

KARELA-ANTI DIABETIC AGENT

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Abstract

In an attempt to screen the hypogly-caemic activity of *Momordica charantia* fruit (Karela) the proximate analysis, alkaloidal tests and its effects on blood glucose levels of normal male albino rabbits were determined. Whole dried and powdered *Momordica charantia* fruit was used for this purpose and blood glucose was determined by O-toluidine method at various time intervals.

The proximate analysis and alkaloidal detection studies revealed that the *Momordica charantia* fruit is rich in proteins and nitrogenous substances and also contains some alkaloid. In normal rabbits, the 0.25g/kg dose did not decrease blood glucose. However, 0.5 g/kg dose caused decrease in blood glucose. The effect was maximum at 10 hours interval after which it decreased gradually. The 1.0 and 1.5 g/kg doses lowered the blood glucose of these rabbits. The maximum decrease was produced by these doses at 10 hours interval.

From the results, it could be concluded that *Momordica charantia* fruit possessed significant and consistent hypoglycaemic effect in normal rabbits. The results obtained are discussed in the light of the available literature. It is conceivable that probably *Momordica charantia* contains some alkaloid which exerts hypoglycaemic action. It is however also possible that synergistic compounds are present in the whole fruit, as used in indigenous medicinal preparations, which are capable of producing hypoglycaemia. Further studies are suggested to isolate the active principle(s) and in order to prove the mechanism of hypoglycaemic effect, experiments should be carried out in diabetic animals (JPMA 30:181, 1980).

Introduction

Although considerable developments have been made in the management of diabetes mellitus, yet a wide scope is still left in the search of newer orally effective antidiabetic agents. Medicinal plants have been used for centuries to treat various diseases including diabetes mellitus. Said (1969), Farnsworth and Segelman (1971) have described many herbs and plants which exhibit hypoglycaemic activity. Some of these plants have also been pharmacologically tested by the modern scientific methods and have shown to be useful in diabetes. Among the more recent folklore plants investigated for their antidiabetic properties, the following have shown encouraging results: Myrtillin, isolated from the leaves of blue berry (*Sambucus glauca*) and various extracts like that of devil's club (*Fatsia horrida*) roots; rose periwinkle (*Vinca rosea*) and raw cabbage (*Brassica oleracea*) (Marquis et al., 1977). The plant alkaloids, vindoline and leucosin have demonstrated a high degree of hypoglycaemic activity. The fruit of *Momordica charantia* Linn commonly known as "Karela" is widely cultivated in Pakistan and has been considered an effective antidiabetic agent for centuries. Many patients add the fruit to their diabetic diet and various preparations of *Momordica charantia* plant are still being administered to the diabetic patients (Nadkarni, 1945; Said, 1969). In addition, various parts of this plant are also used for many other medicinal purposes. In view of the great medicinal importance of this plant and its easy and abundant availability in our country this work was undertaken to determine (1) proximate analysis, (2) presence of alkaloids and (3) its hypoglycaemic activity.

Material and Methods

Plant Material: Fresh green fruit of the *Momordica charantia* Linn popularly known as Karela was obtained in sufficient quantity from the local vegetable market of Faisalabad (Punjab) in June, 1979. They were carefully washed with tap water to remove dust and any other foreign material and dried in the mild sun. The completely dried fruit was powdered with an electric grinder and stored in well closed cellophane bags in the refrigerator.

Chemicals: Alloxan-monohydrate (NH-CO-NH. CO-CO- H₂O), Carboxymethylcellulose (CMC), ad-glucose, Xylene, and all other chemicals and reagents used were of the analytical grades prepared either by E. Merck, Darmstadt, West Germany or B.D.H. Laboratories, Poole, England.

Experimental Procedures

1. Proximate Analysis: In order to get an idea of gross chemical constituents of the dried powdered *Momordica charantia* fruit, the proximate analysis was carried out. For this purpose three random samples were taken from the stock powder after thorough mixing. The recommended methods of analysis approved by the Association of Chemical Analysts (AOAC) were employed. The determinations were made for moisture, crude protein (N x 6.25), fats/oils (ether extract), crude fibre and ash (minerals) contents.

