

Hypoglycemic Activity Of Active Principle(S) From Seeds Of Momordica Charantia Mediates Through Pancreatic As Well As Extra-Pancreatic Actions In Type-I Diabetic Animals.

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Abstract

The present study evaluates hypoglycemic activity of active principle(s) from seeds of momordica charantia mediates through pancreatic as well as extra-pancreatic actions in Type-I diabetic animals. Blood glucose levels were evaluated before and after administration of different fractions of the seed extract. Among the three fractions tested, fraction MCK3 at a much lower dose of 15 mg/kg b.wt. showed the maximum hypoglycemic activity and reduced blood glucose levels in alloxan – induced diabetic rats significantly. The activities of the key regulatory enzymes of glucose metabolism (hexokinase, pyruvate kinase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase), serum insulin, liver and muscle glycogen were determined in MCK3 treated diabetic animals. Loss in hypoglycemic activity of the fraction MCK3 upon proteinase-K treatment indicates the proteinaceous nature of the hypoglycemic principle(s). Overall, the results suggest that the hypoglycemic activity of active principle(s) from seeds of Momordica charantia is mediated by pancreatic as well as extra-pancreatic actions in Type-I diabetic animals. Bittermelon (Momordica charantia) contain active hypoglycemic protein(s) which may find application in treatment of diabetes without evident toxic effects.

Keywords:- Hypoglycemic principle(s), Bittermelon (Momordica charantia), Insulin, Type-I diabetes, Pancreatic, Extra-pancreatic.

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I. Introduction

Diabetes mellitus, a metabolic disorder, is a major global health concern with a projected rise in prevalence from 171 million in 2000 to 366 million in 2030 [1]. The disease is caused due to an insufficiency of insulin secretion, insulin action or both. While type 1 diabetes can be easily managed with regulated insulin administration, repeated administration of insulin prior to every meal is not desirable. Insulin treatment, if not managed properly, occasionally can result in severe hypoglycemia, a life-threatening situation. Continued administration of therapeutics such as sulfonylureas, biguanides, thiazolidinediones, and alpha glucosidase inhibitors used for the treatment of type 2 diabetes is also known to cause undesirable effects [2]. For this reason, there is an increased interest among diabetics for complementary and alternative medicine involving the use of traditional medicinal herbs and their products, and other dietary supplements [3]. *Momordica charantia* L., commonly known as bittermelon, is one of the most used plants for the treatment of diabetes and related conditions in traditional system of medicine world over [4-6]. The extracts from fruit pulp, leaves, and whole plants of bittermelon have been reported to exert hypoglycemic activity in experimental diabetic animal models [7-10], or glucose-loaded rats [11, 12]. In addition, the plant has also been reported to possess other therapeutic activities like anti-tumor [13], anti-human immunodeficiency virus (HIV) [14], anti-ulcerogenic [15] and hypolipidemic activities [16]. While a number of reports have been put forth demonstrating the hypoglycemic activity of bittermelon, systematic studies to evaluate the possible mode of action of active hypoglycemic principle(s) present in fractionated seed extracts on the blood glucose levels together with acute toxicity studies have not been carried out. Therefore, the present study focuses on the bioassay guided fractionation of the acid-ethanolic extract of bittermelon seeds to identify the fraction that contains the active principle(s) responsible for the anti-diabetic activity. The study further evaluates the possible mode of action of the active fraction by which it elicits hypoglycemia in alloxan-induced diabetic rats. In addition the prolonged treatment with the active fraction proved to be non toxic, further facilitate the use of bittermelon seeds for the management of Type-I diabetes.

II. Materials And Methods

2.1 Plant Material and Animals. *Momordica charantia* seeds were procured from Indian Agriculture Research Institute (IARI), Pusa, New Delhi in August 2010 [17] in large quantity to maintain the consistency of the stock for extract preparation. All the chemicals were of analytical grade and were procured from Sigma-Aldrich Chemical Co., USA, or Boehringer- Mannheim, Germany, unless otherwise stated. Protamine-Zinc insulin was procured from Boots Pharmaceuticals Ltd., India. Random bred male Wistar rats of 175 – 200 g. b. wt. (12–14 weeks) were housed in the Small Animal Facility of the Department of Biochemistry, Patna University, Patna. The animals were provided with rat feed (Hindustan Lever Ltd, India) and water ad libitum regarding the use of animals institutional guidelines were followed while handling animals.

