Drug Standardisation

Effects of Momordica Charantia in Alloxan Diabetic Rabbits – An Endocrine Aproach

E.N. Sundram*

Abstract

Mother tincture prepared from fruits of Momordica charantia Linn, was studied for its effects on blood sugar, Serum growth hormone (GH) and prolactin (PRL) level in alloxan induced hyperglycaemic rabbits. Increased blood sugar level in alloxanised diabetic rabbits was accompanied with the decrease in GH and PRL. Oral administration of M. charantia Q at a dose level of 0.2 ml./Kg.b.wt. for 30 days produced significant ($p<0.05$) fall in blood sugar level. Serum GH and PRL levels were found to be significantly ($p<0.05$) elevated following the administration of M. charantia Q, when compared with the normal control and control groups. Histopathological studies of pancreas revealed that the drug did not exhibit restoration of â cell c. ls as of Langerhans. However viable â cells were found to be active on the drug treatment. These studies suggest that the hypoglycaemic principles in M. charantia Q may exert a direct effect in the diabetic rabbits probably by a mechanism similar to insulin and also it may have indirect action by increasing insulin secretion from viable â cells. The findings of present study also confirm that the drug is capable of increasing body weight lost due to diabetes. This study was undertaken at Homoeopathic Drug Research Institute, Lucknow.

Introduction

Momordica charantia Linn, commonly known as bitter gourd or Karela (Hindi) is widely cultivated in India. Fruits, leaves and seeds of this plant are used in traditional medicinal system for a number of diseases. Its root is astringent, leaf juice and seeds are anti-helminthic. An alcoholic extract of the plant is used as a stomachic against colic and fever[1].

M. charantia is considered an effective hypoglycaemic agent in the management of diabetes mellitus. The fruits of this plant have been used as anti-diabetic in India[15]. The oral hypoglycaemic activity of the fruit juice of M charantia has been investigated in normal and diabetic laboratory animals [4,7,10,18]. An insulin like protein called "Plant insulin" [11] isolated from M. charantia has shown to possess hypoglycaemic properties when injected subcutaneously in humans [2].

Although substantial work have been done on the hypoglycaemic activity of M.charantia on laboratory animals and in humans, but the hypoglycaemic activity of M. charantia Q (Homoeopathic preparation) has not been probably investigated which is the main objective of this study. Hence the present work was undertaken to investigate the effects of M. charantia Q on blood glucose, serum growth hormone (GH) and serum prolactin (PRL) levels in alloxan induced diabetic rabbits.

Materials and Methods

M. charantia Q was prepared in 45% alcohol on the basis of maximum extractive value on air

*Research Officer (Endocrinology) Drug Standardisation Unit (H) O.U.B–32, Road no.4, Habsiguda, Hyderabad–500 007

dried sample of fruits (supplied by SMPCU, Ooty) as it is not mentioned in Homoeopathic Pharmacopoeia of India. Percolation method has been used in the preparation of the mother tincture.

Healthy, adult rabbits (1000 – 1200 gms.) of New Zealand white color, obtained from Central Drug Research Institute, Lucknow, were used for the study. The animals were offered commercial feed prepared by M/s Ashirwad, Chandigarh and photo-period L/D (Light 12 ±1 hr. and Dark 12 ± 1 hr) was maintained.

Animals were grouped into three of 10 each. Each animal was weighed and blood was collected from the marginal vein of ear lobes for blood sugar, serum GH and PRL estimations before commencement of the experiments. All the animals were injected I.V. 150 mg. /kg. b.w. of alloxan monohydrates (Sigma Chemicals Co. USA) in citrate buffer of ph 4.5. Eight days after alloxan injection , blood from each one of them was again tested. When hyperglycaemia (blood glucose more than 240 mg/dil.) was established, the treatments with M. charantia Q, 45% alcohol (present as a vehicle in the drug) and saline were given in the following fashion:-

Group-I:	Normal Control:	Received 0.2 ml of 0.9% saline/ kg.b.wt. orally once a day for 30 days.
Group- II:	Control:	Received 0.2ml of 45% alcohol / kg.b.wt. orally once a day for 30 days.
Group- III:	Test Drug:	Received 0.2 ml of M.charantia Q / kg.b.wt. orally once a day for 30 days.

