

Study of Nephroprotective and Normoelectrolytic Effect of a Novel Hypoglycemic Active Principle from the Seeds of *Momordica charantia* L. (Bitter gourd) in Experimental Diabetes

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Abstract: Background: Plants with medicinal properties, the gift of Mother Nature to mankind, are in use for centuries in the indigenous system of medicine (Ayurveda, Unani etc.) in India and other countries for the treatment of several diseases including diabetes mellitus. Since ancient time *Momordica charantia* L. (bitter gourd) the fruit-vegetable, fruit pulp, flower, leaves and seeds have been used in complementary and alternative medicine for the treatment of diabetes mellitus and some of its late complications. The present study examines glycemic control and changes in renal panel markers and certain renal 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), kidney function test (KFT) markers, electrolytes, water intake, diet intake, urine volume, urine sugar, kidney weight and glomerular filtration rate (GFR) 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 renal panel markers and certain renal risk indicators determined by drawing blood from the tail vein during the study period of 20 days. 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 treatment with MCK₃P₈-the hypoglycemic active principle of bitter gourd seeds have significantly reduced the blood urea and serum levels of creatinine, water intake, diet intake, urine volume, urine sugar, kidney weight, highly sensitive C reactive protein (hsCRP) glomerular filtration rate along with significant elevation of sodium, potassium, chloride at 3 hour after treatment in type-1 diabetic rats. Conclusion: The results of the current study clearly provided experimental evidence of normoglycemic, nephroprotective and normoelectrolytic effect of novel hypoglycemic active principle (MCK₃P₈) of bitter gourd seeds. MCK₃P₈ exhibited significant hypoglycemic activity and significantly influenced the levels of renal panel markers and certain renal risk indicators by improving glycemic status and is able to alleviate kidney damage and electrolytic imbalance in alloxan-induced experimental diabetic rats when compared with corresponding levels in controls at the end of study period.

Keywords: Bitter gourd, Novel hypoglycemic principle, Glycemic status, Renal risk indicators, Electrolytic imbalance, Alloxan, Diabetes.

1. Introduction

Since time immemorial *Momordica charantia* L. commonly known as “Karela” in Hindi, Bitter gourd 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-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 shown to have their hypoglycemic and antioxidant effects that reduce oxidative stress [12-13]. Previous study shows that an antihyperglycemic active principle of bitter gourd *Momordica charantia* L. 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 renal panel markers and certain renal risk indicators in Type-I diabetic rats. However, there are inadequate information's about regarding the effect of hypoglycemic active principle(s) from *Momordica charantia* on renal risk indicators [15]. 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 bitter gourd on renal panel markers and certain renal risk indicators in experimental diabetes which are at present unclear.

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2. Material and Methods

2.1 Plant Material

Seeds of *Momordica charantia* L. (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 were authenticated by the taxonomist of the botany department, Dr. APJ Abdul Kalam University, Indore (MP) India. A voucher specimen is deposited in the department of biochemistry of the Abdul Kalam Institute of Medical Sciences, Indore.

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 40°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 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 Evaluation of biological activity of novel hypoglycemic active principle MCK₃P₈ on serum levels of renal function test (RFT) markers 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 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 the determinations of biochemical parameters. Glucose level was measured in blood drawn from the tail vein during the study period using Bergmeyer enzymatic method [16]. Biochemical analysis of renal function test markers (urea, creatinine and electrolytes) are determined by automated Johnson & Johnson Vitros 250 chemistry analyzer. Biochemical analysis of renal risk indicators are determined by fully automated turbochem 100 biochemistry analyzer make of CPC Diagnostics, USA. Urine was collected by keeping rats in metabolic cages under a layer of toluene for a period of 24 h. The content of reducing sugar present in urine was measured by 305-dinitro salicylic acid method. [17]. Creatinine was estimated by Folin's method in urine (24 h collection) [18]. Glomerular filtration rate (GFR) was determined [19] using the formula, GFR (ml/min)

$$\text{GFR} = \frac{\text{Urinary creatinine (mg/dl)} \times \text{Urine volume (ml)} \times 1000 \text{ (g)}}{\text{Plasma creatinine (mg/dl)} \times \text{Body weight (g)} \times 1440 \text{ (min)}}$$

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, 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 renal risk 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 alloxan induced experimental diabetic rats.