2. Test for Alkaloids: In order to detect alkaloids, three random samples of *Momordica charantia* were taken. The presence of the alkaloids was checked by using two different alkaloidal reagents, namely the Mayer's reagent and Wagner's. To confirm the results of these experiments, the tests were repeated at least 6 times.

Effect of *Momordica Charantia* on Blood Glucose

Animals Used: Male, adult, healthy, albino rabbits of a local strain, weighing between 750-1000 g were used in these experiments. The animals were kept in an air conditioned animal room of the physiology and pharmacology department at the University of Agriculture, Faisalabad. The animals were offered a commercial feed prepared by M/S Lever Brothers Ltd., Rahim Yar Khan and allowed tap water adlibitum.

Grouping of Rabbits: Rabbits were randomly divided into 5 groups (I-V) of 3 animals each. Group I served as a control. These animals received orally 10 ml of 1 percent carboxymethyl cellulose (CMC) solution in water. A 0.2 ml sample of blood was immediately collected for blood glucose determination. Similar blood samples were drawn at 5, 10 and 24 hours intervals after the administration of 1 percent CMC solution. The animals of groups II, III, IV and V were treated orally with 0.25, 0.5, 1.00 and 1.5 gm/kg body weight of *Momordica charantia* powder suspended in 1 percent carboxymethyl cellulose solution in water respectively.

Collection of blood: After drug administration, the animal was held in a wooden rabbit holder and immediately 0.2 ml of blood was collected from an ear vein. Similar samples of 0.2 ml were also collected at 5, 10 and 24 hours interval. To prevent coagulation of blood heparinized syringe was always used.

Determination of blood Glucose: Blood glucose was determined by the method of Fings et al (1970), using the O-toluidine reagent. O-toluidine method is one of the most widely used manual methods and therefore it was selected.

Statistical Analysis: The blood glucose levels in the various groups were expressed in mg/100 ml (Means±SEM) and the data was statistically analysed by using analysis of variance technique with factorial arrangement. The comparison of decreases in blood glucose level of rabbits produced by the different doses of *Momordica charantia* found at different time intervals were compared by using Duncans Multiple New Range test (Snedecor, 1965).

Tests for Alkaloids: The alkaloidal detection studies performed by using the Mayer's reagent and also the Wagner's reagent were repeated on several representative samples of the stock *Momordica charantia* powder. In the test tubes containing Mayer's reagent a white precipitate was formed which indicated the

presence of alkaloid. In addition a heavy red brown precipitate was formed with the Wagner's reagent which further confirmed this finding that the Momordica charantia fruit contains alkalid(s).

Effect of Momordica charantia on blood glucose in normal rabbits: The mean blood glucose concentrations \pm SEM of control and drug treated animals after oral administration of different doses of Momordica charantia fruit at various time intervals are summarized in Table II.

Table I: Proximate Analysis of Dried and Powdered Momordica Charantia Fruit

<i>Sr. No.</i>	<i>Constituents*</i>	<i>Percentage on dry basis</i> <i>(Mean \pm SEM)</i>	
1.	Moisture	9.62	\pm 0.02
2.	Crude Protein	15.50	\pm 0.02
3.	Fats or oils (other extract)	3.57	\pm 0.014
4.	Crude fibre	12.78	\pm 0.02
5.	Ash (minerals)	6.53	\pm 0.012
6.	NFE	52	(calculated by difference)

*No. of tests for each parameter=6

Table II: Mean Blood Glucose Levels of Normal Rabbits expressed in mg/100 ml \pm SEM at various Time Intervals after Oral Treatment with 1% (CMC) solution and Dried Fruit of *Momordica charantia* 0.25, 0.50, 1.00 and 1.5 g/kg Body Weight, orally suspended in 1% CMC.

