



EFFECT OF A NOVEL HYPOGLYCEMIC ACTIVE PRINCIPLE FROM THE SEEDS OF BITTER GOURD (*MOMORDICA CHARANTIA* LINN.) ON CARDIOVASCULAR RISK INDICATORS IN EXPERIMENTAL DIABETES

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ABSTRACT

Background: Bitter gourd (*Momordica charantia* Linn) whole fruit, fruit pulp, seeds has been used in complementary and alternative medicine for the treatment of diabetes mellitus and some of its late complex abnormalities. The current study evaluates changes in certain cardiovascular risk indicators observed with a novel hypoglycemic active principle (MCK₃P₈) purified from a fraction of the acid-ethanolic extract MCK₃ from the seeds of bitter gourd in alloxan- induced experimental diabetic rats. **Materials and Methods:** MCK₃P₈ purified from a fraction of the acid-ethanolic fraction MCK₃ of the seed extract of bitter gourd (14 ml containing 196 mg of proteins) by gel filtration column chromatography. This hypoglycemic active principle and the protamine zinc insulin were given by intraperitoneal 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 the levels of fasting blood glucose (FBG), total cholesterol (TC), HDL- cholesterol (HDL), LDL- cholesterol (LDL), VLDL- cholesterol (VLDL), triglycerides (TG), troponin- I (Trop-I), creatinine kinase- MB (CKMB), total cholesterol: HDL-cholesterol ratio, highly sensitive C reactive protein (hsCRP) in serum and atherogenic index of plasma (AIP) were studied in control, insulin, fraction MCK₃, MCK₃P₈ treated alloxan-induced diabetic rats by measuring the blood glucose level enzymatically as well as serum levels of cardiovascular risk indicators were determined by drawing blood from the tail vein during the study period. **Results:** Results of the present study unveiled that MCK₃P₈ - the hypoglycemic active principle of bitter gourd seeds when given intraperitoneally at a dose of 15 mg/ kg b. wt. exhibited significant hypoglycemic activity by 3 hours after administration in alloxan-induced diabetic rats. Loss of hyperglycemic activity upon proteinase -K treatment indicates that MCK₃P₈ is proteinaceous in nature. Present study also indicates that MCK₃P₈ - the hypoglycemic active principle of bitter gourd seeds have significantly influenced the serum levels of cardiovascular risk indicators in experimental diabetes. Biochemical parameters related to lipid profile along with troponin I, creatinine kinase MB, atherogenic index of plasma and C reactive protein, upon treatment with MCK₃P₈ resulted in significant reduction in total cholesterol, triglyceride, low density lipoproteins, very low density lipoproteins, troponin I, creatinine kinase- MB, highly sensitive C reactive protein levels, total cholesterol: HDL-cholesterol ratio and atherogenic index of plasma apart from significant elevation in the high density lipoproteins in type - I diabetic rats. **Conclusion:** The novel hypoglycemic active principle (MCK₃P₈) of bitter gourd seeds exhibited significant hypoglycemic activity and significantly reduced the levels of certain cardiovascular risk indicators in alloxan- induced experimental diabetic rats when compared with corresponding levels in controls at the end of study period.

KEYWORDS

Bitter Gourd, *Momordica Charantia*, Novel Hypoglycemic Principle, Insulin, Cardiovascular Risk Indicators. Alloxan, Experimental Diabetes

1. INTRODUCTION

Since time immemorial *Momordica charantia* Linn. the fruit-vegetable commonly known as "Karela" in Hindi, Bitter gourd / Bittermelon / 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, vicine, sterols, alpha and beta-momocharins [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]. Previous study shows that an antihyperglycemic active principle of bittermelon *Momordica charantia* Linn. seeds significantly reduced the glucose transporters 2 and 4 levels in alloxan- induced experimental diabetic rats. The reduction in oxidative stress may improve GLUT2 and GLUT4 translocation and regulation, thereby enhancing glucose uptake and homeostasis [14]. The objective of the present study was to examine the long term effects of a novel hypoglycemic active principle (MCK₃P₈) which was purified from a fractionated bitter gourd seed extract (MCK₃) on cardiovascular risk factors in Type - I diabetic rats. Bitter gourd is one of the most used plants for the treatment of diabetes mellitus [DM] and some of its late complex abnormalities including dyslipidemia leading to an accelerated atherosclerosis (cardiovascular disease) and inflammatory manifestations. However, there are inadequate information's about regarding the effect of hypoglycemic active principle(s) from *Momordica charantia* on cardiovascular risk indicators. Current study has been carried out to evaluate the changes brought about by a novel hypoglycemic active principle (MCK₃P₈) which is obtained from the seeds of *Momordica charantia*, on certain cardiovascular indicators, in alloxan induced diabetic rats which are 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, Dr. APJ Abdul Kalam University, Indore (MP) India.

