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Effects of alcoholic extract of *Momordica charantia* (Linn.) whole fruit powder on the pancreatic islets of alloxan diabetic albino rats

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Abstract: *Alcoholic extract of whole fruit of Momordica charantia was prepared. Adult healthy albino rats were divided into four groups and received a dose of 6 mg/100 gm. body weight of alloxan monohydrate. Animals of group I served as diabetic control group. The animals of II, III, and IV groups received 25 mg, 50 mg and 75 mg doses of the extract respectively for different durations. 75 mg dose showed increase in body weight. All doses of alcoholic extract of M. charantia were able to decrease the blood sugar level significantly. Extract feeding showed definite improvement in the islets of Langerhans. No toxic effect was observed in the liver. The significant features of the study have been blood glucose once lowered by the treatment with M. charantia fruit extract remained static even after discontinuation of drug for 15 days. Blood sugar never fell below normal values even with a high dose, in pancreatic islets, beta cells showed definite improvement.*

Key words: *Alloxan diabetes, Pancreatic islets, Momordica charantia Linn., Alcoholic extract*
PDF of full length paper is available with author ***(*n27singh@yahoo.com)***

Introduction

Momordica charantia, commonly known as "Karela" (Family Cucurbitaceae), is a tropical household vegetable used as daily food and also as folk medicine especially for diabetes. For the first time, Rivera (1941) studied the chemical properties of *M. charantia* and isolated an alkaloid 'momordicine' from the alcoholic extract, which was reported to be hypoglycaemic in nature.

In recent times, this plant was scientifically evaluated by many workers such as Nadkarni (1954), Sharma *et al.* (1960), Pabrai and Sehra (1962), Chatterjee (1963), Lal and Chaudhary (1968) and Jose *et al.* (1976). Akhtar *et al.* (1981) studied and compared the effects of dried whole fruit powder of *M. charantia* on alloxan diabetic and normal rabbits. Singh *et al.* (1989), studied effect of long term feeding of acetone extract of *M. charantia* on alloxan diabetic albino rats.

Patel and Shrinivasan (1995); Sasmal and Lakshmi (2000); Dhaneria and Patil (2000); Biyani *et al.* (2003) have also studied the blood sugar lowering effect of *M. charantia.*

As none of the above mentioned worker has studied the effect of *M. charantia* on histology of the islets of Langerhans of pancreas of the alloxan diabetic test animals, the present paper deals with the study of the effect of alcoholic extract of whole fruit powder of *M. charantia* on islets**.**

Materials and Methods

Chemicals: Alloxan monohydrate (2,4,5,6- tetra oxypyrimidine and 6-Dioxyuracil) was obtained from Sigma chemicals. Alcohol was bought from central distillery. Other chemicals and strains used were bought from standard chemical companies.

Preparation of extract: Fresh unripe fruits of *M. charantia* were taken from the plants, washed, cut into small pieces, dried in shade and powdered. The alcoholic extract was prepared with the help of Soxhlet apparatus and dried at 55^0C and stored at 4^0C in airtight containers.

Experimental Animals: Albino rats (*Rattus rattus*) Wistar strain (7-8 weeks old, weighing 100-150g) were used for the present study. Rats were procured from animal division of Indian Veterinary Research Institute, Izzatnagar. Experimental animals were maintained in the laboratory on standard food pellets and tap water *ad libitum* and acclimatized for laboratory conditions for two weeks. All animals were cared for according to the Institutional Animals Ethics Committee (IAEC).

Experimental design: Experimental animals were divided into four groups of 18 animals each. All experimental animals received dose of 6 mg/100 g-body weight alloxan monohydrate in citrate buffer of pH 4.5 through intravenous route.

Group I: This group served as diabetic control group.

Group II: Animals were given oral dose of 25 mg/100 g body weight of alcoholic extract of *M. charantia* fruit powder.

Group III: Animals were given oral dose of 50 mg/100 g body weight of alcoholic extract of *M. charantia* fruit powder.

Group IV: Animmals were given orally 75 mg/100 g body weight dose of alcoholic extract of *M. charantia* fruit powder.

