IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 5 Ser. VII (Sep. –Oct. 2020), PP 30-35
www.Iosrjournals.Org

Effect of fractionated bittermelon (*Momordica charantia*) seed extract on Nephroprotective Status in Alloxan-Induced Diabetic Rats

Zamiruddin Ansari[a]*, Zeba Parween[b], and Shree Ram Padmadeo[c]
[a] *Department of Biochemistry, Patna University, Patna, Bihar, India*
[b] *University Department of Chemistry, Veer Kunwar Singh University, Ara, Bihar, India*
[c] *Department of Botany, Patna University, Patna, Bihar, India*
Correspondence should be addressed to Zamiruddin Ansari,

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 nephroprotective status in alloxan-induced diabetic rats. To evaluate the nephroprotective status of bittermelon, blood glucose level, urine volume, urine sugar, kidney weight, glomerular filtration rate and important parameter of kidney functions were monitored in experimental animals. Urine volume and urine sugar were significantly higher in diabetic controls compared to normal rats. Nephroprotective hypertrophy, nephrotoxicity, increased glomerular filtration rate 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 nephroprotective status were estimated in MCK3 treated alloxan-induced diabetic rats. MCK3 treatment resulted in reduction of blood glucose level, reduction in urine volume, and urine sugar level, decrease in kidney weight and glomerular filtration rate, reduction in serum urea and creatinine at 3 h after treatment. These results clearly provided experimental evidence of nephroprotective effect of fraction MCK3 from bittermelon seed extract which is comparable to insulin treatment. The active hypoglycemic principle(s) present in bittermelon seeds improved nephroprotective status and is able to alleviate kidney damage caused by alloxan-induced diabetes.

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

Abbreviations: *MCK3: Momordica charantia, Karella, Fraction 3, GFR: Glomerular Filtration Rate, DM: Diabetes mellitus.*

Date of Submission: 26-10-2020 Date of Acceptance: 05-11-2020

I. 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].

II. 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 and creatinine) in blood are subjected to biochemical analysis in automated CPC Turbochem100 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.

III. 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 nephroprotective 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
MCK3-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). 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.

Excretion of urine was monitored weekly up-to 20 days (Table 2). Similar trend in the excretion of urine sugar experimental was followed in fraction MCK3 treated diabetic group, which was consistent with earlier studies [29]. 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 nephroprotective status [30]. 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 [31]. Long term metabolic control is known to reduce kidney filtration in human diabetic subjects [32]. 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 [33-35]. The polyuria condition prevailed in diabetic group and was 75 – 85 ml/day all along the experiment (Table 2). 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 2), 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 3).

Table 2: 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*
MCK3-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 3: 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*
MCK3-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 and creatinine indicative of nephroprotective effect in both fraction MCK3 as well as insulin treated diabetic animals when compared to normal control animals (Table 4).

Table 4: Effect of prolonged MCK3 and insulin treatment on biochemical parameters in diabetic rats.

Parameters	Saline-treated Normal	Saline-treated Diabetic	MCK3 treated Diabetic	Insulin treated Diabetic
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 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, kidney weight, glomerular filtration rate 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.

IV. Conclusion

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

Acknowledgement

We are grateful to the Department of Biotechnology (Government of India) to provide Zamiruddin Ansari the Junior Research Fellowship. Satyanand Choudhary is acknowledged for valuable technical support.

