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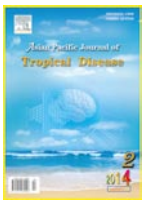

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# Histomorphological and morphometric studies of the pancreatic islet cells of diabetic rats treated with aqueous extracts of *Momordica charantia* (karela) fruits

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ABSTRACT

**Objective:** To investigate the effect of aqueous extract of *Momordica charantia* (karela) (*M. charantia*) fruits on blood glucose level, pancreatic weight changes and histopathology of pancreatic changes in the streptozotocin (STZ) induced diabetic rats.

**Methods:** Thirty-six albino rats were used in the experiment; diabetes mellitus was induced in 30 adult albino rats, using intraperitoneal injection of 55 mg/kg STZ. Six non diabetic rats remained as control (T1). The diabetic rats were randomly assigned into five equal groups: diabetic control (T2) without any treatment, groups T3, T4, T5 and T6 were treated with aqueous extract of karela fruits daily at a doses of 250, 500 and 750 mg/kg and glibenclamide (5 mg/kg) up to 90 d, respectively. At Day 90, all rats were sacrificed, the pancreases of the rats were excised and processed.

**Results:** The results of this study indicate that aqueous extract of *M. charantia* fruits was able to reduce blood glucose level significantly compared with the diabetic control group ($P<0.01$). Histopathologically, STZ resulted severe necrotic changes in pancreatic islets. Tissues sections of pancreas in the treated groups showed regeneration of β cells and increased size of pancreatic islets.

**Conclusions:** The present study suggests that oral feeding of *M. charantia* fruit juice has a significant anti-hyperglycemic effect and may have a role in the regeneration of the β cells in STZ diabetic rats.

## 1. Introduction

Diabetes mellitus is a complex disorder that characterized by hyperglycemia resulting from malfunction in insulin secretion and/or insulin action both causing by impaired metabolism of glucose, lipids and protein. Diabetes mellitus is considered as one of the five leading causes of death in the world[1]. 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[2]. The global estimated that the prevalence of diabetes from 171 million in 2000 to 366 million in 2030[3]. Nowadays, it has been recognized that diabetes mellitus is a major chronic public health problem throughout the world. In the absence of effective and affordable interventions for both types of diabetes, the frequency of the disease will escalate worldwide, with a major impact on the population of developing countries[4].

A recent study has estimated that up to 30% of patients with diabetes mellitus use complementary and alternative medicine[5]. The popularity of complementary medicine to treat diabetes is escalating.

*Momordica charantia* Linn. (*M. charantia*), commonly

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referred to as bitter melon, bitter gourd, balsam pear, or karela, belongs to the Cucurbitaceae family. It is a tropical climbing plant, cultivated throughout in Southern Asia, East Africa and South America. Its fruits are very cheap and available throughout the year in cooking and as a natural remedy for treating diabetes[6]. As many studies report, there has been substantial emphasis on the anti-diabetic compounds and their hypoglycemic properties[7,8]. A number of reported clinical studies have shown that *M. charantia* extract from the fruit, seeds and leaves contains several bioactive compounds that have hypoglycemic activity in both diabetic animals and human[9,10]. There are many traditional herbal remedies that have been used to treated diabetes in Asia and other developing countries[11-14].

*M. charantia* is one of the plants that have been investigated thoroughly for the treatment of diabetes[15]. The possible modes of the hypoglycemic actions of *M. charantia* and its various extracts and compounds are its hypoglycemic effect[16,17], stimulation of peripheral and skeletal muscle glucose utilization[18,19], inhibition of intestinal glucose uptake[20-23], suppression of key gluconeogenic enzymes[24,25] and preservation of islet β cells and their functions[26]. Over 140 different studies worldwide have investigated anti-hyperglycemic and hypoglycemic effects of the different extracts and ingredients of *M. charantia* in both human and animal models till recently[9,27,28].

Our research investigates whether or not aqueous extracts of *M. charantia* fruit could provide lasting hypoglycemic control through regeneration of the destroyed β-cells of the pancreatic islets of experimentally induced diabetic rats.

