Original Full Paper

Evaluation of Hypoglycemic Effect of Momordica charantia Extract in Distilled Water in Streptozotocin-Diabetic Rats

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Abstract

Momordica charantia or Bitter Melon, a tropical vegetable, is a common food in Indian cuisine and has been used extensively in folk medicine as a remedy for diabetes. The present study was undertaken to evaluate the hypoglycemic effect of *Momordica charantia* extract in streptozotocin-induced diabetic rat model for a period of 45 days. The alcoholic extract of *Momordica charantia* was administered orally at the dose rate of 100 and 200 mg/kg body weight in distilled water and compared with standard oral hypoglycemic drug, glibenclamide. In the study a significant ($P \leq 0.001$) improvement in the physiological and biochemical parameters such as body weight, hemoglobin concentration, serum glucose, cholesterol and triglyceride levels was observed in *Momordica charantia* treated rats as compared to diabetic control rats. In *Momordica charantia* treated rats there was gradual and progressive alleviation of streptozotocin effects with *M. charantia* at higher dose rate (200 mg per kg body weight), more effective in normalizing the pancreatic endocrinal architecture, improving the number of β-cells and in enhancing the insulin secretion. Immunohistochemistry and special staining revealed improvement in the insulin secretion in *Momordica charantia* and glibenclamide treated groups.
Key Words: hypoglycaemia, bitter melon, streptozotocin, glibenclamide, immunohistochemistry.

Introduction

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism. These metabolic abnormalities result in part from a deficiency of the blood sugar-lowering hormone insulin or from insulin resistance, a defect in the body's capacity to respond to insulin (7).

The prevalence of diabetes is rising all over the world due to population growth, aging, urbanisation and an increase of obesity and physical inactivity. According to recent estimates, approximately 285 million people worldwide (6.6%) in the 20–79 year-age group are reported to have diabetes in 2010 and, by 2030, 438 million people (7.8%) of the adult population, are expected to have diabetes. The largest increases will take place in the regions dominated by developing economies. Roughly 80% of people with diabetes are in developing countries, of which India and China share the larger contribution (26).

Insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks like insulin resistance (24), anorexia, brain atrophy and fatty liver in chronic treatment (33).

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. *Momordica charantia* (MC), also referred to as bitter gourd or karela, is a member of the Cucurbitaceae family and is commonly used as a traditional remedy for diabetes in India, Asia, Africa and South America. It is commonly consumed as a vegetable in India. The fruit, leaves, seeds and roots of MC have been used in the Indian system of medicine for a number of diseases, besides diabetes (8, 25, 30). The

aqueous extract from MC was demonstrated to be a potent stimulator of insulin release from β-cell rich pancreatic islets isolated from obese-hyperglycaemic mice (34). It has also been reported that the oral administration of different MC extracts show varying pattern of anti-hyperglycaemic effect without altering the insulin response suggesting a mechanism of action which is independent of intestinal glucose absorption and probably involves an extra pancreatic effect (9). Charantin, a peptide resembling insulin isolated from *M. charantia* lowered fasting blood sugar in rabbits gradually beginning from 1st and lasting till the 4th hour and slowly recovering to the initial level (20).

In spite of numerous data available on *Momordica charantia,* its hypoglycemic potential remains largely untapped owing to the inadequate documentation and validation of its activity against known oral hypoglycemic drugs. Hence, the present work was taken up to evaluate the hypoglycemic effect of *Momordica charantia* extract in distilled water in diabetic rats.

Materials and Methods

Experimental animals

Healthy female albino Wistar rats weighing 190±20 g were used for the present investigation. The animals were maintained under standard laboratory conditions, providing standard laboratory animal feed and clean drinking water *ad libitum*. The animals were acclimatized to the experimental conditions for two weeks after procurement. The study was carried out with a prior approval (LPM/IAEC/124/2010, dated 13/09/2010) by the Institutional Animal Ethical Committee (Reg no. 493/01/a/ CPCSEA, dated 31/10/2001), Veterinary College Hebbal, Bangalore.

Preparation of streptozotocin (STZ) solution

The STZ of required quantity (45 mg/kg body weight) was dissolved in fresh 0.1M with pH 4.5 citrate buffer and injected intraperitoneally to rats immediately to avoid degradation.

