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EFFECT OF FRACTIONATED BITTERMELON (*MOMORDICA CHARANTIA*) SEED EXTRACT ON GLYCEMIC STATUS IN ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT
Bittermelon *(Momordica charantia)* or bittergourd commonly known as "Karella" (family: Cucurbitaceae) has been proved for hypoglycemic effects. The objective of the present studies was to examine the long term effect of fractionated bittermelon *(Momordica charantia)* seed extract on glycemic status in alloxan-induced diabetic rats. To evaluate the glycemic control of bittermelon, blood glucose level, urine volume, urine sugar, water intake, diet intake, gain in body weight, kidney weight, glomerular filtration rate and important parameter of kidney functions and serum electrolytes were monitored in experimental animals. Water consumption, urine volume and urine sugar were significantly higher in diabetic controls compared to normal rats. Renal hypertrophy, nephrotoxicity, increased glomerular filtration rate and electrolytic imbalance is observed in diabetic rats. Fraction designated as MCK3 were administered to experimental rats intraperitoneally at a dose of 15mg/kg b. wt. for 20 days while the control group received equivalent volume of saline under ideal condition (n=6 in each case), Biochemical parameters related to glycemic control were estimated in MCK3 treated alloxan-induced diabetic rats. MCK3 treatment resulted in reduction of blood glucose level, water intake, diet intake, gain in body weight, reduction in urine volume, and urine sugar level, decrease in kidney weight and glomerular filtration rate, reduction in serum urea, creatinine and electrolytes level at 3 h after treatment. These results clearly provided experimental evidence of nephroprotective and normoelectrolytic effect of fraction MCK3 from bittermelon seed extract which is comparable to insulin treatment. The active hypoglycemic principle(s) present in bittermelon seeds improved glycamic status and is able to alleviate kidney damage and electrolytic imbalance caused by alloxan-induced diabetes.

KEYWORDS: Bittermelon *(Momordica charantia),* diabetes, insulin, glycemic status, glomerular filtration rate, blood glucose, urine sugar.

Abbreviations: MCK3: Momordica charantia, Karella, Fraction 3, GFR: Glomerular filtration rate, DM: Diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus is considered as one of the five leading causes of death in the world.[1] Diabetes mellitus is a major global health concerning with a projected rise in prevalence from 171 million in 2000 to 366 million in 2030.[2] The disease is caused due to an insufficiency of insulin secretion insulin action or both.[3] Being a major degenerative disease, diabetes is found in all parts of the world and it is becoming the third most lethal disease of mankind and increasing rapidly.[4-5] Diabetes mellitus is a group of metabolic disorders associated with the endocrine system that resulted in hyperglycemic conditions. DM is a well known clinical entity with various late complications like nephropathy, retinopathy, neuropathy *etc.* Ayurveda and other traditional medicinal systems for the treatment of diabetes describe numeral plants used as herbal medicines. Because of low side effects and low cost they play an important role as an alternative drug.[6-9] Complementary and alternative medicine involves the use of herbs and other dietary supplement as alternatives to mainstream western medical treatment. A recent study has estimated that up to 30% of patient with diabetes mellitus use complementary and alternative medicine.[10-13] The alternative medicine system is now gaining momentum with the knowledge of active principles identified from plant species.[14-18] Herbs for diabetes treatment are not new. Since ancient times, plants and plant extract were used to combat diabetes. Many traditional medicines in use

are derived from medicinal plants. *Momordica charantia L.* (bittermelon) is a plant of family cucurbitaceae and is a popular plant used for the treating of diabetes-related conditions amongst the indigenous population of Asia, South America, India, the Caribbean and East Africa, where it is used as a food as well as medicine.[19] Although the seeds, leaves, stem, roots, whole fruit and fruit pulp containing bioactive chemicals, vitamins, minerals, antioxidants, dietary fibers. The main constituents of bittermelon include triterpinoids, lipid and phenolic compounds. The seeds are the most prevalent part of the plant used medicinally as it contains steroidal saponins known as charantin, momordicine, insulin like peptide (polypeptide-P or protein P-insulin), vicin and other active hypoglycemic principle(s).[20-24]

2. MATERIALS AND METHODS

2.1. Plant Material. *Momordica charantia* L. (Cucurbitaceae) seeds purchased from sales counter of Indian Agriculture Research Institute (IARI), Pusa, New Delhi in large quantity in August 2010, to maintain the consistency of the stock for extract preparation and was authenticated by the Taxonomist of University Department of Botany, Patna University,. A voucher specimen is deposited in the Department of Biochemistry of the Patna University. All the chemicals were of analytical grade and were procured from Sigma-Aldrich Chemical Co., USA, or Boehringer- Mannhiem, Germany, unless otherwise stated. Protamine Zinc insulin was procured from Boots Pharmaceuticals Ltd., India.