2.2 Chemical

All the chemicals were of analytical grade procured from Boehringer Mannheim, 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 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. 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 MCK₃ was obtained from ice cold ethanol extract (75% C₂H₅OH 0.2 N HCl 1 mM PMSF), concentrated by centrifugation and in speed vac at 40C. 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 0.25% TCA containing (NH₄)₂SO₄ that resulted in precipitation of all protein. The MCK₃P₈ – the novel hypoglycemic principle was obtained from the fraction MCK₃ (14 ml containing 196 mg of proteins) by gel filtration column chromatography using sephacryl S100 eluting with 0.2 M NH₄HCO₃ (pH 7.2-7.4). At each step of purification, the bioactivity of the fractions was measured.

2.6 Antihyperglycemic Activity of MCK3P8 and its Effects on Serum Levels of Certain Cardiovascular Indicators in Alloxan Induced 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 vacutainers for determination of certain cardiovascular risk indicators by fully automated turbochem biochemistry analyzer manufactured by CPC Diagnostics, USA.

Glucose level was measured in blood drawn from the tail vein during the study period using Bergmeyer enzymatic method [15]. The troponin- I levels were measured through the ELISA method utilizing a kit produced by Moonblind, Incorporation, USA. The VLDL values were computed employing Fried Ewald's formula.

2.7 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 $\pm$ S.E. A probability value that was statistically significant ($P < 0.05$) was taken into consideration. N represents number of experimental male rats.

2.8 Ethical Clearance

The Department of Research and Development's Ethical Committee and Review Board of Dr. A.P.J Abdul Kalam University, Indore, India fully authorized the protocol. The experimental protocol was approved by the Institute's Ethical Committee. 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, that reports purification of an insulin-like proteinaceous hypoglycemic active principle (MCK₃P₈) from *Momordica charantia* seeds. Hypoglycemic activities studied in control, insulin, fraction MCK₃,

Table 2: Effect of Novel Antihyperglycemic Active Principle MCK₃P₈ of Bitter Gourd (*Momordica Charantia*) Seeds on Certain Cardiovascular Indicators in Experimental Diabetes.

Groups $\rightarrow$ Parameters $\downarrow$	Normal control + saline (0.5 ml)	Control Diabetic + saline (0.5 ml)	Diabetic + Insulin (5U/kg b.wt)	Diabetic + fraction K3 (15mg/kg b.wt)	Diabetic + MCK3P8 (15mg/kg b.wt)
Fasting blood glucose(FBG) mg/dl	86.0 $\pm$ 9.5	356.3 $\pm$ 17.2	287.4 $\pm$ 10.4	304.2 $\pm$ 12.9	277.3 $\pm$ 9.1***
Total cholesterol (TC) mg/dl	138.52 $\pm$ 7.33	226.78 $\pm$ 8.58	140.34 $\pm$ 6.45	148.50 $\pm$ 7.45	139.33 $\pm$.35***
HDL- cholesterol (HDLc) mg/dl	54.94 $\pm$ 2.11	40.57 $\pm$ 2.87	49.76 $\pm$ 2.19	47.61 $\pm$ 2.15	50.29 $\pm$ 2.18*
LDL- cholesterol (LDLC) mg/dl	71.68 $\pm$ 9.83	160.19 $\pm$ 17.04	80.28 $\pm$ 9.02	80.28 $\pm$ 9.02	69.09 $\pm$ 15.44***
VLDL- cholesterol (VLDLC) mg/dl	15.10 $\pm$ 0.92	22.10 $\pm$ 2.28	19.64 $\pm$ 1.14	16.18 $\pm$ 0.80	15.65 $\pm$ 0.91*
Triglycerides (TG) mg/dl	160.51 $\pm$ 4.49	210.51 $\pm$ 8.40	178.19 $\pm$ 5.70	185.88 $\pm$ 6.00	176.25 $\pm$ 5.59*
Troponin- I (Trop-I) mg/dl	0.08 $\pm$ 0.02	0.20 $\pm$ 0.09	0.12 $\pm$ 0.04	0.13 $\pm$ 0.06	0.12 $\pm$ 0.03*
Creatinine kinase- MB (CKMB) mg/dl	16.12 $\pm$ 3.48	22.20 $\pm$ 3.57	18.13 $\pm$ 1.28	19.15 $\pm$ 1.30	17.14 $\pm$ 1.28*
Total cholesterol: HDL-cholesterol ratio	2.52	5.20	2.82	3.18	3.07 **
Atherogenic index of plasma	0.106 $\pm$ 0.03	0.234 $\pm$ 0.11	0.218 $\pm$ 0.06	0.214 $\pm$ 0.086	0.211 $\pm$ 0.049*
C reactive protein (hsCRP) mg/L	4.90 $\pm$ 0.13	11.25 $\pm$ 1.28	5.65 $\pm$ 0.31	5.97 $\pm$ 1.41	5.41 $\pm$ 0.26***