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 K3 (15mg / kg b. wt.)	401.6 ± 39.0	304.2 ± 32.9**	< 0.001**
Diabetic + MCK3P8 (15mg / kg b.wt.)	394.4 ± 48.6	277.3 ± 41.1***	< 0.001***
Diabetic + Proteinase-K treated MCK3P8 (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: MCK3P8: *Momordica charantia*, Karella, Fraction3, Peptide 8 Kilo Dalton.

Repeated administration of MCK3P8 did not result in the deterioration of hypoglycemic response in whole of the study period of 20 days which was comparable to that of Protamine-Zinc insulin. In other words MCK3P8 not only reduced the levels of glucose and inhibited them from rising further but also maintain the greatly, a desirable criteria for any potential antidiabetic agent. The significant reduction (69% from the initial level by day 8) in blood glucose level

was observed in MCK3P8 treated alloxan induced diabetic rats. The effect was more pronounced even when compared with the experimental group treated with insulin (5 U/kg b. wt.) The MCK3P8 treated animals had a 100% survival during the study period of 20 days with a normal behavior, whereas the untreated control diabetic animals were lethargic and died after 4 days of experiment. Results are reported in Table 2.

Table 2: Effect of prolonged novel hypoglycemic active principle MCK3P8 treatment on blood glucose levels in alloxan induced experimental diabetic rats during study period.

Groups → Study period ↓	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)
0 Day	91 ± 10.0	457 ± 72	351 ± 58	370 ± 56	368 ± 47
4 Day	88 ± 11.0	655 ± 98	295 ± 38**	293 ± 42**	270 ± 43**
8 Day	85 ± 9.0	All six animals died	251 ± 17***	235 ± 20**	168 ± 14***
12 Day	82 ± 7.0	All six animals died	240 ± 20***	225 ± 17**	175 ± 10***
16 Day	89 ± 10.0	All six animals died	244 ± 18***	216 ± 19**	173 ± 11***
20 Day	87 ± 12.0	All six animals died	247 ± 16***	218 ± 12**	168 ± 14***

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: MCK3P8: *Momordica charantia*, Karella, Fraction3, Peptide 8 Kilo Dalton.

Assessment of nephroprotective and normoelectrolytic effect of a novel hypoglycemic active principle from the seeds of *Momordica charantia* L. (bitter gourd) in experimental diabetes were done in control, insulin, fraction MCK3, MCK3P8 treated alloxan-induced diabetic rats. Results demonstrated significantly reduction of fasting blood glucose (FBG), kidney function test (KFT)

(blood urea and serum creatinine) markers along with significant elevation of serum electrolytes (sodium, potassium and chloride) levels 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 3.

Table 3: Effect of novel hypoglycemic active principle MCK3P8 of bitter gourd (*Momordica charantia*) seeds on certain renal function test markers in experimental diabetes.

Groups → Parameters ↓	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 (mg/dl)	86.0 ± 9.5	356.3 ± 16.2	287.4 ± 10.4**	285.2 ± 11.9**	277.3 ± 9.1***
Urea (mg/dL)	37.2 ± 0.37	61.4 ± 0.51	41.1 ± 1.15**	38.0 ± 1.12**	37.7 ± 1.0***
Creatinine (mg/dL)	1.10 ± 0.10	6.48 ± 2.6	1.74 ± 0.20 **	1.70 ± 0.21**	1.66 ± 0.18***

Sodium (mmol/l)	141.5±2.5	129.86±1.4	142.18±1.17*	144.38±1.29*	142.29±1.20**
Potassium (mmol/l)	4.40±0.20	2.58±0.19	4.87±0.17*	4.90±0.18*	4.59±0.14**
Chloride (mmol/l)	102.0±2.8	92.72±1.28	104.10±1.21*	103.75±1.27*	102.5±1.12**

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: *Momordica charantia*, Karella, Fraction3, Peptide 8 Kilo Dalton, (MCK3P8).

This hypoglycemic active principle MCK₃P₈ from the seeds of bitter melon had favorable effect, not only on blood glucose level but also on key parameters of renal panel test markers and renal risk indicators. Present study also indicates that treatment with MCK₃P₈-the hypoglycemic active principle of bitter melon seeds has significantly

reduced water intake, diet intake, urine volume, urine sugar, kidney weight, highly sensitive C reactive protein, glomerular filtration rate at 3 hours after treatment in type-1 diabetic rats. The data is seen as in Table 4 and 5.