References

[1]. **Joseph B and Jini D. 2011a.** Insight into the hypoglycemic effect of traditional Indian herbs used in the treatment of diabetes, Res J Med Plant. **5(4)**:352 – 376
[2]. **Shaw JE, Sicree RA and Zimmet PZ. 2010.** Global estimates of the prevalence of diabetes for 2010 and 2030, Diabetes Res Clin Prac. **87:**4 – 14
[3]. **Patel DK, Prasad SK, Kumar R and Hemelatha S. 2012.** An overview on antidiabetic medicinal plants having insulin mimetic property, Asian Pac J Trop Biomed, **2:**320 – 330
[4]. **Ansari Z & Nehal M. 2003.** The hypoglycemic effect of Momordica charantia seeds is medicated by extra-pancreatic action, J.Sci Pharm. **4(2):**65 – 70
[5]. **Ansari Z, Zafar E & Nehal M. 2003.** 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
[6]. **Gray AM and Flatt PR. 1998.** Action of the traditional anti-diabetic plant, *Agrimony eutopia* (agrimony): Effect on hyperglycemia, cellular glucose metabolism and insulin secretion,
[7]. Br J Nutr, **80(1)**:109
[8]. **Parmar K, P Subhashchandra, P Japan, P Brijesh and Patel MB. 2011.** Effects of bittergourd *(Momordica charantia)* fruit juice on glucose tolerance and lipid profile in type – II diabetic rats, Int J Drug Dev Res **3(2):**139 – 146
[9]. **Kolawole OT and Ayankunle AA. 2012.** Seasonal variation in the anti-diabetic and hypolipidemic effects of Momordica charatia fruit extract in rats, Eur J med Plants **2(2):** 177 – 185
[10]. **Mahwish, Saeed F, Arshad MS, Mahrun Nisha, Nadeem MT and Arshad MU. 2017.** Hypoglycemic and hypolipidemic effects of different parts and formulations of bittergourd *(Momordica charantia)*, Lipids in Health and Disease. **16:** 211 – 221
[11]. **Namdeo KP, Bodakhe SH, Dwedi and Saifi A. 2013.** A review on anti-diabetic potential of *M.charantia* Linn, Int J Pharm Res Bio Sc. **2(6):** 475 – 485
[12]. **Jini D and Joseph B. 2013.** Anti-diabetic effects of *Momordica charantia* (bittermelon) and its medicinal potency, Asian Pac J Trop Dis **3(2):** 93 – 102
[13]. **Ahmad N, Hasan Noorul, Ahmed Z, Zishan M and Zohrameena S. 2016.** *Momordica charantia* for traditional uses and pharmacological actions, J Drug Delivery & Therapeutics. **6(2)**: 40 – 44
[14]. **Bilal M, Iqbal MS, Shah SB, Rashid T and Iqbal MN. 2018.** Diabetic complication and insight into anti-diabetic potentialities of ethno-medicinal plants: Allergy Drug Discovery **(12):** 7 – 23
[15]. **Joseph B and Jini D. 2011b.** A medicinal potency of *Capparis deciduas* – A harsh terrain plant. Res J Phytochem, **5(1)**:1 – 13
[16]. **Singh U, Singh S and Kochhar A. 2012.** A Theraputic potential of anti-diabetic neutraceuticals. Phytopharmacil, **2(1)**:144 – 169
[17]. **Zohary D and Hopf M. 2000.** Domestication of plants in the old world. Oxford: Oxford University Press, **p.** 122
[18]. **Mahmood MF, Zaheraa FE, Ashry E, Nabila N, Maraghy EL and Famy A. 2017.** Studies on the anti-diabetic activities of *Momordica charantia* fruit juicein zotocin – induced diabetic rats, Pharm Bio **55(1):** 758 – 765
[19]. **Saeed F, Afzaal M, Niaz B, Aeshad MU, Tufail T and Hussain MB. 2018**. A natural healthy vegetable, Int J Food Pro. **21(1):** 1270 – 1290
[20]. **Chanda R, Samadder A and Banarjee J. 2019.** Anti-diabetic activity of *Momordica charantia* or Bittermelon, A review, Acta Sci Pharma Sci. **3(5):** 24 – 30
[21]. **Khanna P, Jain SC, Panagariya A and Dixit VP. 1981.** Hypoglycemic activity of polypeptide-p from a plant source, J Nat Prod **44:** 648 – 655
[22]. **Jia S, Shen M, Zhang F and Xie J. 2017.** Recent advances in *Momordica charantia* : Functional components and biological activites, Int J Mol Scie.**(18)** 2555 – 2580
[23]. **Singh Jaipaul, Cumming E, Manoharan G, Kalasz H, Adeghate E. 2011.** Medicinal chemistry of the anti-diabetic effects of *Momordica charantia* active constitutiona and modes of action, The open Med Chem J. **(14):** 70 – 77
[24]. **Omar SH, Ansari Z and Nehal m. 2007.** Hypoglycemic effect of the seeds of *Momordica charantia*, Fitoterapia. **(78):** 46 – 47
[25]. **Ansari Z, Kumar VB, Padmadeo SR and Mohan A. 2020.** Antidiabetic potential of a novel hypoglucemic active principle from bittergourd *(Momordica charantia)* seeds in alloxan-induced diabetic rats, Biospectra **15(2):** 123 – 126
[26]. **H. U. Bergmeyer and E. Bernt, 1974.** "UV- assay with pyruvate and NADPH," in *Method of Enzymatic Analysis*, H. U. Bergmeyer, Ed., **pp.** 574 – 579, Academic Press, New York, NY, USA
[27]. **Miller GL. 1959.** Use of dinitrosalicylic acid reagent for determination of reducing sugar, Anal Chem **31:**426 – 428
[28]. **Folin O and Wu H. 1919.** A system of blood analysis, J Biol Chem **38:**81 – 110
[29]. **Rasch R, Mogensen CI. 1980.** Prevention of glomeruopathy in streptozotocin induced diabetic rats by insulin treatment, Diabetologia **18:**413 – 416

[30]. **Chethankumar M, Rachappaji KS, Nandini CD, Sambaiah K 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
[31]. **Seyer-Hansen K. 1977.** Nephroprotective hypertrophy in experimental diabetes relation to severity of diabetes, Diabetologia **13:**141 – 143
[32]. **Christainsen JS, Gammelgaard J, Frandsen M and M Parring HH. 1981.** Increased kidney size, glomerular filtration rate and nephroprotective plasma flow in short term insulin dependent diabetics, Diabetologia **20:** 264 – 267
[33]. **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,
[34]. Scand J Clin Lab Invest **51:** 31 – 36
[35]. **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
[36]. **W Sun, X Gao, X Zhao, D Cui and Q Xia. 2010.** Benificial effect of C-peptide an nephroprotective morphologyin diabetic rats. Acta Biochemica el Biophysica Sinica. **vol. 42,** no. 12, pp. 893 – 899
[37]. **AN Kesari, RK Gupta , SK Singh, S Diwakar and G Watal. 2006.** Hypoglycemic and hypoglycemic acticity of Aegle marmelos seed extract in normal and diabetic rats, Journal of Ethnopharmacology, vol. **107, 3, pp** 374 – 379

Zamiruddin Ansari, et. al. "Effect of fractionated bittermelon (Momordica charantia) seed extract on Nephroprotective Status in Alloxan-Induced Diabetic Rats." *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 15(5), (2020): pp. 30-35.