2.2 Seed Extract Preparation and Fractionation. All extraction and purifications were performed at 4°C. Decorticated seeds were extracted in 10 volume (w/v) of 75% ice-cold acid ethanol containing 0.2N HCl and 1 mM PMSF and incubated overnight (O/N) at –20°C to give rise to crude extract (MCKC). This was then centrifuged at 20,000 ×g for 1 h to give rise to the pellet fraction (MCK0) and the supernatant fraction (MCK1). After removal of ethanol by speed-vac concentration, concentrated fraction MCK1 was fractionated by differential salt precipitation using 0.1–1 M ammonium carbonate gradient (pH 7.0) followed by centrifugation at 20,000 ×g for 1 h. The insoluble and soluble fractions were designated as MCK2 and MCK3, respectively. Bioactivity of the fractions was measured at each step of purification.

2.3 Induction of Diabetes in Rats. The male adult Wistar rats (175 – 200 gm) 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.

2.4 Evaluation of Biological Activity of Momordica charantia Seed Fractions. The diabetic animals were grouped into experimental groups each containing minimum 5 – 6 rats. The doses of different fractions are expressed in terms of their protein content. Different groups were treated with different bittermelon seed fractions (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 IU/kg b.wt., s.c.) served as standard reference control. A group of normal saline treated non-diabetic animals was also included in the study. Glucose level was measured in blood drawn from the tail vein during the study period using Bergmeyer enzymatic method [18].

2.5 Dose and Time Kinetics of Hypoglycemic Activity of the Active Fraction. In order to determine the time by which the active fraction is able to bring about hypoglycemic effect, a short-term (0–4.5 h) study was conducted by measuring the blood glucose levels within the indicated periods after administration. Short-term time kinetics of the active fraction in diabetic rats was determined with a dose of 15 mg/kg b.wt. administered intraperitoneally (i.p.) or insulin (5 IU/kg b.wt.). The blood glucose levels were measured at different time intervals. Optimum dose of the active fraction was determined by administering the animals with different concentrations (5–25 mg/kg b.wt.) of the active fraction. Blood glucose levels were measured at 3 h after administration.

2.6 Effect of the Hypoglycemic activity of active fraction of Bittermelon Seed extract on Biochemical Parameters in Diabetic Rats. The rats were divided into different groups (five rats in each group): Group I—saline -treated normal non-diabetic controls, Group II — saline treated diabetic rats, Group III— diabetic rats treated with 15 mg/kg b.wt. of the active fraction, and Group IV—the diabetic rats treated with protamine zinc insulin (5 IU/kg b.wt.). The first two groups of rats were given saline (vehicle) daily. The extract and insulin were administered at the selected dosage to Groups III and IV, respectively, every day for 20 days. The rats were bled prior to sacrifice on the last day of the treatment by cervical dislocation. Serum was collected and subjected to biochemical analysis of glucose and insulin in automated CPC Turbochem 100 chemistry analyzer. Liver excised immediately after sacrifice was washed in chilled PBS and homogenized in 0.025 M sucrose prepared in 0.02 M triethanolamine buffer (TRA-HCl, pH 7.4). After centrifugation at 1,00,000 ×g, supernatant was used for the assay of different enzymes like glucokinase [19], pyruvate kinase [20], lactate dehydrogenase [21], glucose-6-phosphate dehydrogenase [22], and malic enzyme [23]. The glycogen content of the liver and skeletal muscle was estimated as described by Seifter et al. [24].

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

III. Results

3.1 Hypoglycemic activity of Seed Fractions of Momordica charantia The crude acid ethanolic extract upon centrifugation gave rise to fractions MCK0 (insoluble fraction) and MCK1. Since fraction MCK0 contained insoluble material, it was not administered in the animals. The fraction MCK1 and the fractions MCK2 and MCK3 derived from MCK1 were tested for their hypoglycemic potential in experimental diabetic

rats. As evident, fraction MCK1 at the tested dose (15 mg/kg b.wt.) was able to lower blood glucose levels (Table 1). After having established that fraction MCK1 possessed the hypoglycemic activity, its further fractionation was carried out, which resulted in insoluble fraction (MCK2) and supernatant fraction (MCK3). Analysis of the hypoglycemic activity of these fractions demonstrated the activity to be enriched in the fraction MCK3. The fraction MCK3 at a dose of 15 mg/kg b.wt. reduced the blood glucose levels by approximately 36% of the saline-treated diabetic control animals by the 3rd day while only 24% reduction was brought about by equal amounts of fraction MCK1. Fraction MCK2 appeared to have no effect on glucose levels of diabetic animals and 5 out of 7 animals in the MCK2 treated group died by the 3rd day (Table 1). Unlike, saline-treated diabetic animals, serum glucose levels were maintained in the animals treated with insulin, without any further rise.

