Regeneration of β cells in islets of Langerhans of pancreas of alloxan diabetic rats by acetone extract of *Momordica charantia* (Linn.) (bitter gourd) fruits

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Acetone extract of whole fruit powder of *M. charantia* (bitter gourd) in doses 25, 50 and 75 mg/100 g body weight lowered the blood glucose from 13.30 to 50% after 8 to 30 days treatment in alloxan diabetic albino rats, confirming antihyperglycemic effect of this plant in diabetic animals and humans. Histological observations with acetone extract showed different phases of recovery of β cells of the islets of Langerhans of pancreas, which in the untreated diabetic rats were less in number and showed varied degree of atrophy. The most important finding of the present study was observation of the presence of small scattered islets among the acinar tissue in some experimental animals, which may reflect neoformation of islets from pre-existing islet cells. The liver of alloxan diabetic rats showed hydropic degeneration, fatty change and necrosis at some places but liver of extract treated animals was normal. Glycogen localization in liver of diabetic rats was faint but after 30 days treatment with different doses of extract, normal to heavy glycogen localization was observed.

**Keywords**: Acetone extract, Alloxan diabetes, β cells, *Momordica charantia*, Regeneration

Medicinal plants in use in India since ancient times for the treatment of diabetes mellitus are also gaining importance all over the world. *Momordica charantia* (Hindi – Karela, Tamil – Pakal, Kannada – Hagal, Malayalam – Kaippa, Telugu – Kakarakaya) is one of the plants, which has been investigated thoroughly for the treatment of diabetes mellitus. Scientific studies on this plant were first carried out by Rivera1. Since then extensive evaluation has been done on its hypoglycaemic properties, as well as on isolating the active principles from it2-13.

Lotlikar and Rajarama Rao5 isolated charantin from *M. charantia*. Pugazhenthi and Murthy6 reported that *M. charantia* fruit powder and some solvent extracts have hypoglycaemic effect and release insulin from pancreas in intact animals. Khanna et. al.8 observed that *M. charantia* contains plant insulin. Welihinda10 reported that *M. charantia* activates the insulin release from β cells. In 1989, Pugazhenth and Murthy12 isolated three orally active hypoglycaemic principles—Kakara Ib, IIIa and IIIb1 from bitter gourd *M. charantia* and reported their blood sugar lowering potential. Sasmal and Lakshmi14 studied hypoglycaemic effects of *M. charantia* in combination with zinc and ascorbic acid.

These studies point out that *M. charantia* fruit powder and some solvent extracts have hypoglycaemic effects and release insulin from pancreas in intact animals. The present paper reports that treatment with acetone extract of *M. charantia* not only regenerates pancreas as reported by others but also leads to the formation of new islet cells of Langerhans and improves glycogen deposition in liver.

**Materials and Methods**

Unripe fruits of *M. charantia* were procured during the season (April to June), washed, cut into small pieces, dried in shade and powdered. They were identified by the Botany Department of Meerut College and a voucher specimen has been kept in the laboratory. Acetone extract of the powder was prepared with soxhlet apparatus. The extract was dried at 35°C in a hot air oven and stored at 4°C, in airtight containers; 100 g of crude powder yielded 3.2 g of acetone extract.

Albino rats procured from IVRI, Izzatnagar, Bareilly, were acclimatized to laboratory conditions and randomly divided into 5 groups of 18 animals each. All the animals were weighed before and after the experiments. The animals of groups II – V were
injected, iv, 6 mg/100g of alloxan monohydrate (Sigma Chemicals Company) in citrate buffer (pH 5.0). Fasting blood samples were collected from caudal vein and tested for the presence of sugar before starting the experiments and then at specific time intervals (after 8, 15, 30 and 45 days) at 0900 hrs during the period of experimentation. Group I served as normal control, group II served as untreated diabetic control group and animals of group III, IV and V were administered 25, 50 and 75 mg/100 g body weight extract once daily orally, along with normal food (carbohydrates 69%, proteins 16%, fats 7%, fibres 6%, mineral salt mixture 2% and vitamins). Six animals from each group were sacrificed after 15 and 30 days of extract feeding respectively and the remaining 6 rats were kept on normal diet for 15 more days and were then sacrificed.

Pancreas was fixed in Bouin’s fixative (without acetic acid) and liver was fixed in Hollende Bouin’s fixative. After routine processing the tissues were embedded in paraffin wax with cerasin (Emerk) of 58°–60°C. Sections (6 μm thick) of pancreas were stained with Chrome-Haematoxyline-Phloxine. The sections of liver were stained with Haematoxyline-Eosin (HE) stain. For glycogen localization liver was fixed in cold alcohol and sections were stained with Periodic Acid Schiff (PAS) method using diastase treated slides as control for confirmation of glycogen (Mc Manus in Pearse).18

### Table 1—Effect of different doses of *M. charantia* (Linn.) acetone extract on blood glucose level (mg/dl) in alloxan diabetic albino rats along with diabetic control group.