In group II, III and IV six rats were given extract for 15 days, 6 rats for 30 days and 6 rats were fed on extract for 30 days, then extract feeding was discontinued for 15 days (for a total period of 45

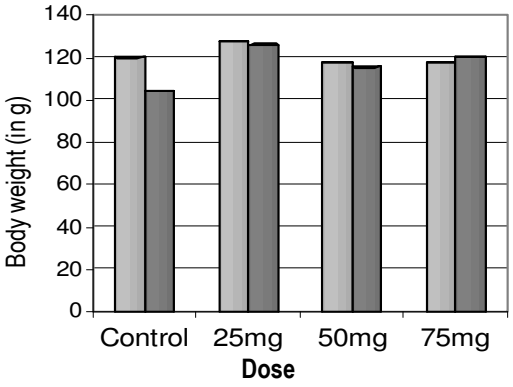

Fig. 1: Bar diagram showing comparison of initial and final body weight of alloxan diabetic rats and effects of different doses of *M. charantia* alcoholic extract for 15 days

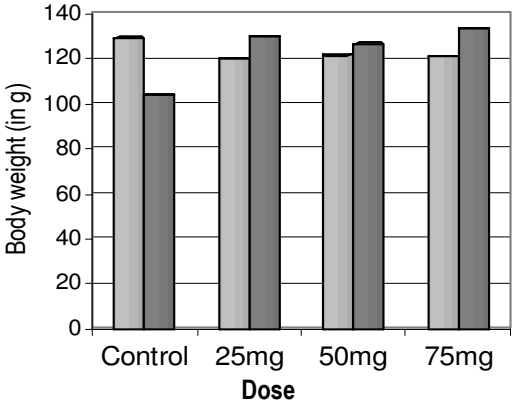

Fig. 2: Bar diagram showing comparison of initial and final body weight of alloxan diabetic rats and effects of different doses of *M. charantia* alcoholic extract for 30 days

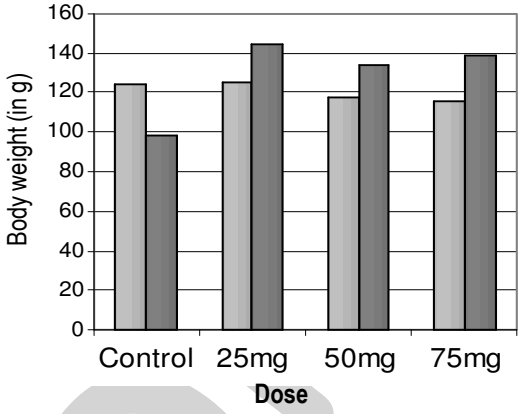

Fig. 3: Bar diagram showing comparison of initial and final body weight of alloxan diabetic rats and effects of different doses of *M. charantia* alcoholic extract for 30 days + 15 days normal diet

Plate - I

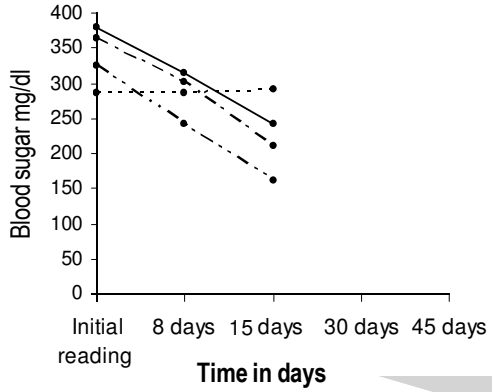

Fig. 1: Graph showing mean fall in blood sugar level of alloxan diabetic albino rats after 15days feeding of different doses of *M. charantia* alcoholic extract and comparison with the blood sugar level of diabetic control group of 15 days

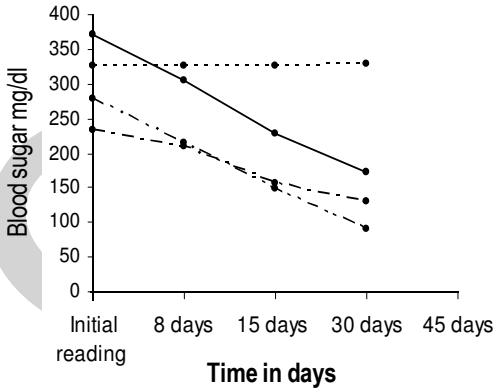

Fig. 2: Graph showing mean fall in blood sugar level of alloxan diabetic albino rats after 30 days feeding of different doses of *M. charantia* alcoholic extract and comparison with the blood sugar level of diabetic control group of 30 days