## 2. Materials and methods

### 2.1. Animals

Thirty-six apparently healthy mixed albino rats, long evens strain (*Ratus norvegicus*) weighting between 150-200 g used in this experiment were procured from the International Center for Diarrheal Disease Research, Bangladesh, Mohakhali, Dhaka, The rats were kept in the departmental animal house in grilled cages at room temperature 21-23 °C with 45%-50% relative humidity and constant 12 h light/dark cycle. Standard rat feed and water were provided *ad libitum*. Prior to commencement of the experiment, all the rats were acclimatized to the new environment for a period of 15 d. The experiment protocols were conducted in accordance with internationally accepted principles for laboratory animal.

### 2.2. Chemicals, drug and plant material

Streptozotocin (STZ) was obtained from Sigma Chemical Co., St Louis, U.S.A and tablets dibenol® from Square Pharmaceuticals, Bangladesh, each dibenol® tablet contains 5 mg glibenclamide. The fresh unripe fruits of *M. charantia* were procured from the Kamal Ranjit Market, Bangladesh Agricultural University campus, Mymensingh. Plant material was identified taxonomically by the botanist of the Botanical Garden, Bangladesh Agricultural University, Mymensingh.

### 2.3. Induction of diabetes by STZ in rats

Diabetes rats were made diabetic by single intraperitoneal injection of fresh solution of STZ (Sigma Chemical Co., St Louis, U.S.A) at the dose of 55 mg/kg body weight in a volume of 1 mL/kg body weight[29]. The control rats were injected the same amount of 0.1 mol/L sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to reduce the drug-induced hypoglycemic mortality. After a week of STZ administration, fasting blood glucose levels were determined by Accu-check (strip method) to confirm the development of diabetes. The blood glucose levels of the rats were between 240 mg/dL and 250 mg/dL in the experiment.

### 2.4. Preparation of the aqueous extract of M. charantia fruits

To obtain karela fruits aqueous extract, this unripe fresh karela fruits were carefully and thoroughly washed in tap water. The fruits were sliced into two halves and the seeds were removed manually, then the fleshy parts were cut into small pieces. Then 1 kg seedless flesh was put into an electric juicer to make juice and it is then filtered through a piece of clean silk cloth. The filtered juice was evaporated to slurry in vacuum rotatory evaporator and temperature was maintained around 45 °C, and the concentrated slurry mass was completely lyophilized by continuous freeze drying operation for 72 h and kept at -20 °C in refrigerator in a air tight container until used.

### 2.5. Experimental designs

Thirty-six diabetic rats were divided into six groups, each consists of six animals. In Group T1, six rats were given only normal feed and water. In Group T2, diabetic control rats received normal feed and water but no tested drug. The aqueous extract of *M. charantia* fruits was given orally

at the doses of 250, 500 and 750 mg/kg orally in a volume of 10 mL/kg to Group T3, T4 and T5, respectively, and Group T6 received dibenol® (glibenclamide) 5 mg/kg body weight suspended in water (10 mL/kg), up to Day 90.

### 2.6. Determination of fasting blood glucose level

Blood samples were obtained from the tail tip of each rat before STZ dosing, 7 d after STZ dosing and 15 d intervals up to Day 90 and their fasting blood glucose level was determined in mmol/L using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany). The rats were fasted for a period of 12 h before their blood glucose levels were measured.

### 2.7. Pancreatic weight changes

At Day 90, all rats were sacrificed by decapitation under pentobarbitone sodium (60 mg/kg body weight) as anesthesia. Fat, lymph nodes were removed and pancreas (including duodenal and splenic lobes) was collected. Pancreatic weight was calculated at gram levels and the relative weight (%) was calculated using body weight at sacrifice and absolute weight as following equation:

Relative pancreas weight (%)=[(absolute pancreas weight/ body weight at sacrifice) ×100]

### 2.8. Histological procedures

Samples from the splenic lobes of pancreas of each rat were fixed in Bouin's solution and processed via the paraffin wax embedding method[30]. After paraffin embedding, 5 μm thickness serial sections were prepared and stained with hematoxylin and eosin for microscopic examination.

### 2.9. Photo micrographic studies

The number of islets and islets cells of each islet were counted by using compound microscope with photographic digital camera (Olympus, OM-20 model) and the islets diameters were measured using calibrated micrometer by taking fixed number of islets from the five experimental groups and one normal group.