Source of plant extracts

Alcoholic fruit extract of *Momordica charantia* (75% purity) used in the present study was obtained from Himalaya Herbals Bangalore, India.

Glibenclamide solution

Glibenclamide (Daonil®, 5 mg), an oral hypoglycemic drug was dissolved in distilled water (82.33 ml) to give a concentration of 60 µg/ml and administered orally at a dose of 600 µg/ kg daily for a period of 45 days.

Administration of plant extract and glibenclamide

For a period of 45 days of experimentation the plant extract and glibenclamide were administered orally to the respective groups during morning hours of the day.

Experimental design

Fifty female albino rats of Wistar strain were weighed and randomly distributed into five groups of ten rats each. Care was taken to maintain the intra-group weight variation to be less than 25 g and inter-group weight variation by 35 g.

The groups and treatments used were as follows,

Group I	Normal control: Used for studying the base line values of the parameters
Group II	Diabetic control: Streptozotocin induced diabetic rats
Group III	Diabetic rats supplemented with glibenclamide
Group IV	Diabetic rats supplemented with extracts of *Momordica charantia* at the dose rate of 100 mg/kg body weight in distilled water
Group V	Diabetic rats supplemented with extracts of *Momordica charantia* at the dose rate of 200 mg/kg body weight in distilled water

Experimental induction of diabetes

The animals were fasted overnight and diabetes was induced in Groups II to V by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (45 mg/kg body weight) in 0.1 M cold citrate buffer having a pH of 4.5 (6). The control animals received citrate buffer alone.

Confirmation of diabetes

The diabetic state was confirmed by estimating the blood glucose levels after 72 h of STZ injection. The animals that showed the blood glucose level above 200 mg/dl were considered as diabetic. After confirmation of diabetic state, the treatment was commenced.

Collection of serum samples

To evaluate the biochemical parameters, serum was collected for which blood was drawn from the retro-orbital plexus of the rats under ketamine anaesthesia at different time intervals on Day 3, 15, 30 and 45 post ST injection of the study.

Sacrifice of animals

To study the progressive effects of the treatments given to different groups, two rats from each group were sacrificed using ketamine overdose on Day 15 and 30 and the remaining rats on Day 45 of the experiment.

Biochemical analysis

The serum samples collected at various intervals were subjected for biochemical estimation of serum levels of glucose, cholesterol and triglycerides using Semi Automatic Biochemical Analyzer.

Immunohistochemical detection of insulin in the pancreatic islets

Sections of pancreas were subjected to immunohistochemistry to demonstrate insulin in the β-cells of islets of Langerhans using polyclonal antibody raised against insulin antigen. Ready to use FlexPolyclonal Guinea Pig Anti-Insulin (Code No. IS002) was used as primary antibody and was procured from

DakoCytomation, Denmark.For secondary antibody, polyclonal Rabbit Anti-Guinea Pig Immunoglobulins conjugated with HRP (Horse Raddish Peroxidase) known to detect guinea pig immunoglobulins bound to antigen in tissue sections was procured from DakoCytomation, Denmark and was used at a dilution of 1:75.

To determine the percentage positivity for insulin production, the number of insulin positive cells immunohistochemically in 1000 β-cells (approximately 10-12 islets) were counted under high magnification and was expressed in percentage.

Special staining for Islets of Langerhan

Pancreatic sections were stained by Gomori's chrome alum hematoxylin and phloxine stain for demonstration of alpha and β-cells as already described (13).

Statistical analysis

Statistical analysis was performed using the statistical software Graph Pad Prism, version 5 for Windows. Mean values and standard error were calculated and all values were expressed as Mean (±SE). The data were analysed by two way analysis of variance (ANOVA).

Results and Discussion

Treatment of rats with streptozotocin is an established model for Type I or insulin-dependent diabetes. All the rats of Group-II, III, VI and V became diabetic and showed hyperglycaemia with an increase in mean serum glucose levels ranging from 397.83±7.95 mg/dL to 403.67±8.99 mg/dL by 72 hours after STZ administration.

Streptozotocin (STZ) is a naturally occurring compound produced by the bacterium *Streptomyces achromogenes* that exhibits broad spectrum antibacterial properties (32). It is assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnitrosourea moiety, especially at the O_6 position of guanine and the transfer of the methyl group from streptozotocin to the DNA molecule that causes damage, which along a defined chain of events, results in the fragmentation of the DNA. The DNA damage induces activation of poly ADP-ribosylation which leads to depletion of cellular NAD+ and ATP. The depletion of the cellular energy stores ultimately results in β-cell necrosis (17).