2.2. Animals Random bred male Wistar rats, 175 – 200 g (12–14 weeks) were housed in standard laboratory conditions, in the Small Animal Facility of the Department of Biochemistry, Patna University. The animals were provided with rat feed (Hindustan Lever Ltd, India) and water ad libitum.

2.3 Tested Material From decorticated bittermelon (Momordica charantia) seeds, fraction MCK3 was obtained from ice cold ethanol extract (75% C2H5OH, 1 mM PMSF, 0.2 N HCI), centrifuged and concentrated in speed vac at 4℃. 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.

2.4. Induction of Diabetes in Rats. 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 body weight (b.wt.). Animals were stabilized for three days by insulin administration, 1-2 units per day for 2 days. Only those animals having blood glucose level more than 300 mg per 100 mL blood were selected for further analysis.

2.5. Evaluation of Biological activity of Fraction MCK3 of Momordica charantia Seed Extract. The diabetic animals were grouped into experimental groups each containing minimum 6 rats. The doses of fraction MCK3 are expressed in terms of their protein content. Different groups were treated with fraction MCK3 (15 mg/kg b.wt.). Diabetic animals treated with saline were included in the study as diabetic control. A group of diabetic animals treated with protamine zinc insulin (5 U/kg b. wt.). A group of normal untreated non-diabetic animals was also included in the study. Blood glucose level was estimated enzymatically in blood drawn from the tail vein during the study period using Bergmeyer enzymatic method method.[25] 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.[26] Creatinine[27] was estimated by Folin's method in urine (24 h collection). Glomerular filtration rate (GFR) was determined[28] using the formula,

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

Kidney function markers (urea, creatinine and electrolytes) in blood are subjected to biochemical analysis in automated chemical analyzer (Johnson & Johnson Vitros 250 chemistry analyzer).

2.6. Statistical Analysis. All the results were analyzed statistically using one-way ANOVA or Student's paired *t*-test for paired data of deferent levels of significance. All the results were expressed as mean ± S.E. *P* values less than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

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 fraction MCK3 of bittermelon *(Momordica charantia)* on glycemic status in alloxan-induced diabetic rats. Fraction MCK3 of bittermelon was very effective in reducing blood glucose level in alloxan-

induced diabetic rats. The activity of fraction MCK3 (15 mg/kg b.wt.) started within 3 hours of intra peritoneal injection. Repeated administration of fraction MCK3 did not result in the deterioration of hypoglycemic response in whole of the study period which was comparable to that of Protamine-Zinc insulin. In other words fraction MCK3 not only reduced the levels of glucose and inhibited them from rising further but also maintain the greatly, a desirable criteria for any potential anti diabetic agent. The control rats had blood glucose of 70 – 110 mg/dl (Table 1) At the end of the experiment, the blood glucose level is reduced 368 ± 57 mg/dl to 168 ± 24 mg/dl in fraction MCK3 (15 mg/kg b.wt.) treated diabetic rats (Table 1). Thus, significant reduction (69% from the initial level by day 8) in blood glucose level was observed in fraction MCK3 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.) (Table 1).

Table 1: Effect of prolonged MCK3 treatment on blood glucose levels.

Period (days) of treatment	0	4	8	12	16	20
Saline-treated normal	90 ± 10	88 ± 11	85 ± 9.1	82 ± 7	89 ± 10	87 ± 12
Saline-treated diabetic	457 ± 72	655 ± 98	All	Six	Animals	Died
MCK-3-treated diabetic	368 ± 57	270 ± 30**	197 ± 24**	173 ± 14**	170 ± 07**	168 ± 24**
Insulin-treated diabetic	351 ± 68	295 ± 18**	255 ± 16**	252 ± 41*	290 ± 12*	278 ± 17**

Diabetic animals (n = 6) were administered with MCK3 (15 mg/kg b.wt.) in saline or protamine zinc insulin (5 U/kg b.wt.) once daily for 20 days. Blood glucose levels (mg/dL) were measured on the days indicated. Control diabetic animals (n= 6) were treated with equal volume of saline. The data represent mean ± S.E. * $P < 0.05$; ** $P < 0.01$; compared with serum glucose levels on 0 day.