### 2.10. Statistical analyses

All recorded and calculated data were subjected to ANOVA in a completely randomize design using MSTAT computer package[31]. Multiple range test was performed to compare mean differences among treatments[32]. The effect of dose was evaluated using linear regression analysis.

## 3. Results

### 3.1. Effect of aqueous extract of *M. charantia* fruits on the blood glucose

The effect of the aqueous extract of *M. charantia* fruits on the blood glucose in the diabetic rats are shown in Table 1. The blood glucose concentration in the untreated diabetic rats was significantly higher at all intervals after intraperitoneal administration of STZ in comparison to those of the normal rats ($P<0.01$). While the blood glucose concentration of the untreated diabetic rats remained high at all intervals, administration of aqueous extract of *M. charantia* fruits in Group T3, T4, T5, and T6 tended to bring the blood glucose levels significantly ($P<0.01$) and dose-dependently decreased toward normal values from Day 15, while the normal rats did not show any significant alterations in their blood glucose concentration during the course of the study.

### 3.2. Pancreatic weight changes

Absolute weight of pancreas was increased in all dosing groups (T3, T4, and T5), in controls and in glibenclamide treated group. However, increases in relative pancreas

**Table 1**

Effects of aqueous extract of *M. charantia* fruits and glibenclamide on blood glucose (mmol/L) level in STZ treated diabetic rats.

| Group | Pre-treatment | Post-treatment | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 0 | Day 15 | Day 30 | Day 45 | Day 60 | Day 75 | Day 90 |
| T1 (Normal control) | 4.53±0.08 | 4.58±0.08$^{c}$ | 4.60±0.14$^{d}$ | 4.62±0.17$^{g}$ | 4.63±0.22$^{k}$ | 4.65±0.12$^{i}$ | 4.73±0.25$^{i}$ |
| T2 (Diabetic control) | 14.08±0.23 | 15.42±0.42$^{a}$ (+9.50) | 16.73±0.33$^{a}$ (+18.80) | 17.42±0.42$^{a}$ (+23.70) | 18.22±0.39$^{a}$ (+29.40) | 18.82±0.24$^{a}$(+33.60) | 20.05±0.56$^{a}$ (+42.40) |
| T3 (Diabetic+MCFEt 250 mg/kg) | 14.63±0.37 | 14.05±0.42$^{b}$ (−3.90) | 13.23±0.38$^{c}$ (−9.70) | 13.00±0.38$^{b-f}$ (−11.10) | 12.53±0.29$^{c-f}$ (−14.30) | 12.00±0.21$^{cd}$(−17.90) | 11.70±0.32$^{cd}$ (−20.00) |
| T4 (Diabetic+MCFEt 500 mg/kg) | 15.15±0.33 | 14.05±0.14$^{b}$ (−7.20) | 13.15±0.14$^{c}$ (−13.20) | 12.13±0.20$^{f}$ (−19.90) | 11.88±0.13$^{fgh}$ (−21.50) | 11.00±0.23$^{ef}$ (−27.30) | 10.15±0.16$^{f}$ (−33.00) |
| T5 (Diabetic+MCFEt 750 mg/kg) | 15.50±0.62 | 14.17±0.82$^{b}$ (−8.58) | 13.57±0.83$^{bc}$ (−12.45) | 12.28±0.79$^{ef}$ (−20.77) | 10.47±0.75$^{j}$ (−32.45) | 9.33±0.83$^{g}$ (−39.81) | 8.58±0.40$^{g}$ (−44.65) |
| T6 (Diabetic+clibenclimide 5 mg/kg) | 15.47±0.65 | 14.12±0.61$^{b}$ (−8.73) | 13.60±0.61$^{bc}$ (−12.08) | 12.52±0.63$^{def}$ (−19.07) | 10.68±0.60$^{ij}$ (−30.96) | 8.38±0.60$^{h}$ (−45.83) | 7.43±0.43$^{h}$ (−51.97) |
| Level of significance | NS | ** | ** | ** | ** | ** | ** |

NS: Not significant; **: $P<0.01$, values in each column bearing dissimilar letter(s) differed significantly according to Duncun's Multiple Range Test (DMRT); Data are expressed as mean±SD; MCFEt: *M. charantia* fruits aqueous extract.

weight were detected in all groups in the present study (Table 2).