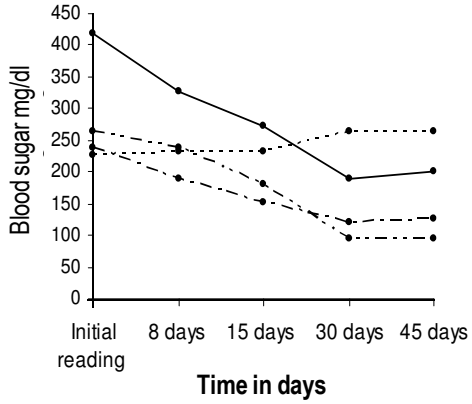

Fig. 3: Graph showing mean fall in blood sugar level of alloxan diabetic albino rats after 30 days feeding of different doses of *M. charantia* alcoholic extract followed by 15 days normal diet and comparison with the blood sugar level of diabetic control group of 45 days

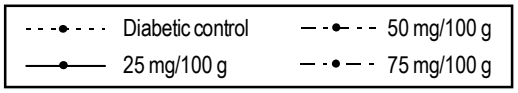

Plate - II

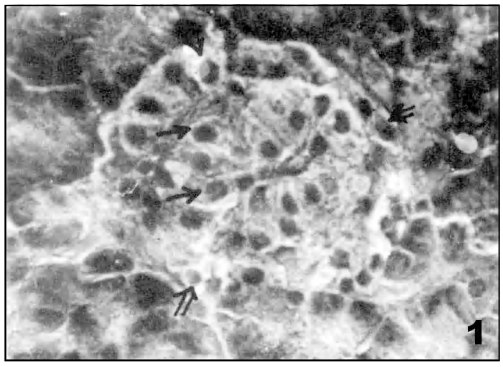

Fig. 1: A normal islet showing alpha cells (⇒) at the periphery and beta cells (→) with granular cytoplasm in the centre (x600)

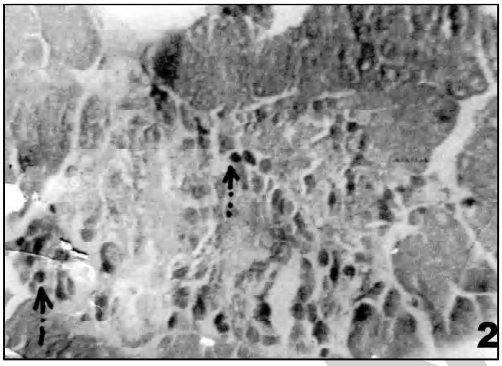

Fig. 2: An islet, totally disfigured, showing clumping (⋯→) and degranulation (–·→) of beta cells after 15 days of alloxan injection (x475)

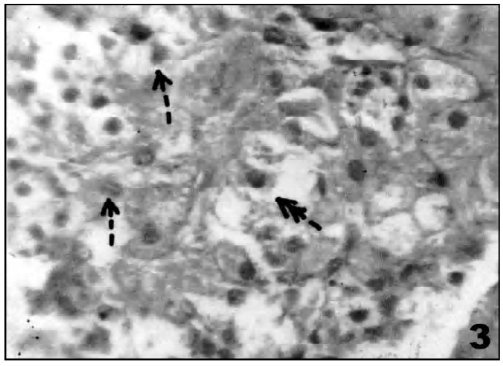

Fig. 3: Hyperplasia of the islet with hydropic degeneration (– – →) and ballooning (↠) of occasional beta cells after 30 days of alloxan injection (x600)

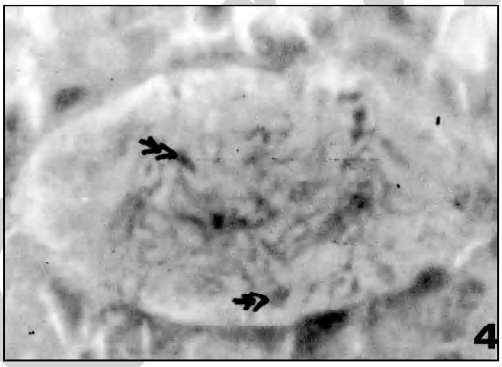

Fig. 4: Complete fibrosis of the islet, showing fibroblasts (→>) after 45 days of alloxan injection (x600)

Plate - III

days). The animals were sacrificed at the end of 15 days, 30 days and 45 days of treatment.