Active hypoglycemic principle(s) present in fraction MCK3 of bittermelon seed extract had favorable effect not only on blood glucose level but also on key parameters of kidney function test(s) including serum electrolytes. The MCK3 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. Water intake is a characteristic symptoms of diabetes. Water consumption increased in diabetic group and bittermelon incorporation in the diet showed significant reduction in water intake during diabetes (Table 2). Diabetic rats showed polyphagic condition and consumed higher quantity of diet compared to control (Table 2). Higher consumption of diet was observed in both the normal and diabetic rats fed with fraction MCK3 bittermelon seed extract. Body weight was monitored and normal control rats gained weight over a study period of 20 days (Table 2). Our result clearly demonstrated that increased body weight observed in fraction MCK3 of bittermelon seed extract treated diabetic group, while there was significant reduction in the body weight of diabetic control. Excretion of urine was monitored weekly up-to 20 days (Table 2).

Table 2: Effect of prolonged MCK3 and insulin treatment on water intake, diet intake and bodyweight in diabetic rats.

Group	Water Intake (mL/24 h)	Diet Intake (g/24 h)	Initial body Weight (g)	Final Body Weight (g)	Gain in weight (g)
Saline-treated normal	29.90 ± 1.4	12.58 ± 0.44	176.0 ± 5.2	237.3 ± 5.5	61 ± 4.9
Saline-treated diabetic	89.84 ± 4.4*	17.46 ± 0.55*	123.7 ± 2.6*	107.1 ± 2.6*	-16 ± 1.9*
MCK-3-treated diabetic	27.60 ± 1.3**	15.74 ± 0.36**	171.0 ± 4.9	223.0 ± 7.6	52 ± 5.7
Insulin-treated diabetic	56.96 ± 2.3*	16.42 ± 0.25*	173.0 ± 2.0	232.0 ± 11.5	59 ± 12.7

Diabetic animals (n = 6) were administered with MCK3 (15 mg/kg b.wt.) in saline or protamine zinc insulin (5 U/kg b.wt.) once daily for 20 days. Control diabetic animals (n= 6) were treated with equal volume of saline. The data represent mean ± S.E. * $P < 0.05$; ** $P < 0.01$;

The earlier studies had shown that rise in body weight in the bittermelon treated diabetic rats compared to well controlled normal rats.[29] Dietary fiber rich foods are known to be consumed in higher amounts, which may be due to better palatability.[30] Fraction MCK3 of bittermelon seed extract was effective in preventing polyuria and polydepsia conditions during experimental diabetes. Similar trend in the excretion of urine sugar experimental was followed in fraction MCK3 treated diabetic group, which was consistent with earlier studies.[31] 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.[32] We observed partial yet significant reduction in the kidney weight in fraction MCK3 of bittermelon seed extract treated diabetic rats. Hyperfunctional kidney with increase in GFR is reported in the early stages of

diabetes.[33] Long term metabolic control is known to reduce kidney filtration in human diabetic subjects.[34] In our study, GFR increased considerably in diabetic rats. Treatment of diabetic rats with fraction MCK3 showed significant reduction in kidney filtration. Earlier studies with dietary fiber (guar gum) have also shown significant improvements in GFR during diabetes.[35-37] The polyuria condition prevailed in diabetic group and was 75 – 85 ml/day all along the experiment (Table 3). Fraction MCK3 treatment showed significant reduction in urine excretion during diabetes. Excretion of urine sugar was monitored weekly upto 20 days. Normal excreted reducing sugar in milligram quantities. The untreated diabetic rats excreted between 6.3 – 8.5 g of reducing sugar per day (Table 3), where as fraction MCK3 treated diabetic rats excreted 3.0 – 4.2 g during the course of the experiment. Significant improvement of about 35% reduction in urine sugar excretion was observed in fraction MCK3 treated diabetic rats during the experimental period. Kidney hypertrophy was assessed as the ratio between the kidney weights per 100 g b.wt. (Table 4).

Table 3: Effect of prolonged MCK3 and insulin treatment on urine volume and urine sugar in diabetic rats.

Group	Urine Volume (mL/24 h)	Urine Sugar (g/24 h)
Saline-treated normal	12.5 ± 2.5	2.7 ± 1.0
Saline-treated diabetic	80.0 ± 5.0*	7.4 ± 2.2*
MCK-3-treated diabetic	15.0 ± 2.0	3.6 ± 1.2
Insulin-treated diabetic	16.5 ± 2.5	3.7 ± 1.3

Diabetic animals (n = 6) were administered with MCK3 (15 mg/kg b.wt.) in saline or protamine zinc insulin (5 U/kg b.wt.) once daily for 20 days. Control diabetic animals (n= 6) were treated with equal volume of saline. The data represent mean ± S.E. $P < 0.05$; $P < 0.01$.