Group-I (Normal control)

In the present investigation, all animals in the normal control group remained healthy at different intervals of the study. All the values of various parameters analysed were within the normal range and indicated their healthy status (Table 1-6; Figure 1, 2, 6, 9).

Group-II (Diabetic control)

In the diabetic control animals (Group-II), the several parameters analysed indicated hyperglycaemic and hypoinsulinemic effects. The statistical analysis indicated a significant reduction (P≤ 0.001) in body weight, haemoglobin (Hb) concentration and significant increase (P≤ 0.001) in serum glucose values, cholesterol and triglyceride levels (Table 1-5).

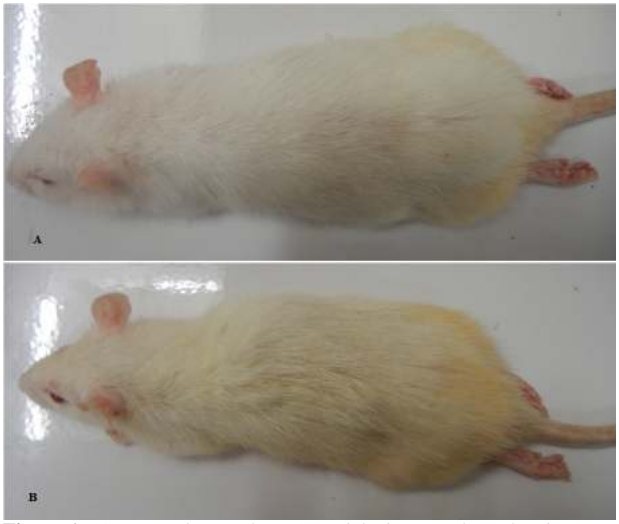

Figure 1. (A) Normal control rat, (B) Diabetic control rat showing poor body condition, emaciation, dehydration and ruffled hair coat on 45th day of the study.

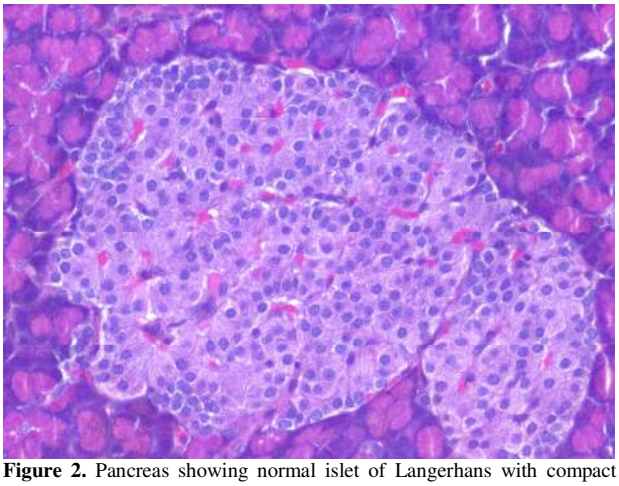

Figure 2. Pancreas showing normal islet of Langerhans with compact arrangement of β-cells with abundant eosinophilic granular cytoplasm occupying core of the islet and α-cells with scanty cytoplasm at the periphery (H&E 100X).

The increased catabolism due to hypoinsulinemia and the fluid loss through glycosuric polyuria and altered uptake of glucose and glycogenesis could account for the decreased body weight (10, 25). Glycosylation of several plasma proteins including Hb is a feature of diabetes which contributes for significant reduction (P≤ 0.001) in Hb concentration in diabetes (21, 23). Hyperglycaemia, which characterises diabetes, is directly related to insulin deficiency caused by relative destruction of pancreatic β-cells by STZ which causes loss of glucose homeostasis (14). In addition various metabolic derangements that

Table 1. The mean (±SE) animal body weight (g) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	Days post-treatment			
	3	15	30	45
Group I(Normal control)	165.67±2.76	176.67±3.37	183.33±3.62	192.83±2.82
Group II(Diabetic control)	170.00±1.52	157.00±1.26 [a]	138.50±1.94 [a]	121.50±2.09 [a]
Group III(Glibenclamide treated rats)	165.50±1.60	166.00±1.91	173.00±3.02 [b]	182.50±3.54 [b]
Group-IV(MC 100mg /kg BW)	168.00±1.98	155.16±3.15 [a]	162.33±3.15[ab]	169.67±1.89[abc]
Group-V(MC 200mg/kg BW)	175.83±5.97[abc]	169.50±5.39 [b]	176.50±5.60 [b]	187.83±5.23 [b]

All values are mean (± SE), Mean values with superscript differ significantly, [a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001.

