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Haematological Changes Observed with a Novel Active Hypoglycemic Principle from the Seeds of Bittermelon in Experimental Diabetes

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Abstract
The current study evaluates the haematological status observed with a novel hypoglycemic active principle MCK_3P_8 obtained from a fraction of the ethanolic extract MCK_3 from the seeds of bittermelon (Momordica charantia Linn) in alloxan-experimental diabetes. Bittermelon whole fruit, fruit pulp, flower, leaves and the seeds are reported for having hypoglycemic activity, haematinic potential, nutritional elements and prevents various forms of diseases. The seeds are the most prevalent part of the plant used medicinally, as it contains steroidal saponins, vicin, proteins, active hypoglycemic principle(s), rich in essential nutrients specifically vitamins C and A and important minerals (potassium, zinc, magnesium and iron) constituents. A hypoglycemic active principle (MCK_3P_8) obtained from a fraction of the acid-ethanolic fraction MCK_3 of the seed extract of bittermelon seeds (14 ml containing 196 mg of proteins) by gel fitration CC. This hypoglycemic active principle and the Protamine Zinc Insulin were given by intraperitonial injection to alloxan-induced male wistar diabetic rats at a dose of 15 mg/kg body weight of experimental animals. Hypoglycemic activities and haematological changes studied in control, insulin, MCK_3, MCK_3P_8 treated alloxan-induced diabetic rat by measuring the blood glucose level enzymatically and haematological parameters red blood cell count (RBC), hemoglobin (Hb), Haematocrit (HCT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were determined by drawing blood from the tail vein during the study period. Based on the results of this study, we conclude that MCK_3P_8 the hypoglycemic active principle of bittermelon seeds when given intraperitoneally at a dose of 15 mg / kg b. wt. exhibited significant hypoglycemic activity along with improvement in selected haematological status in experimental diabetes.

Key Word: *Bittermelon, Active Hypoglycemic principle, Haematological changes, Experimental diabetes.*

Abbreviations: *MCK_3: Momordica charantia, Karella, Fraction$_3$, MCK_3P_8: Momordica charantia, Karella, Fraction$_3$, Peptide $_8$ Kilo Dalton.*

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I. Introduction

Ayurveda and other traditional system of medicine for the treatment of diabetes describe numerous plants used as herbal medicines. Because of nontoxic and with low side effects and low cost they play an important role as an alternative drug. The alternative complementary medicinal system is now gaining momentum with the knowledge of active principles identified from plant parts (fruits, seeds) and their extracts as an alternative to mainstream western medical treatment. Since ancient times *Momordica charantia* Linn. the fruit-vegetable commonly known as "Karella" in Hindi, Bittergourd / 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 for cytotoxic effect on Plasmodium falciparum trophozoites thus inhibiting their development to the schizont stage [7]. Earlier investigations had confirmed the larvicidal property of *M.charantia* against three mosquito species, Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti that causes dengue fever [8] Previously isolated constituents from *Momordica charantia* include sterols, charantin, momordicine [9-10], cardenolides [11], and polypeptide-P [12]. However, there are some inadequate information's about regarding the effects of hypoglycemic active principle(s) on some hematological parameters in diabetic animals. Therefore, there is scope to search the long term beneficial effect of active hypoglycemic

principle(s) present in bittermelon, the fruit- vegetable, especially those of seed and evaluate their effect on selected hematological parameters in experimental diabetes which is at present unclear. Hence the present research has been done to explain the alterations in the haematological status linked with the bittermelon the herbal medicine for the treatment of diabetes in experimental animals.

II. Material And Methods

2.1 Plant Material: *Momordica charantia* Linn. (Cucurbitaceae), seeds purchased from sales counter of Indian Agriculture Research Institute (IARI), Pusa road, New Delhi, in large quantity to maintain the consistency of the stock for extract preparation and were authenticated by the Taxonomist of the University Department of Botany, Aryabhatt Knowledge University, Bihar, India. A voucher specimen is deposited in the department of biochemistry, Shyamlal Chandrasekhar Medical College, Khagaria-851205, Bihar, India.

