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Efficacy of *Momordica charantia* in alleviation of streptozotocin- induced diabetes effects in rats

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Abstract

The pathomorphological evaluation of antidiabetic effect of hydroalcoholic extract of *Momardica charantia (M. charantia)* plant's fruit pulp was studied in streptozotocin (STZ) induced diabetes in Wistar albino rats against glibenclamide. Hydroalcoholic extract of *M. charantia* at 200 mg/kg was administered to the diabetic subjects for 45 days. The antidiabetic effect was evaluated by estimation of serum glucose, cholesterol, triglyceride, ALT, AST and serum insulin. Detailed histopathologic evaluation of islets of Langerhans, liver and other organs was performed to assess its pathomorphological effects. The diabetic rats which received the hydroalcoholic extract of the *M. charantia* fruit pulp showed significant decrease ($P \leq 0.001$) in the serum levels of glucose, cholesterol, triglyceride, ALT and AST and the serum insulin was significantly increased ($P \leq 0.001$) compared to diabetic control animals. The effect observed was almost comparable to those of glibenclamide. The microscopic evaluation of islets of Langerhans indicated either the regeneration or repair of the affected beta cells and also the extract found to have marked hepatoprotective effect against STZ induced liver injury

Key words: *Momordica charantia,* streptozotocin, rat, insulin, diabetes mellitus

Introduction

Diabetes mellitus, a metabolic disorder, characterised by hyperglycaemia is associated with either absolute or relative deficiency in insulin secretion and /or insulin action and clinically characterised by polyurea, polydypsia, polyphagia and weight loss. There is an increased concern over the prevention and treatment of diabetes among the common folk. Along with use of routine allopathic medicines, the herbal therapeutic agents are gaining more importance owing to their relative less side effects and patient compliance. *Momordica charantia* which belongs to the family of Cucurbitaceae, is a

common folklore remedy for diabetes and the extract of fruit pulp, seed, leaves and the whole plant have been used for their hypoglycemic effect. Different workers have tested various parts of *Momordica charantia* plant like aqueous extract of the plant, ether extract of leaves, dried whole fruit, powdered seeds of the fruit with respect to their hypoglycemic potential (Lal and Chaudhary, 1968, Vimladevi *et al*. 1977, Akhtar *et al*. 1981 and Kedar and Chakrabarti 1982). Though extensive literature is available on anti hyperglycemic and hypolipidemic effects of there is paucity in the information regarding the pathomorphological details at tissue level. Hence to understand and ascertain the pathomorphological and biochemical effects of hydroalcoholic extract of *Momordica charantia* in STZ induced diabetes the present study was designed.

Material and Methods

Male Wistar albino rats weighing 180-260 g were procured from Central Animal Facility, Indian Institute of Science, Bangalore. The animals were maintained under standard laboratory condition and offered *ad libitum* feed and water. The experiment was carried out for a period of 45 days with prior permission from Institutional Animal Ethics Committee.

Streptozotocin

Streptozotocin to induce experimental diabetes in rats was procured from Sigma Chemicals, St.Louis, USA.

Momordica charantia plant extract

The hydroalcoholic extract of *Momordica charantia* (identified by HPTLC finger printing and assayed by Gravimetric method) was procured from Ms. PLANTEX, Vijayawada, India.

Glibenclamide solution

Glibenclamide (Daonil®, 5 mg) an oral hypoglycemic drug was administered orally at a dose of 600 µg/ kg (Babu *et al*., 2003)

Administration of plant extract and glibenclamide

Throughout the study period the plant extract and glibenclamide to their respective groups were administered orally by using clean rat feeding needle attached to an appropriate disposable syringe during morning hours of the day.

Experimental design

The rats were divided into four different groups (Group-I - normal control, Group-II- diabetic control, Group-III - diabetic animals treated with glibenclamide and Group-IV - diabetic rats supplemented with hydroalcoholic extract of *M. charantia* fruit pulp) with ten animals each based on body weight. Care was taken to maintain the intra group weight variation to be less than 20 g and inter-group weight variation by 30 g.