Table 2: The mean (±SE) serum glucose (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	Days post-treatment			
	3	15	30	45
Group I(Normal control)	110.83±4.15	109.33±3.52	105.33±3.45	108.00±5.58
Group II(Diabetic control)	403.66±8.99 [a]	437.66±9.30 [a]	462.33±7.86 [a]	485.16±6.89 [a]
Group III(Glibenclamide treated rats)	400.50±6.23 [a]	331.83±4.20[ab]	248.00±3.17[ab]	193.50±2.74[ab]
Group-IV(MC 100mg /kg BW)	399.50±7.82 [a]	329.66±1.70[ab]	269.00±6.02[abc]	222.00±2.30[abc]
Group-V(MC 200mg/kg BW)	397.83±7.95 [a]	326.00±2.64[ab]	259.50±5.01[ab]	206.83±2.95[ab]

All values are mean (± SE), Mean values with superscript differ significantly, [a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001.

Table 3: The mean (±SE) hemoglobin (g/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	Days post-treatment		
	15	30	45
Group I(Normal control)	13.80±0.20	13.75±0.18	14.08±0.21
Group II(Diabetic control)	11.73±0.16 a	9.78±0.130 a	8.03±0.20 a
Group III(Glibenclamide treated rats)	11.81±0.20 a	12.26±0.15ab	13.10±0.17ab
Group-IV(MC 100mg /kg BW)	11.63±0.19 a	12.33±0.19ab	13.30±0.17ab
Group-V(MC 200mg/kg BW)	11.88±0.23 a	12.48±0.04ab	13.58±0.15 b

All values are mean (± SE), Mean values with superscript differ significantly,[a](Comparison with Group I), [b](Comparison with Group II),[c](Comparison with Group III), Values are statistically significant at P≤ 0.001.

Table 4: The mean (±SE) serum cholesterol (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	Days post-treatment			
	3	15	30	45
Group I(Normal control)	80.68±0.61	75.84±0.88	77.82±0.61	78.16±0.58
Group II(Diabetic control)	146.17±1.05 [a]	166.20±0.79 [a]	177.21±0.88 [a]	186.46±0.74[a]
Group III(Glibenclamide treated rats)	147.25±0.80 [a]	128.87±1.71[ab]	116.63±1.34[ab]	106.80±1.13[ab]
Group-IV(MC 100mg /kg BW)	147.02±0.79 [a]	130.52±1.51[ab]	120.81±1.31[ab]	107.31±0.96[ab]
Group-V(MC 200mg/kg BW)	146.72±0.95 [a]	124.14±1.14[abc]	113.45±0.88[ab]	103.73±0.92[ab]

All values are mean (± SE), Mean values with superscript differ significantly,[a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001.

Table 5: The mean (±SE) serum triglyceride (mg/dL) values of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	Days post-treatment			
	3	15	30	45
Group I(Normal control)	94.41±1.19	95.33±0.79	94.71±0.96	93.82±0.93
Group II(Diabetic control)	186.49±1.02 [a]	197.70±0.89 [a]	216.72±1.38 [a]	238.23±0.81 [a]
Group III(Glibenclamide treated rats)	185.98±0.69 [a]	165.92±0.74[ab]	157.05±0.86[ab]	146.46±0.88[ab]
Group-IV(MC 100mg /kg BW)	186.02±0.71 [a]	166.16±0.63[ab]	156.11±0.76[ab]	145.09±1.15[ab]
Group-V(MC 200mg/kg BW)	186.09±0.88 [a]	164.52±1.03[ab]	149.67±0.60[abc]	135.60±0.76[abc]

All values are mean (± SE), Mean values with superscript differ significantly,[a](Comparison with Group I), [b](Comparison with Group II), [c](Comparison with Group III), Values are statistically significant at P≤ 0.001.

occur in response to hypoinsulinemia in diabetes are contributable for hypercholesterolemia and hypertriglyceridemia(11).