TABLE 1: Effect of different fractions of *M. charantia* seed extract on blood glucose levels (mg/dL) in Type-I diabetic rats.

Period (days) of treatment	0	1	3
Saline Treated Normal Control Rats	90 ± 10	91 ± 8	88 ± 11
Saline Treated Diabetic Control Rats	457 ± 72	578 ± 112	655 ± 98
MCK1 Treated Diabetic Rats	412 ± 48	362 ± 27*	331 ± 14*
MCK2 Treated Diabetic Rats	366 ± 72	493 ± 92	NM
MCK3 Treated Diabetic Rats	368 ± 57	299 ± 29*	270 ± 30***
Insulin Treated Diabetic Rats	351 ± 68	324 ± 25*	295 ± 18**

The diabetic rats were treated with different fractions (MCK1, MCK2, and MCK3) at a dose of 15 mg/kg b.wt. and protamine zinc insulin (5 IU/kg b.wt), once daily for 3 days. Normal control and diabetic control animals were treated with corresponding volume of saline (0.5 ml). The data represent mean ± S.E. Each group consisted of at least 5-6 animals. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 compared with the respective group on day 0. NM indicates “not measured.”

3.2 Dose and Time Kinetics of Hypoglycemic Effect of active fraction MCK3. Since fraction MCK3 was found to possess enriched hypoglycemic activity, it was desirable to study how quickly fraction MCK3 could exert its hypoglycemic effect after administration. Therefore, blood glucose levels of the MCK3 treated animals were measured at different time after administration for a short period of 4.5 h. The fraction MCK3 was able to bring down the blood glucose levels significantly by three hours and maintain the same even at 4.5 h after administration. Blood glucose levels were significantly reduced (~40%) in the diabetic animals treated with insulin within 1.5 h, which continued to remain lower during the experimental period. In order to assess the optimum concentration of MCK3 that was able to bring about significant reduction in blood glucose levels of diabetic animals, the animals were administered with different concentrations of the fraction MCK3 (5–25 mg/kg b.wt.) and the blood glucose levels were determined at 3 hr after administration (the time point determined earlier for visualizing the effect). It was observed that MCK3 showed an increased reduction in blood glucose levels till 15 mg/kg b.wt. No further reduction in the blood glucose levels was observed when the animals were treated with a higher concentration (20 mg/kg b.wt.) of fraction MCK3 (Table 2).

3.3 Effect of Long-Term Fraction MCK3 Treatment of Diabetic Animals. In order to assess the long-term effect of fraction MCK3, the diabetic animals were maintained on fraction MCK3 for a period of 20 days in order to assess if the continued administration of the active fraction MCK3 had some toxic or undesirable effect. As expected, the blood glucose levels of the animals treated daily with MCK3 were lower when compared to initial levels prior to the treatment (Table 3). A single daily injection of protamine zinc insulin was able to maintain blood glucose levels in diabetic animals and resulted in slightly reduced blood glucose levels after one week of treatment. Unlike, the MCK3 and insulin-treated group, the PBS-treated diabetic animals showed continued increase in blood glucose levels. Mortality of the diabetic animals was also significantly reduced by administration of different fractions of *M. charantia* seed extracts. The MCK3 treated animals had a 100% survival during the study period of three weeks with a normal behaviour, whereas the untreated control diabetic animals were lethargic, weak and showed only about 40% survival by the end of three-week period.