Animals were kept under observation and their body weight was noted at the beginning and end of experiments. The blood was collected for sugar estimation from the caudal vein of control and treated animals before and after the alloxan injection and at regular intervals (Asatoor and King, 1954).

Histological studies: Pancreas was fixed in Bouin's fixative (without acetic acid) for histopathological studies. Sections (6 μm) were stained with chromium haematoxylene-phloxine for differential staining of alpha and beta cells. (Gomori, 1941). Liver was fixed in Bouin's Hollande fixative and stained with HE stain. Sections were studied under Olympus binocular research microscope.

Results and Discussion

Body weight: Almost no weight gain was observed after 15 days of extract feeding in any dose group. After 30 days period there was 8.33, 4.26 and 10.79% increase in body weight in 25 mg, 50 mg and 75mg dose groups respectively. After 30 days extract feeding and the discontinuation of the extract for 15 days (*i.e.* after 45 days of experiment) there was 15.89, 14.50 and 19.74% increase in body weight in all three dose groups respectively (Fig. 1,2,3 Plate I).

Blood sugar: Injection of alloxan results in increase in blood sugar level. Administration of the alcoholic extract of *M. charantia* to alloxan diabetic rats, was able to bring down the elevated level of blood sugar to normal level with all the three doses *i.e.* 25, 50 and 75 mg/ 100 g body weight in 15 to 30 days. Mean fall in blood sugar level is plotted in graph (Fig. 1, 2, 3, Plate II).

Sharma *et al.* (1960) tested the juice of *M. charantia* on normal and alloxan diabetic rabbits and found its blood sugar lowering effect in both groups. Pabrai and Sehra (1962) reported single dose of both fresh juice (10 ml/kg) as well as dried extract (5 mg/kg) of *M. charantia* lowered blood sugar by 4.9% and 10.8% in 5 hr in alloxan diabetic rabbits. Gupta and Seth (1962) found significant decrease in blood sugar levels after intake of 5 mg/Kg dose of *M. charantia*. They stressed that juice did not influence the absorption of glucose from intestine. Krishnamurthy (1962) also reported that fresh extract of *M. charantia* was able to reduce blood sugar levels at the end of 3rd hr in dogs and at the end of 2nd hr in rabbits.

Later Chatterjee (1963), reported that single dose of pressed extract of *M.charantia* without seeds (3 ml/kg) caused 20% fall in blood sugar level of alloxan diabetic rabbits. On the other hand seeds did not show any hypoglycaemic activity.

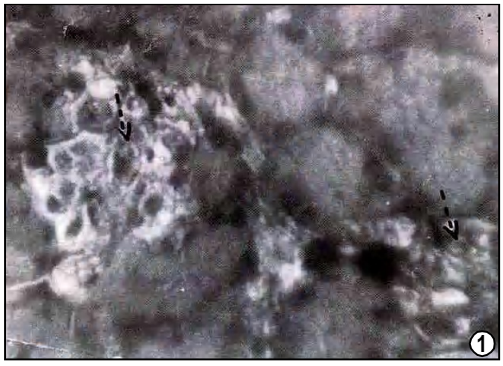

Fig. 1: Some small disfigured islets showing hydropic degeneration of beta cells (– – →) after 15 days extract feeding (25 mg/100 g dose of *M. charantia* alcoholic extract) (x600)