Blood samples were collected from the marginal ear veins on 15th & 30th day of treatment for determination of blood glucose, serum GH and PRL levels. All the blood sugar estimations were carried out on 12 hrs. fasted animals using spectrophotometer (Systronis). Serum GH and PRL concentrations were determined by Chemiluminescence immunoassay (Luminometer LB 9501/16) and using the kits supplied by Nichols Institute Diagnostics, USA. All assays were performed in duplicate and in a single run.

Body weights lost and gained over per kg. initial body weight of each animal were recorded. For â cells study, animals from each group were sacrificed. Pancreatic tissues were dissected out, quickly fixed in bouin's fixative and processed. The paraffin sections 4 ì thickness were stained in Haematoxylin- eosin and Gomoris Aldehyde Fuchsin stain[6]. The â cells per islet area in cross sections were examined under light microscope (Olympus). All the data were analysed statistically and level of significance was calculated by student's 't' test.

Results

Blood sugar concentrations of normal, diabetic and drug treatment alloxan-diabetic rabbits are presented in **Table –I**. It is evident from the data that initial blood sugar level values in groups of rabbits injected with alloxan monohydrate had significantly elevated above the range of 240 mg./ dil. showing that they were diabetic. Administration of the M charantia Q at a dose level of 0.2ml./ kg.b.wt. for 30 days exhibited significant ($p<0.05$) fall in blood sugar level when compared with control groups of animals receiving an equal dose of saline and alcohol. The hypoglycaemic activity came into prominence after 15th day of drug treatment. GH and PRL level measured in samples taken from the group of noemal, diabetic and drug treatment animals are shown in **Table-II & III.** The lowered levels of GH and PRL associated with alloxan induced diabetic rabbits were significantly ($P<0.05$) increased on 30th day treatment of M. charantia Q as compared to that of controls.

