum.*

2.4 Induction of Diabetes

The male wistar rats were made diabetic by using alloxan. Briefly, alloxan was administered i.p. 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.

2.5 Tested Material

In the present study, *Momordica charantia* seeds were used for the consistency of the stock for extract preparation in order to identify the hypoglycemic principle(s). From decorticated seeds, fraction MCK_3 was obtained from ice cold ethanol extract (75% C_2H_5OH 0.2 N HCI 1 mM PMSF.), concentrated by centrifugation and in speed vac at 4^0C. The supernatant was neutralized with $(NH_4)_2CO_3$ to pH 7.2 and centrifuged with liquid ammonia. The supernatant, fraction MCK_3 was further subjected to differential precipitation with 0.25% TCA containing $(NH_4)_2SO_4$ that resulted in precipitation of all protein. The antihyperglycemic principle MCK_3P_8 was obtained from the fraction MCK_3 (14 ml containing 196 mg of proteins) by gel filtration column chromatography using sephacryl S100 eluting with 0.2 M NH_4HCO_3 (pH 7.2-7.4). At each step of purification, the bioactivity of the fractions was measured.

2.6 Antihyperglycemic activity of MCK_3P_8 and its effects on glucose transporter concentration in diabetic rats

The rats were divided into different groups (six rats in each group): Group I— saline -treated normal non-diabetic controls, Group II- saline treated diabetic rats, Group III— the diabetic rats treated with protamine zinc insulin (10 IU/kg b.wt.) and Group and IV and V diabetic rats treated with 15 mg/kg b.wt. of active principle(s), The first two groups of rats were given saline daily. The insulin and active principle(s) were administered at the selected dosage to Groups III, IV and V, respectively, every day for 20 days. The rats were bled prior to sacrifice on the last day of the treatment by cervical dislocation. The blood sample were collected through cardiac puncture and immediately transferred into plain vacuitainers for glucose estimation as well as serum levels of determination GLUT2 and GLUT4 levels were done enzymatically using sandwich ELISA method.

2.6 Statistical Analysis

All the results were analyzed statistically using Student's paired t-test for paired data with different levels of significance. The data of determinations were expressed using mean ± S.E. A probability value that was statistically significant (< P 0.05) was taken into consideration. N represents number of experimental male rats.

2.7 Ethical Clearance

The experimental protocol was duly approved by the Institute's Ethical Committee and Review Board. [Reference No.: IECRB/2025/02, BC/FM-BUHS] Experiments on animals were conducted in accordance with the Guidelines for use of laboratory animals in medical college, Indian Council of Medical Research (ICMR), New-Delhi, India.

3. Results

This solitary study as per the best of our knowledge, conducted in Bihar, state in India, that reports purification of an insulin-like proteinaceous antihyperglycemic active principle (MCK_3P_8) from *Momordica charantia* seeds. Hypoglycemic activities studied in control, insulin, fraction MCK_3, MCK_3P_8 treated alloxan-induced diabetic rat by measuring the blood glucose level enzymatically [15] during the study period. The antihyperglycemic active principle given by intraperitoneal injection to alloxan- induced diabetic rats at a dose of 15 mg/kg b.wt. exhibited significant hypoglycemic activity by 3 hours after administration. Results are reported in Table 1.

Table 1: Hypoglycemic activities of insulin, fraction MCK_3 and novel antihyperglycemic active principle MCK_3P_8 of *Momordica charantia* seeds in experimental diabetes.