The pathological examination of diabetic rats in the present study showed progressive atrophic changes in the pancreas grossly and microscopically necrosis of β-cells specifically due to toxic effects of STZ (5, 21) (Figures 1, 3, 7, 10).

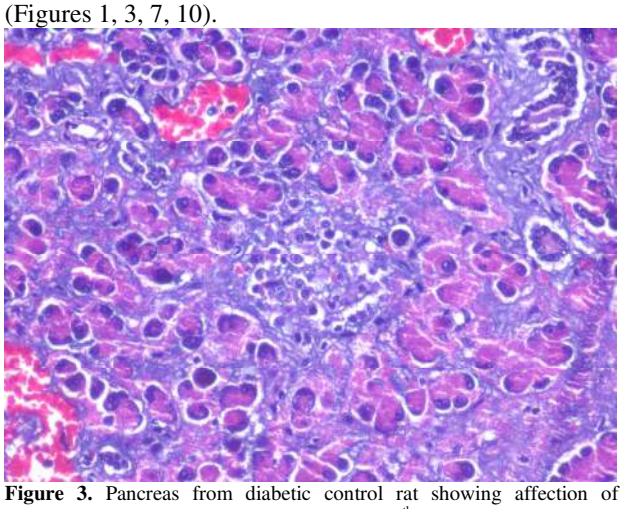

Figure 3. Pancreas from diabetic control rat showing affection of exocrine as well as endocrine pancreas on 45th day of the study. Note hypocellularity with vacuolated and necrotic cells in the islet (H&E 100X).

Group III (Diabetic rats treated with glibenclamide)

Glibenclamide is a second generation sulfonylurea used in the treatment of noninsulin dependent diabetes. Its hypoglycemic effect is mainly due to stimulation of insulin release from pancreatic β-cells and sensitization of the peripheral tissues to insulin (31).

In the present study, there was significant progressive improvement (P≤ 0.001) in several parameters analysed in comparison with those of diabetic control rats such as body weight, mean Hb concentration, mean serum glucose, and cholesterol and triglyceride levels (Table 1-5). In addition, the severity of clinical manifestation and gross changes were reduced. Microscopically, an improvement in the architecture of pancreatic islets with regeneration of β-cells was observed (1, 21) (Figure 4). The improvement could be attributed to the effect of glibenclamide in enhancing insulin secretion by β-cells of the pancreas and also increasing sensitization of the peripheral tissues to insulin (15).

Glibenclamide has been shown to bind to the surface receptors of β-cell membrane inhibiting ATP-sensitive K^+ channels and cause depolarization of the cell membrane. Depolarization leads to opening of K^+ channels which enables extracellular Ca^{2+} to enter the cell. Increased intracellular Ca^{2+} concentration enhances the binding of Ca^{2+} to the transport protein calmodulin which leads to microfilament contraction and release of insulin containing granules. Increased insulin causes subsequent reduction in serum glucose levels which improves β-cells sensitivity to glucose and potentiates insulin secretion (18).

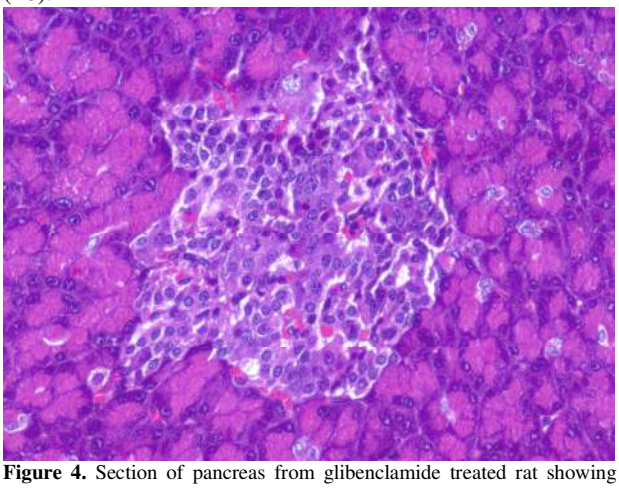

Figure 4. Section of pancreas from glibenclamide treated rat showing improvement in the architecture of the islet with hypercellularity mainly comprising α-cells on 45th day of the study (H&E 100X).