**Table 2**

Mean pancreatic weight (g) in normal and diabetic rats after treatment with aqueous extract of *M. charantia* fruits and glibenclamide.

| Treatment (mg/kg body weight) | Body weight | Pancreas weight | |
|---|---|---|---|
| | | Absolute weights | Relative weight |
| T1 (Normal control) | 241.51±12.71 | 1.20±0.06$^{bcde}$ | 0.49±0.02 |
| T2 (Diabetic control) | 149.15±12.90 | 0.70±0.08$^{g}$ | 0.47±0.02 |
| T3 (Diabetic+MCFEt 250 mg/kg) | 195.22±12.63 | 0.94±0.24$^{abcd}$ | 0.48±0.12 |
| T4 (Diabetic+MCFEt 500 mg/kg) | 216.38±12.20 | 0.99±0.03$^{abc}$ | 0.46±0.02 |
| T5 (Diabetic+MCFEt 750 mg/kg) | 224.53±8.70 | 1.03±0.04$^{ab}$ | 0.46±0.02 |
| T6 (Diabetic+glibenclamide 5 mg/kg) | 231.35±14.80 | 1.08±0.10$^{cdef}$ | 0.47±0.02 |
| Level of significance | NS | ** | NS |

NS: Not significant; **: $P<0.01$, values in each column bearing dissimilar letter(s) differed significantly according to DMRT; Data are expressed as mean±SD; MCFEt: *M. charantia* fruits aqueous extract.

### 3.3. Morphometric analysis

The results of the morphometric analysis revealed a significant reduction in the numerical density of islets (number of islet/pancreas), islet area, islet diameter, numerical density of β-cells (number of β-cells per islet) in the untreated diabetic group in comparison with the control group. However, these morphometric parameters were significantly increased in all treated groups of rats (T3, T4, T5, and T6) when compared with those of the untreated diabetic control (Table 3).

**Table 3**

Effects of aqueous extract of *M. charantia* fruits on the number of islets, number of islets cells and islets diameters in pancreatic tissues in experimental rats.

| Group | Islets | Islets cell | Islets diameters (μm) |
|---|---|---|---|
| T1 (Normal control) | 36.67±3.06$^{a}$ | 109.31±4.75$^{a}$ | 114.55±1.83$^{a}$ |
| T2 (Diabetic control) | 16.33±2.31$^{g}$ | 73.31±0.77$^{fg}$ | 99.31±1.48$^{i}$ |
| T3 (Diabetic+MCFEt 250 mg/kg) | 26.67±2.08$^{bcd}$ | 79.66±1.32$^{e}$ | 104.74±2.46$^{d-g}$ |
| T4 (Diabetic+MCFEt 500 mg/kg) | 27.67±2.52$^{bc}$ | 85.48±1.52$^{cd}$ | 105.38±4.43$^{de}$ |
| T5 (Diabetic+MCFEt 750 mg/kg) | 29.67±0.58$^{abc}$ | 89.06±2.81$^{c}$ | 105.95±3.35$^{de}$ |
| T6 (Diabetic+glibenclimide 5 mg/kg) | 18.68±1.53$^{efg}$ | 81.64±3.38$^{de}$ | 78.50±0.65$^{j}$ |
| Level of significance | ** | ** | ** |

NS: Not significant; **: $P<0.01$, values in each column bearing dissimilar letter(s) differed significantly according to DMRT; Data are expressed as mean±SD; MCFEt: *M. charantia* fruits aqueous extract.

### 3.4. Histopathological changes of pancreas

The histological appearance of the pancreatic islet cells of the normal control (T1) had normal architecture (Figure 1A). Microscopic examination of the pancreatic islet cells of the untreated diabetic control group revealed a breakdown of micro-anatomical features decrease of pancreatic islet numbers, islets cells, islets diameters and their size, atrophy and vacuolation including degenerative and necrotic changes in the pancreatic islet of Langerhans, β-cell degranulation, pycnotic β-cell nuclei and decreased islet cellular density, though the pancreatic acinar epithelium, ductal and connective tissues appeared normal (Figure 1B). But these abnormal histological signs were dramatically and dose-dependently improved in all treated group (T3, T4, and T5), compared to that of control. There was an increased size of pancreatic islets, islet cellular density with an increase in granulation and regeneration of the β-cells but vacuolation was reduced or absent in many islets (Figures 1C, 1D and 1E).