MOMORDICA CHARANTIA

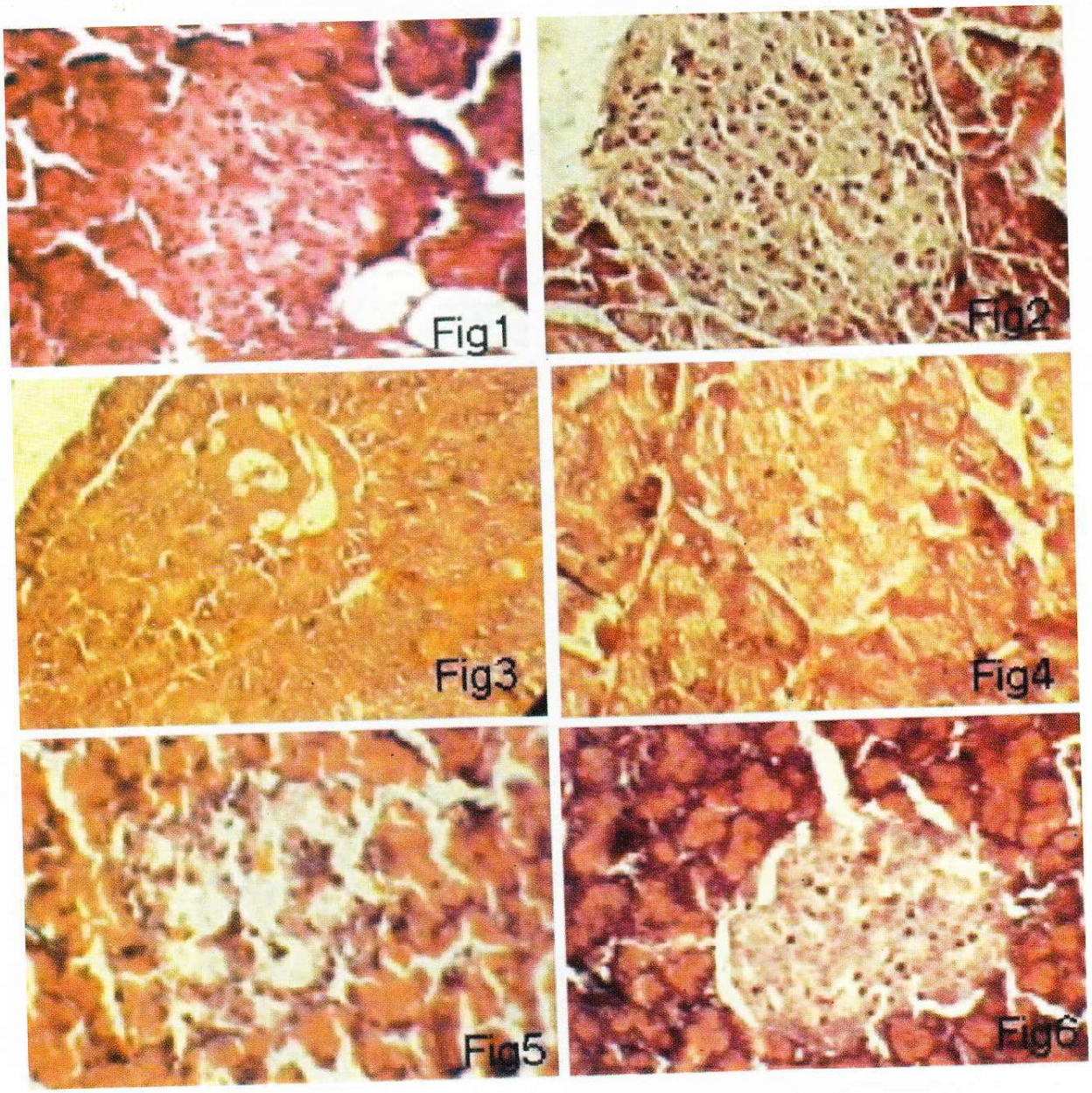

Fig. 1&2. Islet of Langerhans showing histological features before alloxan treatment.
Fig. 3&4. Islet of Langerhans showing necrosis of β cells, viable β cells degranulated after alloxan treatment.
Fig. 5&6. Islet of Langerhans after receiving M. charnatia Q showing darkly granular viable β cells.

Histological examination of pancreas showed that the 30 days administration of M. charantia Q did not restore the cells of Islets of Langerhans which were destroyed by alloxan. There was no evidence of â cells regeneration. However, viable â cells of Islets of Langerhans were found to be more active and granulated on drug treatment. The treatment with drug helped in maintaining body weight whereas saline and vehicle failed to check weight loss.

Discussion

From the data obtained, it is obvious that the administration of M. charantia Q caused a decrease in blood glucose level of alloxan-diabetic rebbits. (Table-I). The mother tincture of drug produced significant hyperglycaemic effect in animals with chemically induced insulin deficiency whereas hyperglycaemia persisted throughout the experiment in the animals which received saline and vehicle. It has been reported that sulphonylurea compound produced hypoglycaemia in normal animals by stimulating the pancreatic â cells to produce more insulin and by increasing the glycogen deposition in the liver. These drugs however, do not decrease blood glucose in alloxan-diabetic animals[8]. In contrast to the oral anti-diabetic agents, the exogenous administration of insulin is well known to produce hypoglycaemia both in normal and in alloxan-diabetic subjects[12]. It is therefore, conceivable that hypoglycaemic principles in the M. charantia Q exert direct effect in the diabetic-rabbits probably by a mechanism similar to insulin.

In the light of strong evidence that alloxan-induces diabetes in laboratory animals[13], it is also clear in the present study that alloxan produced hyperglycaemia by specific destruction of â cells of Islets of Langerhans. On regular administration of M. charantia Q, it produced significant fall in blood sugar although the drug did not exhibit restoration of â cells of Islets of Langerhans. The viable â cells which were found more active on drug treatment provide evidence that M. charantia Q, by stimulating the â cell to enhance insulin secretion, may facilitate the decrease of blood glucose in diabetic-animals. The possibility of M. charantia Q interacting with the â cells of pancreas is further supported by invitro studies [18].