Group-IV (Diabetic rats treated with *M. charantia* 100 mg/kg body weight) and Group-V (Diabetic rats treated with *M. charantia* 200 mg/kg body weight)

To determine the dose dependent antidiabetic effect of *M. charantia* extract, the STZ diabetic rats were treated with *M. charantia* at 100 mg/kg body weight (Group IV) and 200 mg/kg body weight (Group V) for a period of 45 days.

In the present study, significant improvement (P≤ 0.001) was observed in the body weight, Hb concentration, serum glucose, cholesterol and triglyceride levels of *M. charantia*-treated rats in comparison with diabetic control rats, observations made in previous studies (11, 21) (Table 1-5). The improvement could be attributed to the hypoglycemic effect of *M. charantia* preparation by promoting insulin release, enhancing uptake of glucose and potentiating the effect of insulin leading to better utilization of nutrients, glucose, amino acids, fatty acids and other macromolecular components (29). In addition, the hepatoprotective property of the plant and induction of various metabolic enzymes also could be responsible which improved uptake of nutrients and their metabolism (16). There was a dose dependent effect with 200 mg/kg body weight of *M. charantia* better than 100 mg/kg body weight of *M. charantia* in improving various parameters (Table 1-6).

The hypoglycemic effect of *M. charantia* fruit extract could be because of its property of enhancing insulin production from pancreatic β-cells and improving peripheral glucose utilization resulting in increased glycogen storage by liver (34). Some previous studies have also revealed that MC increases the glucose uptake by the liver via promoting glucose-6-phosphate dehydrogenase and declining glucose-6- phosphatase activities (22). In addition, *M. charantia* is also supposed

to increase the mRNA expression of glucose transporter 4 (GLUT4) proteins in skeletal muscles (28).

Several hypoglycemic components of MC have been identified, which consist of a steroidal glycosides (charantin), insulinomimetic proteins (p-insulin or v-insulin) and alkaloids that are concentrated in the fruit of the plant (16), which all could contribute for the antidiabetic effect of *M. charantia*.

There was a gradual decrease in severity of clinical signs such as polyuria, polydypsia, polyphagia and weight loss in diabetic animals treated with the *Momordica charantia* extract (Figure 1). The improvement of the condition could be attributed to the hypoglycemic, hypolipidemic, hepatoprotective, insulinomimetic and insulin secretagogue effects of the plant extract in the diabetic subjects (11, 21).

Histologically, there was a progressive improvement in the architecture of the pancreas from Day 15 to 45 of the study (Figure 5). However, a dose dependent improvement was observed which included formation of more numbers of compact islets with high cellularity, consisting of moderate number of β cells identified by IHC for insulin, with 200 mg/kg body weight of *M. charantia* better than 100 mg/kg body weight dosage (4, 21) (Figure 8). The improvement in architecture of pancreatic islets could be attributed to the regeneration and repair of damaged β-cells by the stimulating effect of *M. charantia* (4). Various phytochemicals of *M. charantia* like polypeptide-P a plant insulin, charantin, vicine, glycosides and karavilosides, improve insulin release from β cells and repair or promote regeneration of insulin secreting β-cells (16).

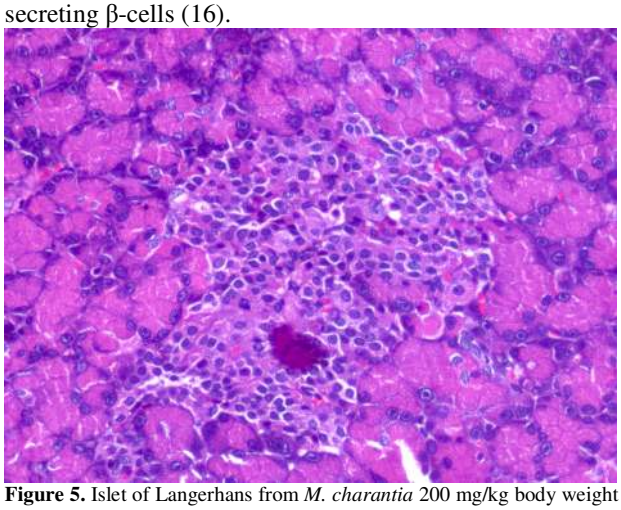

Figure 5. Islet of Langerhans from *M. charantia* 200 mg/kg body weight in distilled water treated rat showing improvement in the size and number of β-cells on 45th day of the study. Note the presence occasional vacuolated β-cells (H&E 100X).