3.4 Effect of Fraction MCK3 on Tissue Glycogen and Enzymes of Glucose Metabolism to evaluate its Possible mode of Action: In order to determine if the reduction in blood glucose levels by MCK3 treatment in alloxan – induced diabetic rats is due to increased glucose utilization, liver and muscle glycogen levels were measured. A significant reduction in both the liver and muscle glycogen levels was observed in the diabetic animals when compared to normal controls. MCK3 treatment resulted in an increase of ~53% and ~77% in glycogen content of liver and muscle when compared to the saline-treated diabetic animals. Both the liver and muscle glycogen levels were also found to be elevated to almost normal levels in the diabetic animals treated with insulin. The effect of MCK3 treatment on key enzymes of glucose utilization in the livers of treated animals was also evaluated. To understand the possible mechanism of action, the diabetic animals were

administered with either fraction MCK3 or same volume of saline and maintained for 3 days on respective treatment. On day 4, the animals were injected with the test samples and were sacrificed after 3 hr of injection. Activities of the various regulatory enzymes were estimated in the livers of the control (saline treated) and MCK3-treated diabetic animals. Control diabetic animals showed reduced levels of all the enzymes when compared to normal controls. It was observed that fraction MCK3 resulted in a significant increase (~2 fold) both in pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase (G6PDH) activities, whereas only ~1.3 and ~1.6 fold increase in the specific activity of hexokinase (HK) and malic enzyme (ME) was noted. However, no significant change in lactate dehydrogenase (LDH) activity was observed. Insulin-treatment also resulted in an increase in the activities of all the enzymes and restored their levels to that observed in normal non-diabetic animals. Earlier reports have indicated that the bittermelon fruit/seeds contain polypeptides that are capable of reducing blood glucose levels. Loss of activity upon heat treatment and proteinase-K treatment of fraction MCK3 confirmed the principle(s) to be of proteinaceous in nature.

TABLE 2: Determination of the optimum dose of fraction MCK3 for hypoglycemic activity in Type-I diabetic rats.

Period (hr) of treatment	0	3	Reduction %
Saline Treated Normal Control Rats	90 ± 10	86 ± 11	4.6
Saline Treated Diabetic Control Rats	414 ± 16.5	428 ± 18.7	3.27
MCK3 (5 mg/kg b.wt.) Treated Diabetic Rats	436 ± 37.0	344 ± 9*	26.74
MCK3 (10 mg/kg b.wt.) Treated Diabetic Rats	401 ± 13.8	300 ± 17.1***	33.66
MCK3 (15 mg/kg b.wt.) Treated Diabetic Rats	521 ± 34.3	285 ± 21.7***	82.80
MCK3 (20 mg/kg b.wt.) Treated Diabetic Rats	537 ± 39.0	296 ± 21.0***	81.41
MCK3 (25 mg/kg b.wt.) Treated Diabetic Rats	534 ± 16.1	442 ± 13.0	20.81
Insulin (5 IU/kg b.wt.) Treated Diabetic Rats	506 ± 19.8	282 ± 16.5	79.43
Proteinase-K + MCK3 (15 mg/kg b.wt.) Treated Diabetic Rats	414 ± 32.4	378 ± 39.1	9.5

Diabetic animals were treated with different doses of fraction MCK3 (0.5 ml). Blood glucose (mg/dL) was measured before and 3 h post-administration. Saline- treated normal and diabetic animals were included as controls. Protamine zinc insulin-treated animals were included as positive controls. The data represent mean ± S.E. Each group consisted of at least 5-6 animals. *P < 0.05; ***P < 0.001, compared with the respective group at 0 hr.

TABLE 3: Effect of prolonged MCK3 treatment on blood glucose levels in Type-I diabetic rats.

Period (days) of treatment	0	4	8	12	16	20
Saline Treated Normal Rats	83.4 ± 8.3	81 ± 6.4	83 ± 7.2	85 ± 7.7	87 ± 7.7	88 ± 7.6
Saline Treated Diabetic Rats	407 ± 73	491 ± 42	5.6 ± 47	467 ± 44	456 ± 67	543 ± 80
MCK3 Treated Diabetic Rats	367 ± 106	250 ± 98**	240 ± 68**	238 ± 77**	210 ± 55**	189 ± 66***
Insulin Treated Diabetic Rats	418.92 ± 23	334.2 ± 61**	346.8 ± 70	285.4 ± 46***	263.9 ± 47***	255.3 ± 8***

Diabetic animals (n = 6) were administered with saline, MCK3 (15 mg/kg b.wt.) and protamine zinc insulin (5 IU/kg b.wt.) once daily. Blood glucose levels (mg/dL) were measured on the days indicated. Control diabetic animals (n = 10) were treated with equal volume of saline (0.5 ml). The data represent mean ± S.E. *P < 0.05; **P < 0.01; ***P < 0.001, compared with blood glucose levels on 0 day.