Treatments→ Groups ↓	Blood glucose level (mg/dl) at 0 hour	Blood glucose level (mg/dl) at 3 hour	P - Value
Normal control +saline (0.5 ml)	91.0 ± 10.0	86.0 ± 9.5	> 0.05
Control Diabetic+ saline (0.5 ml)	348.6 ± 43.9	356.3 ± 17.2	> 0.05
Diabetic + insulin (10 IU / kg b.wt.)	353.8 ± 17.0	287.4 ± 32.4***	< 0.001***
Diabetic + fraction K_3 (15mg / kg b. wt.)	401.6 ± 39.0	277.3 ± 41.1**	< 0.001**
Diabetic + MCK_3P_8 (15mg / kg b.wt.)	394.4 ± 48.6	304.2 ± 32.9***	< 0.001***
Diabetic + Proteinase-K treated MCK_3P_8 (15mg / kg b.wt.)	410.2 ± 67.4	387.1± 45.5	> 0.05

Note: Values are shown as Mean ± S.E., Number of experimental rats N = 5; with *indicating significance at P < 0.05, ** at P < 0.01, and *** at P < 0.001.
Abbreviations: MCK_3P_8: Momordica charantia, Karella, Fraction$_3$, Peptide $_8$ Kilo Dalton.

The hypoglycemic activity brought about by the MCK_3P_8 was comparable to that observed with insulin treatment of the diabetic rats. In order to assess long term consequences of experimental diabetes on the levels of GLUT2 and GLUT4 in insulin and MCK_3P_8 treated alloxan- induced diabetic rats, a dose of 10 IU/ kg b.wt insulin and 15 mg/kg

b.wt. MCK_3P_8 respectively were given by intraperitoneal injection for 20 days. The data is seen as in Table no 2.

Table 2: Effect of novel antihyperglycemic active principle MCK_3P_8 of bittermelon (*Momordica charantia*) seeds on allergic and inflammatory markers in experimental diabetes.

Groups → Parameters ↓	Normal control + saline (0.5 ml)	Control Diabetic + saline (0.5 ml)	Diabetic + insulin (5U/kg bwt)	Diabetic + fraction K3 (15mg/kg wt)	Diabetic + MCK3P8 (15mg/kg b.wt)	P – Value
Glucose Transporter 2 (GLUT2)	15.81 ± 0.96 (<150)	22.51 ± 1.31	16.49 ± 1.62	17.90 ± 1.41	15.98 ± 1.66	< 0.05*
Glucose Transporter 4 (GLUT4)	16.23 ± 1. 16 (<6)	20.94 ± 1.23	17.23 ± 1.21	18.97 ± 1.22	16.89 ± 1.22	< 0.05*

Note: shown as Mean ± S.E., Number of experimental rats N = 5; with *indicating significance at $P < 0.05$,
Abbreviations: *Momordica charantia,* Karella, $Fraction_3$, Peptide $_{8\ Kilo\ Dalton,}$ (MCK_3P_8),

Analysis of GLUT2 levels demonstrated that administration of novel antihyperglycemic active principle MCK_3P_8 resulted in a value of 15.98 ± 1.66, which was lower and significantly different ($p < 0.05$) compared to the control diabetic group (22.51 ± 1.31). Meanwhile, the GLUT4 level in novel antihyperglycemic active principle MCK_3P_8 treated group was 16.89 ± 1.22, lower and significantly different ($p < 0.05$) compared to control diabetic group (20.94 ± 1.23**).**The serum levels of GLUT2 as well as GLUT4 mere significantly reduced ($P < 0.05$) in alloxan- induced diabetic rats when treated with novel antihyperglycemic active principle MCK_3P_8 when compared with corresponding levels in controls at the end of study period.

4. Discussion

Diabetes is the world's largest endocrine disorder and is one of the major killers in recent times.[16] It is essentially *a fulminating clinical catastrophe* and *a silent chemical deviation from the norm,* commonly referred to as a syndrome or a disorder rather than a disease. An elevated plasma glucose concentration, under certain clearly defined conditions, remains in the *sine qua non* of the diagnosis. Insulin therapy affords effective glycemic control, yet its short comings such as ineffectiveness on oral administration, short self-life, requirement of constant refrigeration, and in the event of excess dosage fatal hypoglycemia- a life-threatening situation- limits its usage. Other drawback of insulin therapy include insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment [17].The evaluation of medicinal plants, herbs and especially, of their active principle(s) is a logical way of searching for new drugs to treat diabetes and its late complications. Some phytochemical studies have revealed that *Momordica charantia,* the fruit-vegetable, is sufficiently rich in proteins. It is believed that both fruits and seeds of MC contain numerous phytochemicals, saponins, polysaccharides, triterpenes, proteins, vitamins, minerals, flavonoids, ascorbic acid, steroids and orally active insulin-like or insulinomimetic compounds. Besides, multiple biological characteristics have also been confirmed, such as antioxidant, antitumor, antibacterial, skin care, [18 - 19].