2.2 Chemical: 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.

2.3 Animal: Randomly bred male wistar rats, 175-200 g (12-14 weeks), were housed in standard laboratory conditions, in the small animal facility of department of biochemistry, Shyamlal Chandrasekhar Medical College, Khagaria, Bihar, India. The animals were provided with rat feed (Hindustan Liver Ltd, India) and water *ad libitum.*

2.4 Induction of Diabetes: The male wistar rats were made diabetic by using alloxan. Briefly, alloxan was administered i.p. after starving the animals for 36 hrs. at a dose of 150 mg/kg b.wt. Animals were stabilized for 3 days by insulin administration, 1-2 units per day for 2 days. Only those animals having blood glucose level more than 300 mg/100 ml. blood were selected for further analysis.

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

2.6 Effect of the Hypoglycemic activity of active fraction of Bittermelon Seed extract on Haematological Parameters in Diabetic Rats: The rats were divided into different groups (six rats in each group): Group I— saline -treated normal non-diabetic controls, Group II — saline treated diabetic rats, Group III— diabetic rats treated with 15 mg/kg b.wt. of the active fraction, and Group IV—the diabetic rats treated with protamine zinc insulin (10 IU/kg b.wt.). The first two groups of rats were given saline daily. The active fraction 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. The blood sample were collected through cardiac puncture into EDTA (Ethylene diamine tetra acetic acid) vacuitainers for glucose estimation enzymatically and evaluation of selected hematological parameters determined by using the automated hematology analyzer (Sysmex KX-21N^{TM}).

2.6 Statistical Analysis: All the results were analyzed statistically using 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. N represents number of experimental animals.

2.7 Ethical clearance: 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, New Delhi, India.

III. Results

The present study reports purification of an insulin-like hypoglycemic active principle from Bittermelon *(Momordica charantia)* seeds. Hypoglycemic activities studied in control, insulin, fraction MCK_3, MCK_3P_8 treated alloxan-induced diabetic rat by measuring the blood glucose level enzymatically [13] during the study period. The hypoglycemic active principle MCK_3P_8 given by intraperitoneal injection to alloxan- induced diabetic rats at a dose of 15 mg/kg b.wt. showed significant hypoglycemic activity *(<P 0.001)* by 3 hours after administration. The hypoglycemic activity brought about by the MCK_3P_8 was comparable to that observed with insulin treatment of the diabetic rats. Results are reported in Table 1.

Table 1: Hypoglycemic activity of insulin, fraction MCK_3 and novel hypoglycemic active principle MCK_3P_8 of bittermelon (*Momordica charantia)* seeds in experimental diabetes.

Groups Treatment i.p ↓	Blood glucose level (mg/dl) at 0 hour	Blood glucose level (mg/dl) at 3 hour
Normal control +saline (0.5 ml)	91.0 ± 10	86.0 ± 10.0
Control Diabetic+ saline (0.5 ml)	329.0 ± 17.7	315.0 ± 17.2
Diabetic + insulin (10 IU / kg b.wt.)	511.0 ± 38.0	287.0 ± 32.4***
Diabetic + fraction K_3 (15mg / kg b. wt.)	473.0 ± 79.0	277.0 ± 41.1***
Diabetic + MCK_3P_8 (15mg / kg b.wt.)	540.0 ± 67.0	304.0 ± 32.9***

Values are mean ± *S.E.,* Number of experimental rats *N* = 5; ****P <0.001* (Student's *t-test*) vs. control diabetic + saline (0.5 ml).

In order to assess long term consequences of experimental diabetes on selective hematological markers RBC, Hb, HCT, MCV and MCH were estimated in whole blood samples of MCK_3P_8 (hypoglycemic active principle) treated alloxan- induced diabetic rats at a dose of 15 mg/kg b.wt given by intraperitoneal injection for 20 days. The levels of red blood cell (RBC) count, hemoglobin (Hb.) level, haematocrit (HCT) value with mean corpuscular volume (MCV) as well as mean corpuscular hemoglobin (MCH) values, mere significantly elevated *(P <0.01*) in diabetic rats treated with novel hypoglycemic active principle MCK_3P_8 of bittermelon (*MC)* seeds among diabetic rats when compared with corresponding levels in controls at the end of study period. The data is seen as in Table no 2.

Table 2: Effect of novel hypoglycemic active principle MCK_3P_8 of bittermelon (*Momordica charantia)* seeds on haematological changes (RBC Indices) in experimental diabetes.