Time interval (Hours)	Groups and dosage of <i>Momordica charantia</i>				
	1% CMC (Group I)	0.25 g/kg (Group II)	0.5 g/kg (Group III)	1.00 g/kg (Group IV)	1.5 g/kg (Group V)
Zero	95 \pm 1.5	97 \pm 3.5	100 \pm 2.2	92 \pm 4.3	99 \pm 1.9
5	95 \pm 1.6	91 \pm 3.1	86 \pm 2.1	74 \pm 3.7	62 \pm 1.3
10	95 \pm 1.7	92 \pm 2.9	77 \pm 0.9	62 \pm 2.4	46 \pm 0.80
24	95 \pm 2	95 \pm 1.8	92 \pm 1.8	78 \pm 3.7	70 \pm 0.66

Number of assays for each observation = 3

CMC— Carboxymethylcellulose

SEM— Standard Error of Means.

The blood glucose levels of animals treated with 1% carboxymethylcellulose (CMC) solution at zero hour after administration was found to be 95 \pm 1.5 mg/100 ml. The CMC did not affect the blood glucose levels of rabbits as they were found to be statistically the same at 5, 10, and 24 hour intervals. The blood glucose level of animals treated with 0.25 g/kg of *Momordica charantia* powder at zero hour interval after drug administration was recorded to be 97 \pm 3.5 mg/100 ml. The drug produced a slight decrease in blood glucose level at 5 hour interval when the level was 91 \pm 3 mg/100 ml. This decrease was, however, found to be statistically non-significant. In addition, the blood glucose levels at 10 and 24 hour intervals were also found to be statistically non-significant from the zero hour level as well as from their preceding values. The mean blood glucose level of the animals treated with 0.5 g/kg *Momordica charantia* was found to be 100 \pm 2.2 mg/100 ml just after the administration of drug. The glucose level after 5 hours of drug administration was 86 \pm 2 mg/100 ml. It was found to be significantly lower than at zero hour. The blood glucose level at 10 hours interval was 77 \pm 0.9 mg/100 ml which was also significantly lower than the zero hour level. After 24 hours of drug administration, the mean glucose level was 92 \pm 1.8 mg/100 ml which was though still significantly lower than at zero hour interval but had significantly increased from the preceding value. The blood glucose level of animals treated with 1 g/kg body weight was found to be 92 \pm 4.3 mg/100 ml at zero hour interval. The glucose level after 5 hours of drug administration was reduced to 74 \pm 3.7 mg/100 ml and it was found to be significantly lower than at zero level. The lowering of blood glucose level continued even at 10 hours interval when the level recorded was 62 \pm 2.4 mg/100 ml. After 24 hours of drug administration, the blood glucose level was found to be 78 \pm 3.7 mg/100 ml which was though statistically higher than at 10 hours interval but still significantly lower than at zero hour. The blood glucose levels of animals treated with 1.5 g/kg of the drug at zero 5, 10 and 24 hours intervals were found to be 99 \pm 1.9, 62 \pm 1.3, 46 \pm 0.80 and 70 \pm 0.66 mg/100 ml respectively. The values at 5, 10 and 24 hours intervals were found to be significantly different than at zero hour and also from the preceding values.

Discussion

It is well known that insulin promotes the transfer of glucose from tissue fluids into body tissue (Guyton, 1975). Thus diabetes usually occurs when B-cells in the pancreatic islets of langerhans are unable to produce insulin. The treatment of uncompleted diabetes is usually an individualized problem and depending upon the severity of the symptoms may involve either the use of orally active antidiabetic agents or one or more injections per day of insulin (Lamer and Haynes, 1975). Insulin is obtained from the animal pancreas and is a replacement therapy only. It does not completely cure or

prevent the disease. The oral hypoglycaemic drugs including sulphonylureas are effective only in patients who still have active islets which for some reason are not secreting adequate quantities of insulin. Obviously, these drugs are of no value in the treatment of severe diabetes of any type and for juvenile diabetics for their islets have already lost all potential ability to secrete insulin (Guyton, 1975). Therefore, till today the search for more effective and safe antidiabetic agents continues as a potential area of investigation. Although diabetes is a complicated metabolic disease involving all aspects of the intermediary metabolism, changes in blood glucose level are a convenient and useful tool in screening of antidiabetic drugs. They give indirect evidence of increase in insulin release (Said, 1969). One of the most useful screening method is based on depression of blood glucose values in intact animals, since the intact animals theoretically possess all the mechanisms involved in blood glucose regulation (Sard, 1969).