Table 4: Effect of novel hypoglycemic active principle MCK3P8 of bitter melon (*Momordica charantia*) seeds on water intake, diet intake, urine volume and urine sugar in experimental diabetes

Groups → Parameters ↓	Normal control + saline (0.5 ml)	Control Diabetic + saline (0.5 ml)	Diabetic + Insulin (5U/kg b.wt)	Diabetic + fraction K ₃ (15mg/kg b.wt)	Diabetic + MCK ₃ P ₈ (15mg/kg b.wt)
Water intake (mL/24 h)	29.90 ± 1.8	89.84 ± 7.4	56.96 ± 2.1**	48.59 ± 2.2**	27.60 ± 1.9**
Diet intake (g/24 h)	12.58 ± 2.43	19.46 ± 2.4	15.89 ± 0.25**	15.98 ± 0.28**	15.74 ± 0.26**
Urine volume (mL/24 h)	12.5 ± 2.5	80.0 ± 6.1	16.5 ± 2.4**	16.2 ± 2.1**	16.5 ± 2.2***
Urine sugar (g/24 h)	2.7 ± 1.0	7.4 ± 2.2	3.7 ± 1.3**	3.6 ± 1.2**	3.5 ± 1.2***
Urine creatinine (g/24 h)	12.2 ± 1.2	36.3 ± 5.7	16.9 ± 1.4**	15.5 ± 1.3**	14.2 ± 1.2***

Table 5: Effect of novel hypoglycemic active principle MCK3P8 of *Momordica charantia* L. (bitter melon) seeds on certain renal risk indicators in experimental diabetes

Groups → Parameters ↓	Normal control + saline (0.5 ml)	Control Diabetic + saline (0.5 ml)	Diabetic + Insulin (5U/kg b.wt)	Diabetic + fraction K ₃ (15mg/kg b.wt)	Diabetic + MCK ₃ P ₈ (15mg/kg b.wt)
Glomerular Filtration Rate (ml/min)	1.18 ± 0.16	6.01 ± 0.53	1.32 ± 0.31**	1.30 ± 0.14**	1.26 ± 0.15***
Kidney Weight (g/100 g)	0.68 ± 0.02	1.33 ± 0.07	0.86 ± 0.04**	0.85 ± 0.05**	0.83 ± 0.04***
C reactive protein (hsCRP) mg/L	4.90 ± 0.13	11.25 ± 1.28	5.85 ± 0.30**	5.89 ± 0.32**	5.21 ± 0.26***

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: *Momordica charantia*, Karella, Fraction3, Peptide 8 Kilo Dalton, (MCK3P8).

4. Discussion

Far from being a mere pointer of affluence and physical inactivity until 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 [17]. Diabetes is the world's largest chronic incurable endocrine disorder and is one of the major killers in recent times instead of latest treatment methods and despite tremendous developments in the medical sciences. [18]. 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. Severity of the glucose abnormalities trigger a vast group of diabetic complications, commonly classified as micro vascular (glomerulus & retina), macrovascular (accelerated atherosclerosis), and neuropathic [19] Insulin therapy affords effective glycemic control, yet its short comings such as ineffectiveness on oral administration, short self-life, quite expensive painful, requirement of constant refrigeration, and in the event of excess dosage causes fatal "insulin shock" (hypoglycemia) -a life-threatening

situation-limits its usage [20]. Other drawbacks of insulin therapy include insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment [20]. Disability and death in diabetes much more often relate to so-called its late complications including damage to the kidneys (nephropathy-leading to renal failure [21]. Medicinal plants and their products (active natural principles and crude extracts) have shown experimental or clinical anti-diabetic activity [22]. 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 [23]. 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. Diabetic animals were administered with MCK₃P₈ (15 mg/kg b.wt.) or protamine zinc insulin (5 U/kg b.wt.) once daily for 20 days. Control diabetic animals were treated with equal volume of saline. Each group consisted of 6 animals each. Type I diabetes was induced in male Wistar rats using alloxan. The diabetic animals were maintained for a period of 20 days in order to assess the