RBC Indices → Treatment i.p ↓	RBC (10^{12}/ L)	Hb. (g/dL)	HCT (%)	MCV (fL)	MCH (pg)
Normal control + saline (0.5 ml)	5.16 ± 0.28	14.90 ± 0.33	47.40 ± 1.15	91.80 ± 0.18	29.80 ± 0.42
Control Diabetic+ saline (0.5 ml)	3.75± 0.23	11.25 ± 0.28	33.75 ± 1.29	78.15 ± 0.32	20.93 ± 0.56
Diabetic + insulin (5U / kg b.wt)	5.03± 0.18***	13.65 ± 0.31***	43.38 ± 1.16***	83.36 ± 0.27***	27.23 ± 1.02***
Diabetic + fraction K_3 (15mg / kg b.wt)	4.97± 0.39*	12.25 ± 0.41*	38.58 ± 1.28*	80.13 ± 0.41*	26.31 ± 0.64*
Diabetic + MCK_3P_8 (15mg / kg b.wt)	4.94 ± 0.13***	13.41 ± 0.26***	43.99 ± 1.10***	81.15 ± 0.29***	26.94 ± 0.42***

Values are mean ± *S.E.,* Number of experimental rats *N* = 5; **P < 0.05, ***P < 0.001* (Student's *t-test*) vs. control diabetic + saline (0.5 ml). Abbreviation: RBC = Red Blood Cells; Hb. = Hemoglobin; HCT = Haematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin.

IV. Discussion

Insulin and oral hypoglycemic agents, the insulin secretogogues, though in frequent use, have their own limitations and have also certain undesirable side effects [14]. The evaluation of medicinal plants, herbs and especially, of their active principle(s) is a logical way of searching for new drugs to treat this disease. A recent

study has estimated that up to 30% of patients with diabetes mellitus use complementary and alternative medicine. [15]. *Momordica charantia* (MC) has been used in indigenous and modern system of medicine since long. Some phytochemical studies have revealed that this fruit-vegetable is sufficiently rich in proteins. It is believed that both fruits and seeds of MC contain hypoglycemic components that may include alkaloid, vitamins (A and C), iron, phosphorus, potassium, zinc, and orally active insulin-like or insulinomimetic compound [16]. In the present study a novel insulin-like hypoglycemic active principle MCK_3P_8 was purified from a fraction MCK_3 obtained from the ethanolic seeds extract of *Momordica charantia* Linn. MCK_3P_8 was able to bring down the blood glucose level significantly by 3 hours after administration in alloxan-induced diabetic rats. The hypoglycemic activity brought about by MCK_3P_8 was comparable to that observed with insulin treatment of the diabetic rats. The hematopoietic system of animals is a good indicator of toxicology research since it shows marked sensitivity to variations in the surrounding environment in which increasing metabolic demands rapid cellular production and breakdown [17]. In present study hypoglycemic active principle MCK_3P_8 treatment reinstate impaired hematological parameters toward the normal levels in alloxan - diabetic rats suggesting potential corrective effect. Our results showed a significant improvement of selected haematological parameters such as RBC, hemoglobin concentration (Hb.), HCT, MCV with MCH levels in MCK_3P_8 treated diabetic rats when compared to the normal control group. Anemia is assessed either by measurement of the haematocrit or the haemoglobin concentration. Decreased HCT mirrors hemoglobin reduction since HCT is directly proportional to RBC mass thus suggestive of anemia of chronic disease in long term experimental diabetes [18-19]. Anemia is most typical hematologic condition among late complications of diabetes mellitus [20]. In the present study, diabetic rats' became anemic as characterized by reduction in red blood cells, hemoglobin concentration and haematopacked cell volume. Whereas MCK_3P_8 the hypoglycemic active principle treated alloxan-diabetic rats showed remedy of anemia. This remedy may be related with the significant anti-diabetic effect of *Momordica charantia* seeds. The present result may indicate that bittermelon has brought the promising preventive effect in delay and prevention of late complications of diabetes [21]. These findings can be the sensitive index that could be useful as an indirect predictive or distinctive tool in assessing the haematological status in experimental diabetes.

V. Conclusion

As best to knowledge, this solitary research conducted in Bihar, state in India, that investigates the haematological status observed with novel hypoglycemic active principle MCK_3P_8 treatment in experimental diabetes. Based on the results of this study, we conclude that hypoglycemic active principle MCK_3P_8 obtained from bittermelon (*Momordica charantia* Linn.) seeds when given intraperitoneally at a dose 15mg/kg b.wt. not only brings about significant reduction in blood glucose levels by 3 hours after administration but also significantly influenced the hematological parameters in alloxan-induced diabetic rats.

Conflict of interests

There are no conflicts of interest pertaining to the publishing of this research, according to the authors.

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