In this study, the whole dried finely powdered fruit of *Momordica charantia* was orally administered as suspension in 1% carboxymethylcellulose solution to the normal and alloxan diabetic rabbits. The whole fruit, instead of an extract, was intentionally used on the prediction that the total fruit may contain, in addition to an active principle, some synergistic compounds because the whole fruit is customarily added to the various folkloric medicinal preparations and not an extract of it.

Our data shows that the administration of various doses of *Momordica charantia* fruit caused a decrease in blood glucose level of normal rabbits. It has been reported that sulphonylurea compounds produce more insulin and by stimulating the pancreatic B-cells to produce more insulin and by increasing the glycogen deposition in liver. It may therefore, be suggested that hypoglycaemic principle in the *Momordica charantia* fruit might be acting by stimulating the release of insulin.

The phytochemical studies have revealed that the *Momordica charantia* is sufficiently rich in proteins (Goth, 1974). One of these proteins might be, as already discussed, an orally active insulin-like substance, an alkaloid or a like substance. Nadkarni (1945) has reported that *Momordica charantia* fruit contains some alkaloid. Some alkaloids like vindolining and leuresine have already been shown to exert high degree of hypoglycaemic activity in the normal animals only (Lewis and Lewis, 1977). It is, therefore, hypothesized that the active principle of *Momordica charantia* may be an alkaloid or a like substance producing effect in the normal rabbits. However, production of hypoglycaemia by some synergistic compounds or by some entirely different mechanism cannot be excluded at present. Further investigation for isolation of pure alkaloid and/or other active principles of *Momordica charantia* are suggested. The determination of hypoglycaemic effect in diabetic animals is required to prove the exact mechanism of action.

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References

1. Farnsworth, N.R. and Segelman, A.B. (1971) Hypoglycaemic plants. *Tile. Till.*, 57:52.
2. Fings, C.S., Tatliff, C.R. and Dunn, R.T. Glucose determination by O-toluidine method using acetic acid. *Clinical Chemistry by Toro, C. and P.G. Ackerman. Little Browning and Company, Boston: 115, 1970.*
3. Goth, A. *Medical Pharmacology*. 7th ed. Saint Louis, Mosby, 1974, pp. 472-3.
4. Guyton, A.C. *Text book of Medical physiology*. 4th ed. Philadelphia, Saunders, 1971, pp. 915-28.'
5. Lerner, J., and Haynes, C. *Insulin and hypoglycaemic drugs, glucogen. The Pharmacological basis of*

Therapeutics, 5th ed. New York, Macmillan, 1975, pp. 1507-1528.

6. Lewis, H.W., and Elvin-Lewis, M.P.H. Medical Botany: Plants affecting Man's health New York, John Wiley and Sons, pp. 36, 98, 218 and 515.

7. Marquis, V.O., Adanlawe, T.A. and Olaniyi, A.A. (1977) The effect of feetidin from *Momordica feetida* on blood glucose level of albino rats. *Planta Medica*, 31:367.

8. Nodine, J.H. and Siegler, P.E. Animal and Clinical Pharmacologic techniques in drug evaluation. Chicago, Year book Med. Publ., 1964, p. 515.

9. Nadkarni, K.M. Indian Materia medica. Bombay Popular book depot, 1945, p.p. 805-7.

10. Said, M. Hamdard Pharmacopeca of Eastern Medicine, Hamdard National Foundation, Karachi Times Press, pp. 42. 1969.

11. Snedecor, G.W. Statistical Methods. 5th ed. Ames, Iowa State Univ. Press, 1965.