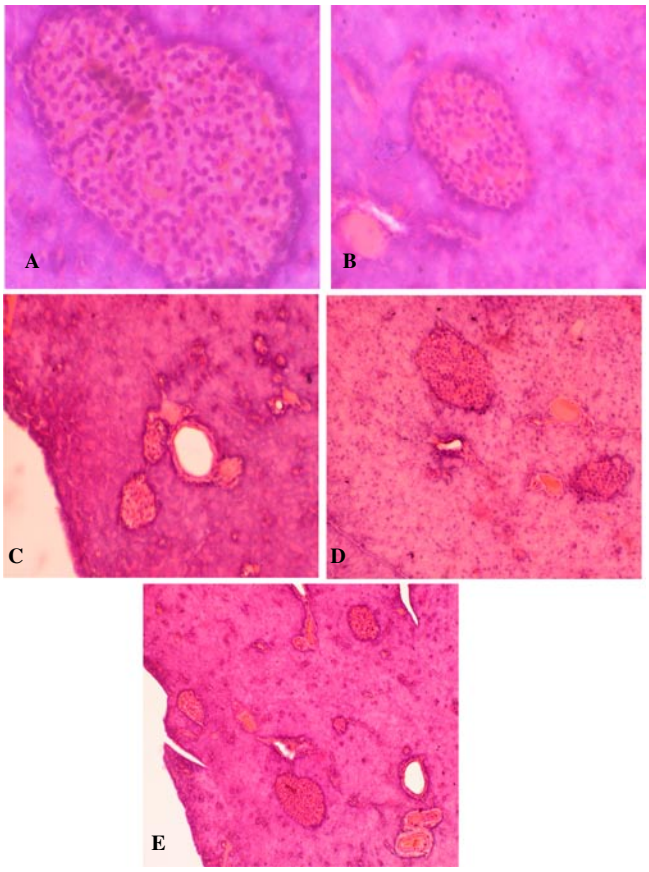


**Figure 1.** Histological sections of pancreas from the normal, untreated diabetic rats and diabetic rats treated with *M. charantia* fruits extract.
A: The pancreas section from a normal rats (T1); B: Pancreas section from an untreated diabetic rats (T2); C: Pancreas section from an treated diabetic rats at 250 mg/kg body weight (T3); D: Pancreas section from an treated diabetic rats at 500 mg/kg body weight (T4); E: Pancreas section from an treated diabetic rats at 750 mg/kg body weight (T5).

## 4. Discussion

STZ is the drug that selectively destroy β cells, insulin producing pancreatic endocrine cells, and thus induce experimental diabetes mellitus[33,34]. In our study we have found that the continuous treatment with aqueous extract of *M. charantia* fruits for a period of 90 d produced a significant decrease in blood glucose levels of the STZ-diabetic rats which inhibits the decrease of insulin-producing cells in

the pancreatic islets.

The dose-dependent hypoglycemic effect of *M. charantia* fruits detected in the present study considered as the evidence that *M. charantia* fruits has relatively favorable anti-diabetic effects. More favorable anti-diabetic effects were replicated in aqueous extract of fruits 750 mg/kg on the hypoglycemic compared to that of untreated diabetic control in this study.

The present findings are supported by those reported by Ragasa *et al.* and Sărăndan *et al.* who confirmed that various extracts and compounds of *M. charantia* have antidiabetic properties[16,17]. Consistent with the findings of the present study, Akhtar *et al.* showed that the *M. charantia* extract had a hypoglycemic effect in rabbits and they attributed this effect to the saponin and glycosidic components present in the plant[19]. A number of reported clinical studies have shown that *M. charantia* extract from the fruits, seeds and leaves contains several bioactive compounds that have hypoglycemic activity in both diabetic animals and human[9,27].

Parkash *et al.* isolated a non-nitrogenous neutral substance from the fruit of *M. charantia* named charantin, a peptide resembling insulin[35]. Ng *et al.* found that insulin-like bioactivity molecules were presented in *M. charantia* seeds[36]. Khanna *et al.* also have isolated polypeptide-P, from the fruits, seeds and tissues of *M. charantia*. These studies also reported that the substances have a potent hypoglycemic effect[37].