TABLE 4: Effect of intraperitoneal administration of fraction MCK3 of bittermelon seed extract on activities of key regulatory enzymes of glucose metabolism in diabetic rat liver.

Group No.	Treatment (Intraperitoneal)	Enzyme Activity (μ moles/min/ing/mg protein)				
		Hexokinase	Pyruvate kinase	Lactate dehydrogenase	Glucose-6-Phosphate dehydrogenase	Malic enzyme
1.	Diabetic control + Saline (0.5ml) (4)	6.8 ± 1.5	35.0 ± 6.0	31.2 ± 4.0	13.0 ± 3.0	3.0 ± 0.2
2.	Diabetic + Fraction MCK3 (15mg/kgBW)(4)	9.05 ± 0.5*	80.0 ± 2.6**	39.0 ± 3.9	26.0 ± 4.0*	5.0 ± 1.0*

Diabetic animals were administered with fraction MCK3 for 3 days. Control animals received equal volume of saline. Animals were killed on day 4, 3 h after injection of test sample and liver homogenate was subjected to various enzyme assays. The values represent the mean S.E. of specific activity of enzymes. One unit of specific activity is defined as μ moles of NADPH/NADH utilized or liberated per minute per mg protein. *p≤0.05, **P≤0.01

TABLE 5: Effect fraction MCK3 on serum insulin, glycogen content of liver and muscle in Type-I diabetic rats :

S.No.	Treatment (Intraperitoneal)	Biochemical parameters		
		Serum Insulin (μ IU/ml)	Liver Glycogen (g/100g)	Muscle Glycogen (g/100g)
1.	Normal Nondiabetic control + saline (0.5ml)(6)	12.0 \pm 7.0	5.70 \pm 0.47	0.93 \pm 0.13
2.	Diabetic Control + saline (0.5ml)(6)	7.2 \pm 0.83	4.03 \pm 0.188	0.47 \pm 0.034
3.	Diabetic + Fraction MCK3 (15 mg/kg BW)(6)	15.6 \pm 4.21**	6.16 \pm 0.182**	0.83 \pm 0.072**

Diabetic animals were injected with fraction MCK3 (15 mg/kg BW, i.p.). Control animal received equal volume of saline. Animals were killed: 3h after injection of test sample and serum was separated from freshly collection blood, and tissues, which are subjected to various biochemical analysis. The values represent the mean \pm S.E. for the number (n) of animals in parenthesis. * $p \leq 0.05$, ** $P \leq 0.01$