Note: Values are shown as Mean $\pm$ 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: *Momordica charantia*, Karella, Fraction3, Peptide 8 Kilo Dalton, (MCK3P8).

3. DISCUSSION

Far from being a mere pointer of affluence and physical inactivity until

MCK₃P₈ treated alloxan-induced diabetic rat by measuring the blood glucose level enzymatically [15] during the study period. The hypoglycemic activity brought about by the MCK₃P₈ 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 cardiovascular indicators in insulin and MCK₃P₈ treated alloxan-induced diabetic rats, a dose of 10 IU/ kg b.wt insulin and 15 mg/kg b.wt. MCK₃P₈ respectively were given by intra-peritoneal injection for 20 days. The hypoglycemic 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 MCK₃ and Novel Antihyperglycemic Active Principle MCK₃P₈ of Bitter Gourd (*Momordica Charantia*) Seeds in Experimental Diabetes.

Treatments $\rightarrow$ Groups $\downarrow$	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 $\pm$ 10.0	86.0 $\pm$ 9.5	> 0.05
Control Diabetic+ saline (0.5 ml)	348.6 $\pm$ 43.9	356.3 $\pm$ 17.2	> 0.05
Diabetic + insulin (10 IU / kg b.wt.)	353.8 $\pm$ 17.0	287.4 $\pm$ 32.4***	< 0.001 ***
Diabetic + fraction K3 (15mg / kg b. wt.)	401.6 $\pm$ 39.0	304.2 $\pm$ 32.9**	< 0.001 **
Diabetic + MCK3P8 (15mg / kg b.wt.)	394.4 $\pm$ 48.6	277.3 $\pm$ 41.1***	< 0.001 ***
Diabetic + Proteinase-K treated MCK3P8 (15mg / kg b.wt.)	410.2 $\pm$ 67.4	387.1 $\pm$ 45.5	> 0.05

Note: Values are shown as Mean $\pm$ 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: MCK₃P₈: *Momordica charantia*, Karella, Fraction3, Peptide 8 Kilo Dalton.

Assessment of diabetic rats prone to cardiovascular risk were done by analyzing fasting blood glucose (FBG), lipid profile parameters, troponin I, creatinine kinase MB, atherogenic index of plasma and C reactive protein levels in control, insulin, fraction MCK₃, MCK₃P₈ treated alloxan-induced diabetic rats. Results demonstrated significantly decreased values FBG, total cholesterol, HDL-cholesterol, LDL- cholesterol, VLDL-cholesterol, triglycerides, troponin-I, creatinine kinase-MB, total cholesterol: HDL-cholesterol ratio, highly sensitive C-reactive protein, atherogenic index of plasma along with significantly elevated level of HDL-cholesterol in insulin, fraction MCK₃, MCK3P8 treated alloxan-induced diabetic rats when compared with corresponding levels in controls at the end of study period. The data is seen as in Table no 2.