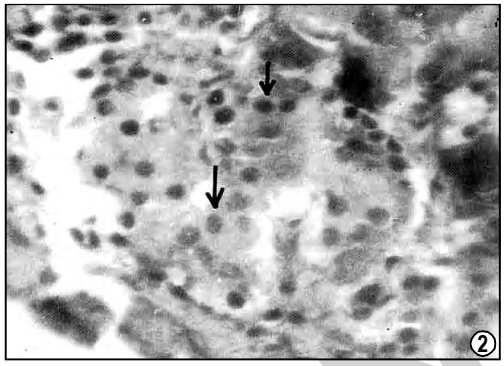

Fig. 2: A large islet showing normal granulation of most of the beta cells (→) after 30 days extract feeding. (25 mg/100 g dose) (x600)

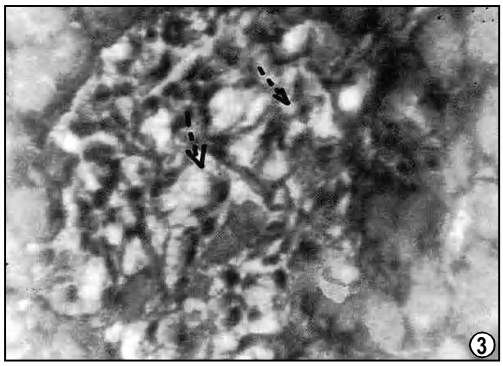

Fig. 3: A medium sized islet with extreme hydropic degeneration (– – →) and ballooning (→) of occasional beta cells after 15 days extract feeding (50 mg/100 g dose) (x600)

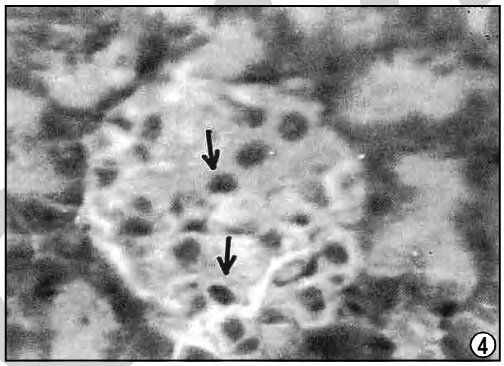

Fig. 4: A medium sized islet with normal beta cell granulation (→) after 30 days extract feeding. (50 mg/100 g dose) (x 600)

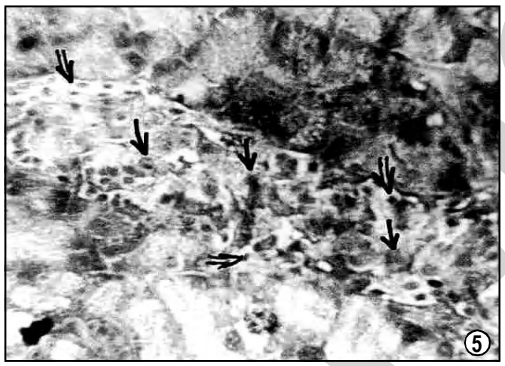

Fig. 5: An elongated islet with alpha cells (⇒) on the periphery and beta cells with normal granulation (→) in the centre after 30 days extract feeding (75 mg/100 g dose) (x400)

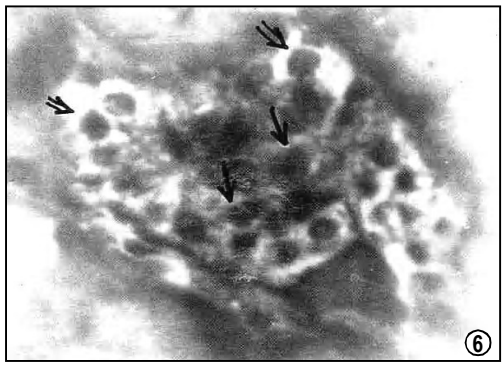

Fig. 6: A disfigured islet with alpha cells (⇒) on the periphery and beta cells in the centre (→) with normal granulation after 30 days extract feeding followed by 15 days normal diet. (75 mg/100 g dose) (x650)

Plate - IV

Lotlikar and Rajaram Rao (1966), observed 23, 32 and 42% fall in fasting blood sugar levels with 10, 20 and 50 mg after 5 hr, where as Akhtar *et al.* (1981) reported 2.51, 7.69, 24.22 and 32.85% fall with 0.25 g, 0.5 g, 1 g and 1.5 g/kg doses of whole fruit powder after 24 hr.