The assessment of various parameters for the effect on *M. charantia* at 100 and 200 mg/kg body weight in distilled water in STZ-induced diabetes revealed presence of a dose dependent effect with the higher dosage superior in alleviation of diabetic effects.

Immunohistochemistry and special staining

In the present study, there was a drastic decline in the number of insulin positive cells in the islets of diabetic control rats owing to the specific destruction of β-cells by STZ (2, 3) (Figure 7).

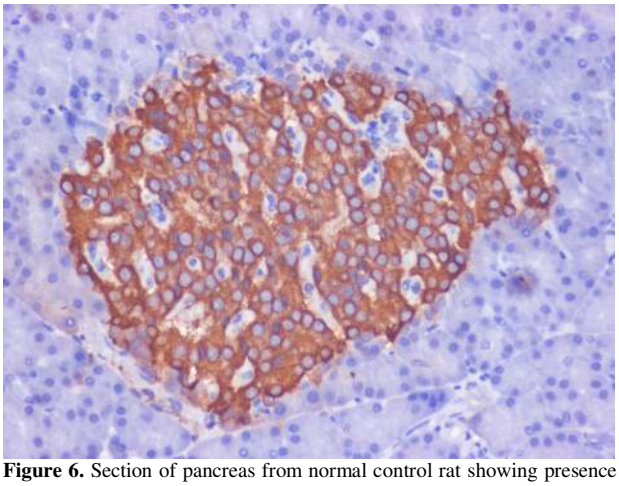

Figure 6. Section of pancreas from normal control rat showing presence of compactly arranged insulin immunopositive β-cells occupying major portion of the islet on 45th day of the study (IHC 100X).

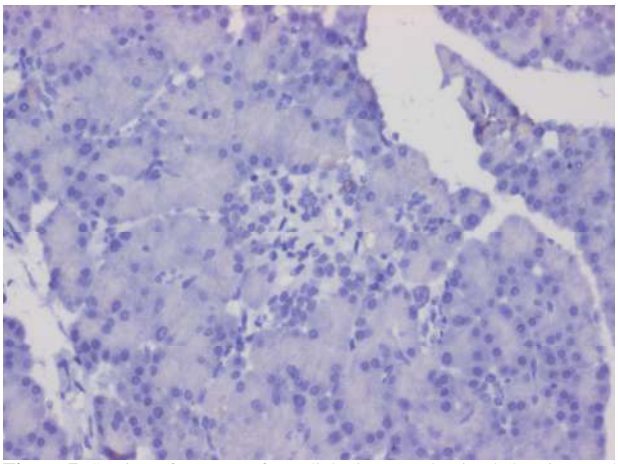

Figure 7. Section of pancreas from diabetic control animal showing total absence of functional β-cells in the islet on 15th of the study (IHC 100X).

There was an improvement in the number of insulin positive cells in various treatment groups with *M. charantia* treated rats showing better response with respect to the increase in the percentage insulin positive β-cell (Table 6; Figure 8). There was also dose dependent improvement in the number of β-cells with *M. charantia* at 200mg/kg body weight showing maximum improvement with mean percentage of insulin positive cells of 20.70 ±0.26 on 45TH day of the study (4, 16, 21) (Table 6).

Special staining in treatment groups revealed hypercellularity with more number of alpha cells and a few normal appearing β-cells. The possible reason for relative increase in the alpha cells could be to stimulate insulin synthesis by increasing glucagon levels (2, 13) (Figure 9, 10).

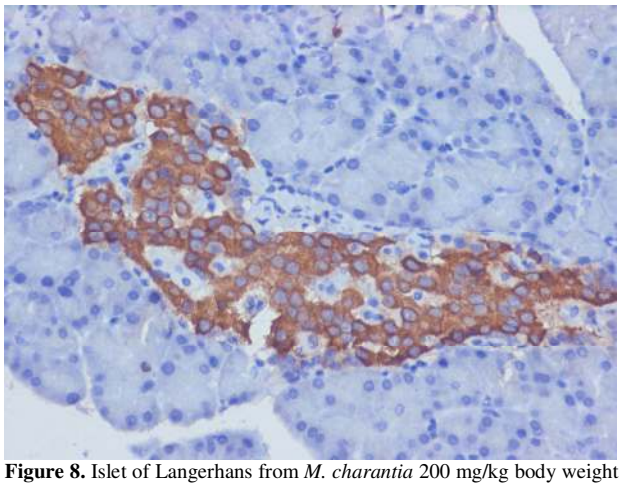

Figure 8. Islet of Langerhans from *M. charantia* 200 mg/kg body weight in distilled water treated rat showing improvement in the number of insulin positive cells forming cords occupying the centre of the islet on 45th day of the study (IHC 100X).