recent past, diabetes, of late has been growing steadily on prevalence scale among all sections of society, manifesting a wide array of metabolic disorders and resulting in postprandial and fasting hyperglycemia, dyslipidemia and hyperinsulinemia. 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 drawbacks of insulin therapy include insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment [17-18]. Evaluation of medicinal plants for novel anti-diabetic compounds with minimal adverse effects is gaining popularity in new drug development, particularly when conventional anti-diabetic drugs fail because of severe adverse effects and adaptation problems. 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. Several studies stated that secondary metabolites in bitter melon, including charantin, polypeptides, napin-like protein, alkaloids, momordicin, flavonoids, and cucurbitane-type triterpenoids, play crucial roles in reducing blood glucose levels [19-20]. In the present study a novel insulin-like hypoglycemic active principle (MCK₃P₈) was purified from a fraction MCK₃ - obtained from the acid-ethanolic bitter melon seed extract. MCK₃P₈ 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 MCK₃P₈ was comparable to that observed with insulin treatment of the diabetic rats. Cardiovascular diseases, once primarily associated with sedentary lifestyles, have now become the most prevalent metabolic condition worldwide, with rising incidence across all socioeconomic groups [21]. A key strength of this research is its novel approach in assessing the longterm and detrimental cardiovascular effects of Type-1 diabetes, which has not been sufficiently explored. This study assesses various vascular risk agents that's include in lipid panel, total cholesterol to HDL cholesterol ratio, plasma atherogenic index, fasting blood sugar, creatinine kinase MB, troponin I. Hyperlipidemia is a significant factor in the development of atherosclerosis and cardiovascular disease. The positive relationship between the TotalC: HDLC ratio and CKMB supports the notion that a higher Total C: HDLC ratio is a major risk factor for mild myocardial damage, potentially leading to myocardial infarction [22]. Similar research showed that the Total Cholesterol to HDL ratio (TC:HDL ratio) is a well-established marker for cardiovascular risk, and its positive correlation with biomarkers like CK-MB (Creatine Kinase-Mycardial Band) in unstable angina highlights its role in tracking active heart muscle stress. [23]. Diabetes is usually associated with derangement in fat metabolism, alterations in the plasma lipids and lipoprotein profile. This certainly contributes to the development of cardio-vascular disease. Treatment with a novel insulin-like hypoglycemic active principle (MCK₃P₈) at a much lower dose of 15 mg/kg b.wt. significantly and greatly alleviate dyslipidemia indicating its potent antihyperlipidemic and anti-atherogenic activity. MCK₃P₈ improved lipid metabolism apart from the direct interaction with glucose homeostasis. The decreased level of serum cholesterol, LDL-cholesterol and increased level of HDL-cholesterol are considered to be favorable, since there is highly positive correlation between serum cholesterol level and incidence of atherosclerosis but negative for HDL-cholesterol [24]. Early studies suggest the antiatherogenic activity of bitter melon. Similar observations also suggest that bitter melon for the treatment/prevention of dietary fatty liver. The triglyceride lowering property of bitter melon could in directly contribute to the overall antihyperglycemic activity through a mechanism of so called glucose-fatty acid cycle [25]. According to it increased supply of plasma triglyceride percent could constitute a source of increased FFA availability and oxidation that than can impair insulin action, glucose metabolism and utilization leading to development of hyperglycemia [26]. Therefore, the reduction of triglyceride following MCK₃P₈ treatment also facilitates glucose oxidation and utilization and subsequently the reduction of hyperglycemia. This is further substantiated by the fact that MCK₃P₈ is effective enough in either, in stimulating insulin release from residual β -cells or due to its insulinomimetic activity or combination of the two, might in tern produce an overall improvement in lipid profile [27]. C-reactive protein is one of the acute phase proteins synthesized by

hepatocytes. C-reactive protein is a protein produced by the liver that serves as a primary marker for inflammation in the body. While traditional cardiovascular risk checks focus heavily on cholesterol, inflammation plays an equally critical role in how heart disease develops and progresses. [28]. MCK₃P₈ treated alloxan-diabetic rats showed reduced levels of highly sensitive C- reactive protein – an inflammatory marker. This remedy may be related with the significant anti-diabetic effect of *Momordica charantia* seeds. These findings can be the sensitive index that could be useful as an indirect predictive or distinctive tool in assessing the inflammatory consequences in experimental diabetes.

4. CONCLUSION

The novel hypoglycemic active principle MCK₃P₈ of bitter melon seeds when given intraperitoneally exhibited significant hypo-glycemic activity by 3 hours after administration in alloxan-induced diabetic rats when compared with corresponding levels in controls. The repeated administration of MCK₃P₈ did not result in the deterioration of hypoglycemic response in diabetic rats (in terms of blood glucose level), even one week after discontinuation of treatment a desirable criteria for any novel hypoglycemic principle with therapeutic potential. MCK₃P₈ at much lower concentration (15mg/kg. b.wt.) helped to attain nearly normal normolipidemic status, shown anti-inflammatory response in vivo and is able to decrease cardiovascular risk indicators in alloxan – induced experimental diabetic rats No visible side effect or toxicity was observed with MCK₃P₈ in treated Type – I diabetic group of animals during entire experimental period of 20 days.

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.

Acknowledgment

I, Zamiruddin Ansari, am extremely grateful for the financial support (Junior Research Fellowship) by the Department of Biotechnology, Ministry of Science and Technology, Government of India during this study.

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