GH and PRL are polypeptides with similar amino acid sequences. As they share some biological activity, they are grouped together as lactogenic hormone of the GH family. The regulation of GH and PRL gene expression and the pathway of GH and PRL synthesis are partly analogous[16]. It is well established and also clear in the present study that increasing blood sugar level in diabetic animals was accompanied with reduced GH and PRL[3]. Exogenous insulin, even when administered after long-term diabetes in rats, partially/totally reversed these alterations,

Table-1. Effect of Momordica charantia Mother Tinture on Blood Sugar Level in Alloxan Diabetic Rabbits. (Mean±S.E.M.)

Dose of 0.2 ml/Kg. b.wt. once a day for 30 days, under following groups	Blood Sugar (mg./dl.)			
	Before Alloxan Injection	After Alloxan Injection	After treatment 15th day	30th day
Normal Control (0.9% saline)	99.4 ± 6.11	260 ± 19.18	291.6 ± 3.56	311.4 ± 7.10
Control (45% alcohol)	93.1 ± 9.56	255.7 ± 8.04	293.1 ± 3.60	315.1 ± 4.25
Test Drug M.charantia Q	97.2 ± 6.94	258.9 ± 18.72	246.7 ± 17.32	105.1 ± 5.60 *

*P<0.05: Significant

Table-II. Effect of Momordica charantia Mother Tinture on Serum Growth Hormone in Alloxan Diabetic Rabbits. (Mean±S.E.M.)

Dose of 0.2 ml/Kg. b.wt. once a day for 30 days, under following groups	Serum Growth Hormone Level (ng./ml.)			
	Before Alloxan Injection	After Alloxan Injection	After treatment 15th day	30th day
Normal Control (0.9% saline)	6.274 ±0.467	1.144 ± 0.119	0.994 ± 0.015	0.747 ± 0.119
Control (45%alcohol)	6.503 ± 0.492	1.150 ± 0.169	0.942 ± 0.091	0.729 ± 0.103
Test drug M.charantia Q	6.11 ± 0.338	1.097 ± 0.115	3.059 ± 0.163	6.121 ± 0.353*

* P<0.05: Significant

Table-III. Effect of Momordica charantia Mother Tinture on Serum Prolactin Hormone in Alloxan Diabetic Rabbits. (Mean±S.E.M.)

Dose of 0.2 ml/Kg. b.wt. once a day for 30 days, under following groups	Serum Growth Hormone Level (ng./ml.)			
	Before Alloxan Injection	After Alloxan Injection	After treatment 15th day	30th day
Normal Control (0.9% saline)	8.597 ± 0.705	2.479 ± 0.441	1.782 ± 0.223	0.919 ± 0.118
Control (45%alcohol)	7.929 ± 0.354	2.484 ± 0.463	1.889 ± 0.144	1.030 ± 0.308
Test drug M.charantia Q	8.017 ± 0.532	2.147 ± 0.086	3.646 ± 0.393	8.128 ± 0.393*

* P<0.05: Significant

proving that they result from diabetes[19]. Infusion of insulin treatment does normalize the reduced GH secretion in diabetic rats[5]. Under some circumstances, insulin can stimulate GH gene expression in the rat pituitary gland invitro [9] and this stimulation could be lacking in the diabetic animals leading to reduced GH. The data obtained in the present study showed that M. Charantia Q treatment is able to increase GH and PRL levels in alloxan induced hyperglycaemic rabbits which is in close conformity with the findings of insulin infusion. It is therefore, supposed that the existence of insulin-like molecules in M. charantia [14] is capable of increasing GH and PRL secretions probably by stimulation on somatotroph and lactotroph cells of pituitary gland or on the hypothalamic cells.

Despite the fact that diabetic rabbits have virtually twice the food intake of normal rabbits, most of the calories in this food are not available to support body weight gain. During the present study, we found that administration of drug daily for 30 days caused gain in body weight. This may also throw some light as the possible mechanism of hypoglycaemic activity either by direct stimulation of glucose uptake/utilization or via the mediation of enhanced insulin secretion from existing β cells.

Acknowledgements

The author is thankful to Director, Central Council for Research in Homoeopathy, for facilities provided and to Prof. M. Prabhakar, Project officer, DSU (H), Hyderabad for his encouragement in the preparation of this article.