prolonged effect of MCK₃P₈ on glycemic status in alloxan-induced diabetic rats. The MCK₃P₈ treated animals had a 100% survival during the study period of 20 days with a normal behavior, whereas the untreated control diabetic animals were lethargic and died after 4 days of experiment. The activity of MCK₃P₈ (15 mg/kg b.wt.) started within 3 hours of intra peritoneal injection. Repeated administration of MCK₃P₈ did not result in the deterioration of hypoglycemic response in whole of the study period, even one week after discontinuation of treatment which was comparable to that of Protamine-Zinc insulin. In other words, MCK₃P₈ not only reduced the levels of glucose and inhibited them from rising further but also maintain the greatly, a desirable criterion for desirable criteria for any potential anti-diabetic hypoglycemic principle. In-addition significant reduction (69% from the initial level by day 8) in blood glucose level was observed in treated alloxan induced diabetic rats. The effect was more pronounced even when compared with the experimental group treated with insulin. This hypoglycemic active principle MCK₃P₈ from the seeds of bitter gourd had favorable effect, not only on blood glucose level but also on key parameters of renal panel test markers and renal risk indicators. Serum creatinine levels rise when renal function is compromised. An indicator that the kidneys are not functioning at their best is a little rise of the creatinine level over the usual range. All around the body, blood contain proteins. Urea is the waste product that remains after a body cell uses the proteins and returns to the circulation. Healthy kidneys remove urea from blood, however if it isn't functioning all right, the amount of urea rises in the blood [24]. Our results show the increase of urea in diabetic animals which perhaps as a result of kidney unwillingness to extract them from the blood. The clinical presentation of type 1 diabetes is usually acute with classical osmotic symptoms such as increased thirst, polyuria, despite increased appetite, fatigue respectively [25]. Excretion of urine was monitored weekly up-to 20 days. The polyuria condition prevailed in diabetic group and was 65-70 ml/day all along the experiment. Our result clearly demonstrated reduction of urine excretion in the MCK₃P₈ treated diabetic rats compared to diabetic rats. MCK₃P₈ of bitter gourd seed was effective in preventing polyuria, polydipsia and polydipsia conditions during experimental diabetes over a study period of 20 days. Diabetic rats showed glycosuria condition and excrete higher quantity of sugar in their urine when compared to normal controls. Similar trend in the excretion of urine sugar in experimental diabetic rats and MCK₃P₈ treated diabetic group was followed, which was consistent with earlier studies [26]. Kidney enlargement is an early feature in both experimental and human diabetes due to an increase in the capillary length and diameter and was correlated with the degree of glycemic control [27]. We observed partial yet significant reduction in the kidney weight in MCK₃P₈ of bitter gourd seeds treated diabetic rats. Hyperfunctional kidney with increase in GFR is reported in the early stages of diabetes [28]. Earlier studies with dietary fiber (guar gum) have also shown significant improvements in GFR during diabetes. [29]. During diabetes, the kidney weight nearly doubled indicating kidney hypertrophy [30]. Treatment with MCK₃P₈ prevented the hypertrophy partially, yet significantly by about 25%. Glomerular filtration rate (GFR) was significantly higher in diabetic

controls in comparison to the control rats. Significant reduction (27%) in GFR was observed during study period in MCK₃P₈ treated diabetic rats. Significant change was observed in urea, creatinine, electrolyte levels indicative of nephroprotective and normoelectrolytic effect in both MCK₃P₈ as well as insulin treated diabetic animals when compared to normal control animals. No visible side effect or toxicity was observed in MCK₃P₈ treated diabetic group of animals during entire experimental period of 20 days. The active hypoglycemic principal(s) present in MCK₃P₈ had favorable effect on urine volume, urine sugar, water intake, diet intake, when comparable to insulin treated alloxan induced diabetic rats. MCK₃P₈ treatment not only normalizes blood glucose level but also resulted in reduction in urea and creatinine level-the key parameters of kidney function test. MCK₃P₈ treatment also normalizes the imbalance of serum electrolytes apart from reducing kidney weight and GFR of diabetic rats. C-reactive protein is one of the acute phase proteins synthesized by hepatocytes. While traditional renal risk checks focus heavily on renal panel markers, inflammation plays an equally critical role in how kidney disease develops and progresses [31]. MCK₃P₈ treated alloxan-diabetic rats showed reduced levels of highly sensitive C-reactive protein-an inflammatory marker. This remedy could 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. Similar research showed that the diabetes is usually associated with derangement in renal function markers [32]. This certainly contributes to the development of kidney disease [33-34]. 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 nephrotoxicity indicating its potent nephroprotective and normoelectrolytic activity.