Table 4: Effect of prolonged MCK3 and insulin treatment on kidney weight and GFR in diabetic rats.

Group	Kidney Weight (g/100 g)	Glomerular Filtration Rate (ml/min)
Saline-treated normal	0.68 ± 0.02	1.18 ± 0.16
Saline-treated diabetic	1.33 ± 0.07*	6.01 ± 0.53*
MCK-3-treated diabetic	0.61 ± 0.03	1.26 ± 0.15
Insulin-treated diabetic	0.83 ± 0.04	1.32 ± 0.31

Diabetic animals (n = 6) were administered with MCK3 (15 mg/kg b.wt.) in saline or protamine zinc insulin (5 U/kg b.wt.) once daily for 20 days. Control diabetic animals (n= 6) were treated with equal volume of saline. The data represent mean ± S.E. $P < 0.05$; $P < 0.01$.

During diabetes, the kidney weight nearly doubled indicating kidney hypertrophy. Treatment with fraction MCK3 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 fraction MCK3 treated diabetic rats. Significant change was observed in urea, creatinine, electrolyte levels indicative of nephroprotective and normoelectrolytic effect in both fraction MCK3 as well as insulin treated diabetic animals when compared to normal control animals (Table 5).

Table 5: Effect of prolonged MCK3 and insulin treatment on biochemical parameters in diabetic rats.

Parameters	Saline-treated Normal	Saline-treated Diabetic	MCK-3 treated Diabetic	Insulin treated Diabetic
Electrolytes				
Sodium (mmol/l)	141.5± 6.5	129.86 ± 1.44	144.38 ± 1.29**	142.18 ± 1.17**
Potassium (mmol/l)	4.40 ± 0.9	2.58 ± 0.196	4.92 ± 0.171*	4.87 ± 1.13*
Chloride (mmol/l)	102.0 ± 4.5	92.72 ± 1.10	102.82 ± 0.90**	101.0 ± 1.21**
Kidney function				
Urea (mg/dL)	37.2 ± 0.37	61.4 ± 0.51	38.0 ± 1.0*	41.1 ± 1.15*
Creatinine (mg/dL)	1.10 ±0.10	6.48 ± 2.6	1.80 ± 0.20*	1.88 ± 0.86*

Diabetic animals were treated with fraction MCK3 (15 mg/kg b. wt.) or protamine zinc insulin (5 U/kg b.wt.) once daily for 20 days. The serum was analyzed for biochemical parameters related with electrolytes and kidney function. The data represent mean ± S.E. The control animals received corresponding volume of

saline. Each group consisted of 6 animals each. * $P \leq 0.05$, * * ≤ 0.01 (student's *t*-test) vs control diabetic (0.5 ml).

In the present study, Fraction MCK3 has been isolated from acid ethanol extract of bittermelon seeds showing significant hypoglycemic activity in type-I diabetic rats in which diabetes was induced using alloxan during 20 days study period. The hypoglycemic effect brought by the MCK3 fraction of bittermelon seeds was comparable to that observed with insulin treatment of diabetic animals with protamine-zinc insulin has abled to prevent the mortality in diabetic animals. Insulin also used to alleviate the increase in blood glucose levels, urine volume, urine sugar, GFR. Over a period of 20 days treatment daily with fraction MCK3 at a much lower dose (15 mg/kg b.wt.) significantly reduced the blood glucose level near to normoglycemia in alloxan-induced diabetic rats. The repeated administration of fraction MCK3 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 potential anti-diabetic hypoglycemic principle(s). No visible side effect or toxicity was observed in MCK3 treated diabetic group of animals during entire experimental period of 20 days. The active hypoglycemic principle(s) present in fraction K3 had favorable effect on urine volume, urine sugar, water intake, diet intake, gaining in body weight of treated diabetic rats. MCK3 treatment not only normalizes blood glucose level but also resulted in reduction in urea and creatinine level – the key parameters of kidney function test. MCK3 treatment also normalizes the imbalance of serum electrolytes apart from reducing kidney weight and GFR of diabetic rats.

CONCLUSION

The hypoglycemic principle present in fraction MCK3 is able to alleviate kidney damage caused by alloxan-induced diabetes and have brought nearly normal glycemic status in alloxan-induced diabetic rats.

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