[Values are mean ±SD from 6 animals in each group]

<table>
<thead>
<tr>
<th>Before experiment</th>
<th>After 8 hrs. Alloxan injection</th>
<th>After 8 days extract feeding</th>
<th>After 15 days extract feeding</th>
<th>After 30 days extract feeding</th>
<th>After 30 days extract feeding + 15 days normal diet</th>
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<tr>
<td>Diabetic control group</td>
<td></td>
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<tr>
<td>105.96 ± 0.79</td>
<td>286.50 ± 49.71</td>
<td>285.27 ± 54.62</td>
<td>290.33 ± 54.07</td>
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<tr>
<td>96.85 ± 13.74</td>
<td>325.43 ± 52.73</td>
<td>326.14 ± 52.73</td>
<td>327.15 ± 55.09</td>
<td>329.69 ± 55.15</td>
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<tr>
<td>103.56 ± 14.49</td>
<td>226.66 ± 50.70</td>
<td>231.66 ± 52.73</td>
<td>231.66 ± 55.09</td>
<td>260.33 ± 55.15</td>
<td>262.50 ± 32.32</td>
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<tr>
<td>25 mg/100g body weight of extract</td>
<td></td>
<td></td>
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<tr>
<td>100.25 ± 0.84</td>
<td>378.75 ± 36.14</td>
<td>312.99 ± 45.04</td>
<td>241.06 ± 80.04</td>
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<tr>
<td>93.28 ± 0.89</td>
<td>370.33 ± 32.13</td>
<td>305.77 ± 28.17</td>
<td>228.03 ± 64.81</td>
<td>173.42 ± 64.81</td>
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<tr>
<td>91.36 ± 22.83</td>
<td>361.388 ± 28.17</td>
<td>326.28 ± 27.36</td>
<td>273.26 ± 190.26</td>
<td>200.82 ± 190.26</td>
<td>125.36 ± 72.66</td>
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<td>75 mg/100g body weight of extract</td>
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<tr>
<td>93.35 ± 0.82</td>
<td>325.25 ± 48.08</td>
<td>240.51 ± 52.16</td>
<td>211.36 ± 52.16</td>
<td>-</td>
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<td>111.55 ± 75.75</td>
<td>277.79 ± 46.47</td>
<td>213.79 ± 50.16</td>
<td>157.45 ± 50.16</td>
<td>130.36 ± 50.16</td>
<td>-</td>
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<tr>
<td>90.26 ± 68.41</td>
<td>263.31 ± 23.73</td>
<td>238.31 ± 26.41</td>
<td>179.67 ± 60.81</td>
<td>94.32 ± 60.81</td>
<td>93.80 ± 44.96</td>
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<tr>
<td>75 mg/100g body weight of extract</td>
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*P values: a<0.05; b0.01; c0.005; d<0.001*
Results

Results of the blood glucose level of different groups are given in Table 1.

Intravenous injection of alloxan monohydrate resulted in damage of islet cells. Islets of Langerhans from the pancreas of diabetic control group showed varying degree of damage. Regular arrangement of α and β-cells was disturbed. β-cells showed clumping, degranulation and hydropic degeneration. In some sections necrosis and fibrosis of the whole islets were also observed. On the whole, there was extreme reduction in the number of islets in alloxan diabetic animals when compared with the normal islets (Fig. 1a–d).

Islets from all the three extract treated groups showed substantial recovery. The lost granules of β cells reappeared, but the necrosed areas of islets remained unchanged. Out of all the experimental animals, pancreatic sections of some animals showed groups of small islets. The size of these islets was very small in the sections of rats treated for 15 days, but islets of larger size were observed in the rats treated for 30 days. The exocrine pancreas is divided into lobules, which are separated from each other by connective tissue septa. It was observed that in a certain lobule all islets were with β cell destruction and other lobules from the same pancreas contained these newly formed islets (Fig. 2).

The liver of the diabetic control group showed hydropic degeneration and mild fatty infiltration and small necrosed areas in some sections after 30 and 45 days of diabetes. In all the treated groups, liver of some rats showed hydropic degeneration after 15 days but after 30 days, liver was normal and remained so even after 15 days discontinuation of the drug (Fig. 3).

Fig. 1—a : normal islet showing α cells (AC) at the periphery and β cells (BC) with granular cytoplasm in the center (× 600) ; b : totally disfigured islet, showing clumping of β cells (CL) after 15 days of alloxan diabetes (× 475) ; c : hyperplasia of the islet with hydropic degeneration (HD) and ballooning (BL) of occasional β cells after 30 days of alloxan diabetes (× 600) ; d : complete fibrosis of the islet, showing fibroblasts (ST) after 45 days of alloxan diabetes (× 600)
Fig. 2—