3. Conclusion

The current investigation presents experimental evidence for the identification of MCK₃P₈-a novel hypoglycemic active principle of bitter gourd seeds, when given intraperitoneally, exhibited significant hypoglycemic 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 criterion for any novel hypoglycemic principle with therapeutic potential. MCK₃P₈ at much lower concentration (15mg/kg. b.wt.) exhibited significant hypoglycemic activity and significantly influenced the levels of renal panel markers and certain renal risk indicators by improving glycemic status and is able to alleviate kidney damage and electrolytic imbalance in alloxan-induced experimental diabetic rats when compared with corresponding levels in controls at the end of study period. 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 were used during the study and writing the manuscript.

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References

- [1] **Sarkar S, Pranava M and Rosalind NA. 1996.** Demonstration of the hypoglycemic action of *Momordica charantia* in a validated animal model of diabetes, *Pharm Res.* **33 (1)**:1-4.
- [2] **Ansari Z & Singh SK. 2007.** Hypoglycemic effect of the seeds of *Momordica charantia*. *Fitoterapia.* **78**:46-47
- [3] **Ansari Z, Zafar E & Nehal M. 2008.** Effect of bittermelon (*Momordica charantia*) on serum levels of key hepatic enzymes and proteins in alloxan diabetic rats. *J Sci Pharm.* **4 (3)**:109-114.
- [4] **Ansari Z and Nehal M. 2008.** The hypoglycemic effect of *Momordica charantia* seeds is mediated by extra-pancreatic action, *J Sc Pharm.* **4 (2)**:65-70.
- [5] **Singh N, Gupta M, Sirohi P and Varsha. 2008.** Effect of alcoholic extract of *Momordica charantia* (Linn.) whole fruit powder on the pancreatic islets of alloxan diabetic albino rats. *J Env Biol.* **29 (1)**:101-106.
- [6] **Chopra RN, Chopra IC, Handa KI and Kapur LD. 1982.** Chopra's indigenous drug of India (Academic Publication, Calcutta).
- [7] **Solomon BA and Akino FO. 2014.** Histopathological and haematological changes observed in adult wistar rats with *Momordica charantia* (bittermelon) aqueous leaf extract. *J Comp Altern Med Res.* **20(2)**: 30-36.
- [8] **Lolitkar MM and Rao MRR. 1996.** Pharmacology of a hypoglycemic principle isolated from the fruits of *Momordica charantia* Linn. *Indian J. Pharmacol.* **28**:129-133.
- [9] **Rivera G. 1941.** Preliminary chemical and pharmacological studies on "*Cuneda Mor*" and "*Charantia L*", *Am J Pharmacol.* Part 11 **113(7)**:281-297.
- [10] **Smith JHC and Benitez A. 1955.** Modern methods of plant analysis, edited by Paech K & Tracey MV, **Vol.4**:142
- [11] **Khanna P, Jain SC, Panagariya A and Dixit VP. 1981.** Hypoglycemic activity of polypeptide-P from a plant source. *J Nat Prod.* **44 (6)**: 648-655.
- [12] **Mahmoud MF, El Ashry FEZZ, El Maraghy NN, and Fahmy A. 2017.** Studies on the antidiabetic activities of *Momordica charantia* fruit juice in streptozotocin-induced diabetic rats. *Pharmaceutical Biology*, **55(1)**: 758-765.
- [13] **Liu Z, Gong J, Huang W, Lu F, and Dong H. 2021.** The effect of *Momordica charantia* in the treatment of diabetes mellitus: A review. *Evidence-Based Complementary and Alternative Medicine*: 3796265.
- [14] **Ansari Z and Ahmad S. 2026.** Effect of a novel antihyperglycemic active principle from the seeds of *Momordica charantia* Linn. on glucose transporters 2 and 4 in experimental diabetes. *I J Sci Res.* **15 (7)**:2463-2467.
- [15] **Ansari Z and Ahmad S. 2025.** Antidiabetic potential of an insulin-like molecule from the seeds of *Momordica charantia* Linn. in experimental diabetes. *J Phar Bio Sci.* **20(5)**. pp. 37-40.
- [16] **Bergmeyer HU., Bernt E., Schmidh F & Strake H. 1974.** Glucose determination with hexokinase and glucose-6-phosphate dehydrogenase, edited by Bergmeyer HU. *Methods of enzymatic analysis.* **3** (Academic Press, NY):1196.
- [17] **Defronzo RA Bondonna RC Perranini E.1992.** Photogenesis of IDDM: a balanced overview *Diabetes care.* **15**: 318-367.
- [18] **Mukhtar Y, Galalain AM and Yunus UM. 2020.** A modern overview on diabetes mellitus: A Chronic Endocrine Disorder. *Eur J Biol.* **5(2)**: 1-14.
- [19] **Ali L Khan AK Mamun MI Rokeya B. 1993.** Studies on hypoglycemic effects of fruit pulp, seed and whole plant of *Momordica-charantia* on normal and diabetic model rat, *Planta Medica.* **59(5)** :408-412.
- [20] **Choudhary S.K., Chhabra G, Sharma D, Vashishta A, Ohri S and Dixit A.2012.** Comparative evaluation of anti-hyperglycemic activity of fractionated *Momordica charantia* seed extract in alloxan-induced diabetic rats. *Evidence-Based Complementary and Alternative Medicine.*
- [21] **Xu B, Li Z, Zeng T, Zhan J, Wang S, Ho CT, Li S. 2022.** Bioactives of *Momordica charantia* as potential antidiabetic/hypoglycemic agents. *Molecules.* **27(7)**:2175.
- [22] **Ziyadeh FN. 2002.** Long term prevention of renal insufficiency, excess matrix gene expression and glomerular mesangial matrix expansion by treatment with monoclonal antitransforming growth factor-b antibody in db/db diabetic mice. *Proc. Natl Acad Sci* **97(6)**: 8015-8020.
- [23] **Ajit Kar Choudhary BK Bandhopadhyaya NG.1999.** Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test *J Ethnopharm.* **64**: 179-184.
- [24] **Mahwish, Saeed F, Arshad MS, Mahrnun Nisha, Nadeem MT and Arshad MU. 2017.** Hypoglycemic and hypolipidemic effects of different parts and formulations of bitter gourd (*Momordica charantia*), *Lipids in Health and Disease.* **16**: 211-221.
- [25] **Yokozawa T, Chung HY, He LQ and Qura H. 1996.** Effectiveness of green tea tannin on rats with chronic renal failure, *Niosci Biotech Biochem*, **60**: 1000-1005.
- [26] **Nandini CD, Sambaiah K and Salimath PV. 2003.**