In the present study a novel insulin-like antihyperglycemic active principle (MCK_3P_8) was purified from a fraction MCK_3 obtained from the ethanolic seeds extract of *Momordica charantia* Linn. MCK_3P_8 was able to bring down the blood glucose level significantly by 3 hours after administration in alloxan-induced diabetic rats. The hypoglycemic activity brought about by MCK_3P_8 was comparable to that observed with insulin treatment of the diabetic rats.

In present study antihyperglycemic active principle MCK_3P_8 treatment. Several studies stated that secondary metabolites in bitter melon, including charantin, polypeptides, alkaloids, momordicin, flavonoids, and cucurbitane-type triterpenoids, play crucial roles in reducing blood glucose levels [20- 21].

Glucose is a primary energy source for cellular activity in living organisms. However, due to its hydrophilic property, glucose transport across the cell membrane requires a specific carrier protein. Glucose transporters (GLUTs) are transmembrane proteins that facilitate the movement of glucose across cell membranes [22]. Elevation of blood glucose level stimulates GLUT2 to transport glucose into β cells and trigger insulin secretion. Increased insulin promotes glucose uptake into muscle and adipose tissues by regulating GLUT4 translocation to the plasma membrane [23]. Therefore, GLUT2 and GLUT4 play crucial roles in the progression of diabetes mellitus. A previous research study demonstrated that several compounds in bitter melon enhance GLUT4 translocation through the AMP-activated protein kinase (AMPK) pathway in muscle cells [24]. Furthermore, another study reported that bitter melon reduces the expression of GLUT2, leading to decreased glucose reabsorption in the kidneys and increased urinary glucose excretion [25]. The bioactive compounds found in bitter melon play vital roles in reducing blood glucose levels. Specifically, charantin, which inhibits the α-glucosidase and α-amylase enzymes [26], whereas alkaloid compounds act through an insulin-like mechanism [27]. Nevertheless, studies investigating the influence of active antihyperglycemic peptides and proteins from bitter melon seeds on serum GLUT2 and GLUT4 levels in alloxan-induced diabetic rats are still scarce Therefore, the present study aimed to assess the effects of bitter melon (Momordica charantia) extract on blood glucose, as well as serum GLUT2 and GLUT4 levels, in experimental diabetes. During the present study, the control group exhibited the highest levels of GLUT2 and GLUT4, possibly due to alloxan triggering oxidative stress and lipid peroxidation, which led to hyperglycemia [28] β-cells are particularly vulnerable to oxidative stress because they contain low concentrations of endogenous antioxidant enzymes, including catalase and glutathione peroxidase [29].

5. Conclusion

The antihyperglycemic active principle MCK_3P_8 of bittermelon seeds when given intraperitoneally at a dose of

15 mg / kg b. wt. exhibited significant antihyperglycemic activity along with significant decrease in the levels of GLUT2, and GLUT4 by 3 hours after administration in alloxan-induced diabetic rats when compared with corresponding levels in controls at the end of study period.

Additionally, future studies should consider measuring GLUT levels in different organs, such as the liver, pancreas, muscles, intestines, and brain.

Conflicts of interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical considerations

The manuscript is original, and it has not been published elsewhere. All authors reviewed the original content of the manuscript. No AI tools was used during the study and writing the manuscript.

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