References

1. *The Wealth of India-Raw Materials.* Vol. VI. L.-M. Council of Scientific and Industrial Research, New Delhi.

2. Baldwa, V.S., Bhandari, C.M., Pangaria, A. & Goyal, R.K. (1977) Clinical trial with patients with Diabetes Mellitus of an insulin like compound obtained from a plant source. Uppsala *Journal of Medical Science* 82, 39-41.

3. Boujon, E.C., Bestetti, G.E., Abromo, F., Locatelli, V. and Rossi, G.L.. The reduction of circulation growth hormone and prolactin in streptozotocin-induced diabetic male rats is possibly caused by hypothalamic rather than pituitary changes. *Journal of Endocrinology,* 1955

4. Cakici,I., Hurmoglu, C., Tunctan, B., Abacioglu, N., Kanzik, I. and Sener, B. (1994) Hypglycaemic effect of Momordica charantia extracts in hormoglycaemic or cryproheptadine – induced hyperglycaemic mice. *Journal of Ethnopharmacology* 44, 117-121.

5. Carlsson, L.M.S., Clarke, R.G., Skottner, A. and Robinson, I.C.A.F. (1989) Growth Hormone and growth in diabetic rats: Effects of insulin and insuline-like growth factor-I infusion, *Journal of Endocrinology* 122, 661-670.

6. Cullings, C.F.A. (1963) *Handbook of Histopathological Techniques.* II Edn., pp. 341, London: Butterworths.

7. Day, C., Cartwright, T., Provost, J. and Bailey, C.J. (1990) Hypoglycaemic effect of Momordica charantia extracts. *Planta medica* 56,426-429.

8. Dulin, W.E. (1964) Basic pharmacological technique for evaluating antidiabetic agent p. 515. *Animal and clinical pharmacologic techniques in drug evaluation.* Year Book Medical Publishers. Chicago.

9. Isscs, R.E, Gardner, D.G. and Bester, J.D. (1987) Insulin regulation of rat growth hormone gene expression. *Endocrinology* 120,2022-2028.

10. Karunanayake, E.H., Welihinda. J., Sirimanna S.R. and Sinnadorai, G. (1984) Oral hypoglycaemic activity of some medicinal plants of Sri Lanka. *Journal of Ethnopharmacology* 11, 223-231.

11. Khanna, P., Jain, S.C. Pangaria, A. and Dixit, V.P. (1981) Hypoglycaemic activity of polypeptide-P from plant source. *Journal of Natural Product* 44, 648-665.

12. Larner, J. and Haynes, C. (1975) *Insulin and Hypoglycaemic drugs*, Glucogen In : *The Pharmacological basis of therapeuties* 5th Edn., 1507-1528. Macmillan Publishing Co., Inc. New York.

13. Lukenes, F.D.W. (1948) Alloxan diabetes. *Physiological Reviews* 28,304-330.

14. NG,T.B., Wong, C.M., Li, W.W. and Yeung, H.W. (1986) Insulin like molecules in Momordica charantia seeds. *Journal of Ethnopharmacology* 15, 107-117.

15. Nadkarni K.M. (1954) *Indian Materia medica.* Popular book Depot. Bombay, pp. 923-924.

16. Seo, H. (1985) *Growth Hormone and prolactin: Chemistry, gene organization,biosynthesis and regulation of gene expression. In The Pitutary Gland,* pp 57-82. Ed H Imura. New York. Raven Press.

17. Welihinda, J., Arvidson, G., Gylfe, E., Hellman, B. and Karlsson, E. (1982) *The insulin releasing activity of the tropical plant Momordica Charantia. Acta Biologica et Medica Germanica* 41, 1229-1240.

18. Welihinda, J. and Karunanayake, E.H. (1986) Extra pancreatic effects of Momordica charantia in rats. *Journal of Ethnopharmacology* 17, 247-225.

19. Yamauchi, K and Shino, M.(1986) Pituitary prolactin cells in diabetic rats induced by the injection of streptozotocin. *Experimental and Clinical Endocrinology* 88, 81-86.