Dietary fibers ameliorate decreased synthesis of heparan sulphate in streptozotocin induced diabetic rats, *J Nutr Biochem*, **14**: 203-210.

- [27] **Chethankumar M, Rachappaji KS, Nandini CD, and Salimath PV. 2002.** Modular effect of butyric acid-a product of dietary fiber fermentation in experimentally induced diabetic rats, *J Nutr Biochem*, **3**: 522-527.
- [28] **Seyer-Hansen K. 1977.** Renal hypertrophy in experimental diabetes relation to severity of diabetes, *Diabetologia*. **13**: 141-143.
- [29] **Christainsen JS, Gammelgaard J, Frandsen M and M Parring HH. 1981.** Increased kidney size, glomerular filtration rate and renal plasma flow in short term insulin dependent diabetics. *Diabetologia*. **20**: 264-267.
- [30] **Feldt-Rammussen B, Hegedus L, Mathiesen ER and Deckert T. 1991.** Kidney volume in type 1 (insulin dependent) diabetic patients with normal or increased urinary albumin excretions: Effect of long term improved metabolic control, *Scand J Clin Lab Invest*. **51**: 31-36.
- [31] **Raman BV, Krishna NV, Rao NB, Saradhi PM and Rao BMV. 2012.** Plants with anti-diabetic activities and their medicinal values. *Int Res J Pharm*. **3(3)**: 11-15.
- [32] **Kesari AN, Gupta, RK, Singh SK, Diwakar S and Watal G. 2006.** Hypoglycemic and hypoglycemic activity of Aegle marmelos seed extract in normal and diabetic rats, *J Ethnopharm*. **107(3)**: 374-379.
- [33] **Li J, Chen J, Lan HY, Tang Y. 2022.** Role of C-reactive protein in kidney diseases. *Kidney Dis*. **9(2)**:73-81.
- [34] **Dong Y, Tang M, Xu Y, Peng Q. 2025.** Predictive value of systemic immune-inflammation index in patients with diabetes mellitus: a systematic review and meta-analysis. *Front Endocrinol*. **16**:01-16