Lal and Chaudhary (1968), observed that continuous administration of fruit extract at a dose of 5 ml/kg for 15 days produced 10% reduction in the fasting blood sugar on 7th day and 23% on 15th day with aqueous extract of *M. charantia* in rabbits made diabetic by oral glucose load and anterior pituitary extract. Biyani *et al*. (2003) have also reported 48% fall in fasting blood sugar with aqueous extract of *M. charantia* and compared its hypoglycaemic activity with that of glibenclamide.

During the course of present study the recorded maximum fall in elevated levels of blood sugar was 25% in 8 days, 53% in 15 days and 71% in 30 days with different doses of alcoholic extract of *M. charantia.*

Histopathological studies: Intravenous injection of alloxan monohydrate results in selective damage of beta cells of islets resulting in elevation of blood sugar level upto 4 times the normal range. Histological examination of islets of Langerhans from the pancreas of diabetic control group showed varying degree of damages. Regular arrangement of alpha and beta cells was disturbed. Islets showed reduced granulation of beta cells, hydropic degeneration, clumping of beta cells, pyknosis and necrosis. (Fig. 1, 2, 3, 4 and Plate-III).

It is well established by Dunn *et al.* (1943), Black (1980), Bhaveja *et al.* (1982) and also clear from diabetic control group in the present study that alloxan produces permanent hyperglycaemia by selective destruction of the beta cells of the islets of Langerhans of pancreas.

After 15 days extract feeding islets showed no improvement. After 30 days feeding of the alcoholic extract of *M. charantia,* no hydropic degeneration was found in all the three dose groups (*i.e.* 25 mg, 50 mg and 75 mg). Large and some medium sized islets were present containing beta cells with cytoplasmic granules and areas of necrosis were very few. Necrosed areas in islets indicate damage caused by alloxan injection. Islets of Langerhans were generally large in size. Small islets were comparatively fewer. Some islets showed disfigurement also but alpha and beta cells were clearly distinguishable and normal in appearance and arrangement. When after 30 days extract feeding, it was discontinued for 15 days, beta cell granulation remained intact along with necrosed areas. (Plate-IV) There was not much difference in the islet picture in all the three dose groups.

The histopathology of pancreatic islets has clearly shown a definite improvement in the granulation of beta cells as well as in the size of most of the islets. This could be the reason that the blood sugar remained normal even when the extract was discontinued for a fortnight.

Although much work has been done on blood glucose lowering effect of *M. charantia* but no work is available on islet histopathology for comparison. Chakravarthy *et al.* (1980), have reported regeneration in islets with a plant, *Pterocarpus marsupium*. Welihinda *et al.* (1982) studied the insulin releasing activity of centrifuged juice of *M. charantia* on isolated pancreatic islets of obese hyperglycaemic mice and concluded that it stimulated beta cells to release the insulin. Chatterjee (1963) expressed the view that the mode of action of *M. charantia* is extra pancreatic because he noted fall in blood sugar level of those alloxan diabetic rabbits, which were kept in hyperglycaemic condition for one month prior to the start of experiment.

But during present study, improvement in beta cell granulation indicates that most probably alcoholic extract of *M. charantia* lowers blood sugar by affecting islets of Langerhans. Most probably the blood sugar lowering effect of alcoholic extract of *M. charantia* is due to the presence of some alkaloid similar to the alkaloid reported in *Pterocarpus marsupium* by Chakravarthy *et al.* (1980), which improved islet histology.

The alcoholic extract of *M. charantia* does not show any toxic manifestation in the liver of rats fed on different doses for as long a period as 30 days as also reported by Pabrai and Sehra (1962). It is therefore concluded that *M. charantia* alcoholic extract may safely be given to diabetics without any fear of toxicity.

In the end it may be concluded that alcoholic extract of *M. charantia* was quite effective in lowering blood sugar levels and islet histopathology also showed improvement. The lowered blood sugar and improvement in islet histology remained as such even after discontinuation of extract feeding for 15 days.

Acknowledgments

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