a: group of small islets (IS) scattered among normal acinar tissue (AT) after 15 days of 25 mg dose of *M. charantia* acetone extract (× 600);
b: an islet showing normal arrangement of α (AC) and β (BC) cells with normal granulation after 30 days of 25 mg dose of *M. charantia* acetone extract (× 600);
c: a small islet showing hydropic degeneration of β cells (BC) after 15 days of 50 mg dose of *M. charantia* acetone extract (× 600);
d: many small and large islets (IS) scattered among normal acinar tissue after 30 days of 50 mg dose of *M. charantia* acetone extract followed by 15 days normal diet (× 150);
e: a section showing two pancreatic lobules with many small and large islets (IS) scattered among normal acinar tissue (AT) after 30 days of 75 mg dose of *M. charantia* acetone extract (× 150);
f: a large islet full of β cells (BC) without any sign of previous injury after 30 days of 75 mg dose of *M. charantia* acetone extract followed by 15 days normal diet (× 450)
Fig. 3—a: hexagonal hepatocytes showing arrangement in hepatic cords (HC), hepatocytes with granular cytoplasm (Cy), prominent nucleus (Nu) and Kupffer cells (Kc) in normal liver (× 600); b: hepatocytes showing cloudy swelling (CS) and pyknosis of nuclei (Pc) after 30 days of alloxan diabetes (× 600); c: hepatocytes showing normal structure and cytoplasm is full of dark basophilic granules (Cy), nucleus (Nu) with prominent nucleolus (nl) after 30 days of 25 mg/100g dose of the extract (× 600); d: hepatocytes showing hydropic degeneration (Hd) after 15 days of 50 mg/100g dose of extract (× 600); e: some hepatocytes show hydropic degeneration (Hd) and others are normal, some nuclei show beginning of pyknosis (Bp) after 15 days of 75 mg/100g. dose of the extract (×600); f: normal hepatocytes with dark basophilic granules (Cg) in cytoplasm and prominent nuclei (Nu) after 30 days of 75 mg/100g dose of extract and 15 days of normal diet (× 600)
Fig. 4—a: section of liver of normal rat showing intense glycogen localization in hepatocytes (× 900); b: adjacent section treated with diastase enzyme showing total lack of any glycogen deposition. Section of normal liver treated with diastase showing no glycogen (×150); c: after 30 days of alloxan diabetes there is very little glycogen localization in the cytoplasm of hepatocytes (× 900); d: Most of the hepatocytes showing almost normal glycogen localization after 30 days of 50 mg/100g. dose of the extract (× 900); e: some hepatocytes showing faint and others showing moderate glycogen localization after 30 days of 25 mg/100g. dose of the extract (× 150); f: all hepatocytes showing very heavy glycogen localization after 30 days of 75 mg/100g. dose of extract (× 600).
In hepatocytes of normal rats adequate amount of glycogen localization is found. In alloxan diabetic rats liver glycogen is depleted and after 45 days of diabetes almost no glycogen localization is observed. In group III, moderate glycogen was observed. After 30 days treatment, in group IV normal glycogen localization was observed in hepatocytes. In group V heavy glycogen localization was observed after 30 days treatment. In all three dose groups no depletion in glycogen localization was observed in the hepatocytes of rats in which blood sugar was normal, after 15 days of discontinuation of extract feeding (Fig. 4).

Discussion

The results of the present study confirm the earlier work of Kedar and Chakrabarti with bitter gourd treatment in streptozotocin diabetic rabbits. Pugazhenthi and Murthy isolated and purified three compounds form M. charantia and observed that one of the three compounds called Kakara III b improved glucose tolerance and increased serum insulin levels. Other compounds (I a and III a) improved glucose tolerance and reduced free fatty acid levels but had no effect on insulin levels in alloxan diabetic rabbits. Akhtar et. al. had reported insulin like mechanism of action of hypoglycaemic principles of M. charantia.

In alloxan diabetic rats there was selective destruction of β cells of islets of langerhans. The histopathological examination showed reduced number of the islets, degranulation of β cells, hydropic degeneration, clumping of β cells, pyknosis and necrosis. The necrosis was mostly found in the centre of larger islets because the central portion of larger islets is the first to be affected by alloxan. The interesting aspect of the present study is the observation of newly formed islets. However there is already some indication of such a change from the studies of Hellerstrom et. al. who also reported the appearance of numerous small islets, probably like the islets of the present study, from the pre-existing islet cells. The stimulation for multiplication of the pre-existing cells is controlled by several factors and dietary composition is one among the others. Since M. charantia contains several alkaloids, some alkaloids present in the acetone fraction of this fruit may be responsible for triggering the multiplication of pre-existing islet cells and their differentiation into new islets. Other alkaloids may be responsible for the recovery of partially damaged β cells and insulin release as reported earlier. Since M. charantia contains several alkaloids, it is worth while investigating some alkaloids present in the acetone fraction which trigger the multiplication of pre-existing islet cells and their differentiation into new islets. However charantin, Kakara I a, III a, III b and other constituents isolated previously, may be responsible for the recovery of partially damaged β cells and insulin release.

Chatterjee had reported that M. charantia can execute its hypoglycaemic action in animals, where insulin secretion was almost stopped. Later Sasmal and Lakshmi had also reported blood sugar lowering effect of M. charantia but they had not studied islet histology. Improvement of liver histology and glycogen localization in the liver and presence of functional β cells in the pancreas are favourable effects. The effect of acetone extract of M. charantia is reported to be permanent. The formation of new islets with functional β cells, which can secrete insulin, can reasonably explain the above mentioned findings.

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References