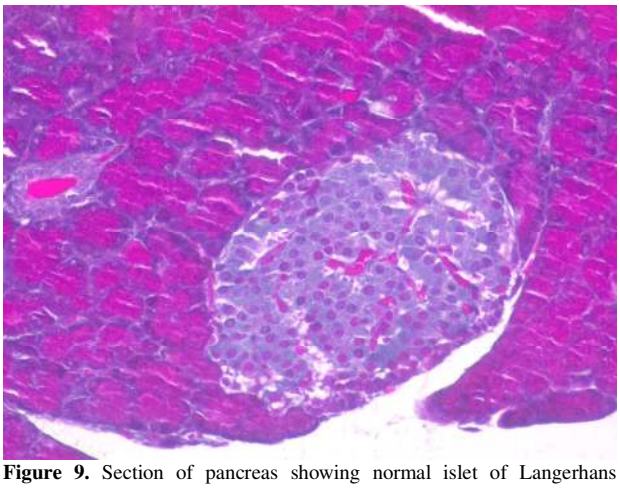

Figure 9. Section of pancreas showing normal islet of Langerhans comprising centrally located β-cells with bluish granular cytoplasm in the form of cords and α-cells with pinkish cytoplasm at the periphery (Gomori's special stain 100X).

Table 6. The mean (±SE) values of positivity of insulin secreting cells (percentage) of normal control, diabetic and diabetic treatment groups at different intervals of time.

Groups	Days post-treatment		
	15	30	45
Group I(Normal control)	79.62 ±0.38	79.05±0.49	78.74 ±0.92
Group II(Diabetic control)	2.36 ±0.60	2.88±0.23	3.50 ±0.33 [a]
Group III(Glibenclamide treated rats)	11.17± 0.85	13.21±0.76	18.55 ±0.28[ab]
Group-IV(MC 100mg /kg BW)	9.58±0.64	12.90±0.12	16.27 ±0.18[abc]
Group-V(MC 200mg/kg BW)	12.93±0.50	15.09 ±0.33	20.70 ±0.26[ab]

All values are mean (± SE), Mean values with superscript differ significantly,a(Comparison with Group I), b(Comparison with Group II), c(Comparison with Group III), Values are statistically significant at P≤ 0.001.

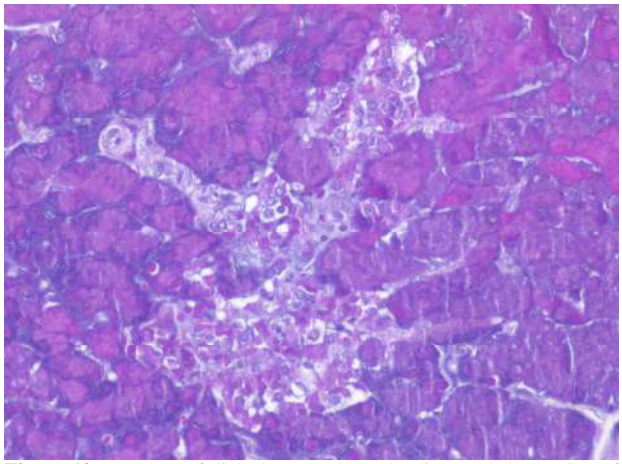

Figure 10. Pancreas of diabetic control rat showing total destruction of functional β-cells on 15th day of the study. Note absence of cells with bluish granular cytoplasm (Gomori's special stain 100X).

Conclusion

The present study has shown that:

- Glibenclamide substantially alleviated the effects of STZ in diabetic rats. However, the improvement was not on par with normal control animals.
- *M. charantia* was as effective as glibenclamide in alleviating STZ induced diabetic effects and has a dose dependent antidiabetic effect.

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