IV. Discussion

Hyperglycemia associated with diabetes mellitus can be controlled by diet management, exercise, oral hypoglycemic agents, and insulin therapy. Both insulin therapy and oral hypoglycemic agents have their own side effects and adaptation problem. The secondary complications of diabetes that appear with lapse of time are actually the major cause of morbidity and mortality. Therefore, development of new approaches for treatment of diabetes that can reduce blood sugar level with better adaptation is desirable. The evaluation of plants and especially of their active principle(s) is a logical way of searching for new drugs to treat the diabetes mellitus. However, the presence of undesirable hyperglycemic substances along with the hypoglycemic components in *Dioscorea dumetorum* extracts has been reported. Thus, the search for a new therapeutic derived from plant for the treatment of diabetes will depend on the proper processing of the plant extracts in order to obtain the desired effect. The detailed toxicological studies both on crude plant extract as well as on the purified substances are also necessary. *Momordica charantia* has been used in indigenous system of medicine since a long time. Several reports have been put forth suggesting different parts of the plant to possess hypoglycemic activity. These have been found to be effective in chemically (alloxan and STZ) induced experimental diabetic rats. Many small molecular weight proteins, polypeptides with antihyperglycemic or hypoglycemic activities have been isolated from *Momordica charantia* [25-27]. Other peptide and protein molecules isolated from seeds of *M. charantia* referred to as Charantin and polypeptide-P have been reported to lower blood glucose level (~25–40%) in experimental diabetic animals [28-33]. In the present study, fraction MCK3 obtained from the acid-ethanol extract of the *M. charantia* seeds has resulted in ~40% reduction in blood glucose levels within 3 h of treatment with an onset of reduction within 1 h of treatment. The hypoglycemic effect brought about by the MCK3 fraction of *M. charantia* seeds was comparable to that observed with insulin treatment of the diabetic animals. Our observations in the insulin-treated animals are in agreement with the earlier reports where in a sharp decrease in the blood glucose levels was observed in protamine zinc insulin within two hours [34]. Like earlier studies, prolonged treatment of diabetic animals with protamine zinc insulin was able to maintain plasma glucose levels [35, 36]. The hypoglycemic activity of the fraction MCK3 is significantly higher than that observed with acid-ethanol extract (fraction MCK1) or other fraction MCK2 derived from MCK1. It appears that fraction MCK2 consists of some toxic compounds that caused skin lesion and necrosis at the site of injection. Over a period of 20 days treatment daily, the blood glucose levels of fraction MCK3 treated diabetic rats were significantly reduced, a desirable criterion for any potential anti-diabetic agent. Also, no hypoglycemic condition was observed in the treated animals. The fraction MCK3 was effective at much lower concentration (15 mg/kg b.wt.) when compared to that of the crude ethanolic extracts of other plants which were found to be effective in the range of 100– 500 mg/kg b.wt. in diabetic rats [37-39]. Thus, fraction MCK3 can work as an effective anti-diabetic agent as it normalized the blood sugar maintenance function, without causing hypoglycemia. Hyperglycemic condition due to partial or total lack of insulin arises because of disturbances in glucose metabolism caused by a decrease in several key enzymes of glycolysis, namely, glucokinase, phosphofructokinase, and pyruvate kinase, thus resulting in impaired peripheral glucose utilization and augmented hepatic glucose production [40]. Also, chronic diabetes results in a decrease in liver weight due to enhanced catabolic processes such as glycogenolysis, lipolysis, and proteolysis [41]. An increase in tissue glycogen levels, the primary intracellular storage form of glucose upon treatment with fraction MCK3 can directly be correlated with its hypoglycemic activity. This could be due to stimulation of the glycogen synthesis and inhibition of glycogen phosphorylase [42, 43]. At the same time, it inhibited the gluconeogenic process that ethanol fractions of *M. charantia* fruit brought down the levels of hepatic gluconeogenic enzymes (glucose-6-phosphatase and fructose-1,6-biphosphotase) significantly in diabetic animals. Similar results with an increase in the hepatic glycogen have also been reported with *S. cordatum* extract, capable of bringing down blood glucose

levels [44]. Also, an increase in the activity of glucose utilization enzymes could contribute to the hypoglycemic effect of fraction MCK3. The present data [45] showed that administration of fraction MCK3 significantly reversed alloxan diabetes, with direct and significant effects on insulin secretion, increasing the glycogen content of liver and muscle and influencing key regulatory enzymes of glucose metabolism, which also suggests bittermelon seeds contain active hypoglycemic principle(s) that enhances the cellular uptake of glucose by promoting insulin release from β -cells of islets of Langerhans of the pancreas and potentiating its effect. Our results are compatible with earlier reports on the effect of bittergourd seed on diabetes mellitus suggested the possible mechanism of hypoglycemic action through the stimulation of insulin release by pancreas in diabetic rabbits. The study observed of the activation of β -cells in mildly STZ-diabetic animals in which some β -cells were found active and granulation returned to normal giving insulinogenic effect of MCK3. Anti-diabetic potential of *M. charantia* have employed extracts of whole fruit, pulp, and leaves in experimental diabetic animals of glucose-loaded rats. The present study reports substantial purification of a proteinaceous active hypoglycemic principle(s) from fractionated acid-ethanolic extracts of *M. charantia* seeds. The active hypoglycemic principle(s) is highly effective in bringing down blood glucose levels to near normoglycemia, at much lower concentrations without causing any adverse effect on the treated animals [46]. Studies are in progress to further purify and characterize the active proteinaceous principle present in fraction MCK3 of bittermelon (*Momordica charantia*), which will be helpful in understanding the molecular mechanisms by which hypoglycemic effect is brought about.

V. Conclusion

The hypoglycemic activity brought about by active proteinaceous hypoglycemic principle(s) present in fraction MCK3 of Bittermelon (*Momordica charantia*) seed extract is mediated by pancreatic as well as extra-pancreatic actions in experimental diabetes.

ABBREVIATIONS

MCK:	Momordica charantia Karella
b.wt.:	Body weight
G-6-PDH:	Glucose-6-phosphate dehydrogenase
HK:	Hexokinase
PK:	Pyruvate kinase
LDH:	Lactate dehydrogenase

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