Sathishsekar and Subramanian[38] and Sekar *et al.*[39] observed that two varieties of *M. charantia* (MCSEt1 and MCSEt2) seeds significantly reduced blood glucose when given orally in STZ induced diabetic rats for 30 d. Similar to the findings of the present study, Grover *et al.* found that aqueous extract of *M. charantia* (200 mg/kg body weight), *Eugenia jambolana* (200 mg/kg body weight), *Mucuna pruriens* (200 mg/kg body weight) and *Tinospora cordifolia* (400 mg/kg body weight) reduced the plasma glucose concentration of 24.4%, 20.84%, 7.45% and 9.07%, respectively, when the extract was given to STZ induced diabetic mice for a period of 50 d[40]. Similar to the findings of the present study, Singh and Gupta stated that oral administration of acetone extract of whole fruits powder of *M. charantia* in doses at 0.25, 0.50 and 0.75 mg/kg body weight lowered the blood glucose from 13.30% to 50.00% after 8 to 30 days' treatment in alloxan induced diabetic albino rats[41]. The hypoglycemic effect of *M. charantia* fruit may be due to two reasons: firstly, its effect over the gluconeogenic enzymes, and secondly, it may exert its action on the transporters of glucose. The mechanisms involved in the hypoglycemic effects of *M. charantia* have not been fully established. However, it has been suggested that the depression of key gluconeogenic enzymes such as glucose-6-phosphatase and fructose bi-phosphatase may be partly involved in the hypoglycemic effects of *M. charantia* fruit juice[24]. Increase in the levels of intestinal $Na^+$/glucose co-transporters, *M. charantia* may cause hypoglycemia via an increase in glucose oxidation through the activation of glucose metabolism and/or the inhibition of glucose absorption in the gut. It has also been reported by Higashino *et al.*[42] and Ahmed[43] that aqueous extract of *M. charantia* decreased the absorptive capacity of fluid across the small intestine and sodium ions and it could also involve a wash out of glucose from the blood stream. Mahomoodally *et al.* and Ahmed *et al.* reported that *M. charantia* could regulate glucose uptake into jejunum membrane brush border vesicles and stimulate glucose uptake into skeletal muscle cells, which was similar to the action of insulin[44,45]. Although, absolute pancreas weights are decreased in diabetic rats compared to non-diabetic, no meaningful changes were detected in the relative weights. Therefore, the decrease of absolute weight detected was due to the effect of the decrease body weights, not due to diabetes.

Decrease in the blood concentration of glucose towards normal value in the treated animals could be related either to the partial regeneration or the preservation of the pancreatic β cell mass by *M. charantia* in rats. The histopathologic sections of the pancreas of the treated animals in the present study showed an increase in the size of the islets, with hyperchromic nucleus and regeneration of the β cells. These findings reveal that the anti-hyperglycemic effect of *M. charantia* is not only through the insulin-like substances such as saponin present in this plant or activation of the insulin receptors, but this plant is able to increase the number of the β cells in the islets of Langerhans which results in a higher production of insulin. The observations from this study are also supported by the report of Ahmed *et al.* who showed that oral administration of this *M. charantia* fruit extract, in the STZ induced diabetes in rats may have a role in the renewal of β-cells or partial regeneration or preservation of the pancreatic β cell mass[46].

Klomann *et al.* observed that significantly decreased blood glucose and increased the body weight in diabetic mice when given daily orally of a whole fruit powder of *M. charantia* at a dosage of 150 mg/kg body weight for 5 weeks[47]. Tripathi and Chandra[48] and Choudhary *et al.*[49] stated that aqueous extracts of *M. charantia* pulp and seed powder of *Trigonella foenum-graecum* significantly reduced blood glucose when given orally in alloxan induced diabetic rats for 30 d.

In conclusion, the present study indicated a significant

antihyperglycaemic/hypoglycemic effect of *M. charantia* fruits. The pancreases of the treated rats showed an improvement in their histological architecture. These results support the traditional usage of the *M. charantia* fruit extract in the treatment of diabetes mellitus.

## Conflict of interest statement

We declare that we have no conflict of interest.

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