Experimental induction of diabetes

Hyperglycemic state was experimentally induced by intra peritoneal inoculation of Streptozotocin at the dose rate of 45 mg/kg in 0.1M citrate buffer (pH 3.5- 4.5) (Babu *et al*., 2004) in rats of Groups II, III and IV fasted for 16 h. The Group I animals received citrate buffer alone. The rats of Group II were maintained as positive control. The Group III rats were treated with glibenclamide at a dose rate of 600 µg/ kg and Group IV rats with extract of *M. charantia* at a dose of 200 mg/kg daily by oral route.

Confirmation of diabetes

The diabetic state was confirmed by estimating the serum glucose level at 72 post STZ injection using Span Diagnostic kit with Semi-Automatic Biochemical Analyser (ARTOS, Bangalore). The animals that showed the serum glucose level above 200 mg/dl were considered diabetic and selected for the study.

Collection of serum samples

About 2 ml of blood from the retro-orbital plexus of the rats in all the groups were collected under light ether anaesthesia separately in clean test tubes at different time intervals of the study such as 3rd, 15th, 30th and 45th days post STZ injection. The serum collected was subjected for glucose, cholesterol, triglyceride, ALT, AST estimation biochemically and insulin by Radioimmuno assay.

Collection of tissue samples

To study the progressive effects of the treatments administered to different groups, two rats from each group were sacrificed humanely on 15th and 30th day of experiment under ether anaesthesia and the remaining rats on 45th day of experimentation. Euthanized animals were subjected for detailed post mortem examination and gross changes. Further, representative tissue samples from pancreas, liver, kidney, lungs, heart, intestine, brain, stomach, skin and muscle were collected in 10 % neutral buffered formalin (NBF) for the pathomorphological evaluation.

Statistical analysis

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

Results

In the present study, STZ at 45 mg/kg i.p induced diabetes with significant (P≤0.001) increase in serum glucose, cholesterol, triglyceride (Table 1,2 & 3) and significant (P≤0.001) reduction in serum insulin level (Table 6). STZ also caused hepatic injury as evidenced by the histopathology with concurrent rise in the marker enzymes viz ALT and AST (Table 4 & 5). Supplementation of *Momordica charantia* to diabetic rats (Group IV) caused progressive reduction in the serum glucose, cholesterol and triglyceride levels. The reduction was significant (P≤0.001) from Day 15 post-treatment and continued till the end of the study. A significant hypolipidaemic effect was also observed in which the serum cholesterol levels on 45th day of the study was 54.74±1.35 mg/dl against 119.28±4.19 mg/dl of diabetes control (Table 2). The extract of *Momordica charantia* also produced significant (P≤0.001) reduction in the serum ALT and AST levels (Table 4 & 5) compared to diabetic control.

The antidiabetic effect of the *Momordica charantia* extract was comparable to that of glibenclamide with respect to serum glucose and serum triglyceride. However, glibenclamide was found superior with respect to hypocholesterolemic effect (Table 2).

Streptozotocin induced significant hypoinsulinism (Table 6) in the diabetic control rats (Group I) was observed till Day 45 post STZ injection. However, administration of *Momordica charantia* extract resulted in a gradual and progressive improvement in insulin levels from 15th day to 45th day post-treatment in Group IV rats.

Light microscopic examination

Microscopically, STZ caused marked damage to islets of Langerhans and liver in diabetes induced rats. The affected islets varied in their number, size and shape. Islets appeared elongated, crescentic or 'starfish' like. The beta cells initially showed degenerative changes in the form of cell swelling, loss of granularity, cytoplasmic vacuolations and later necrosis of beta cells. In addition, mild fibrosis and infiltration of inflammatory cells into islets were also observed as late lesions. Liver showed swelling of hepatocytes decreased sinusoidal spaces, cytoplasmic vacuolations and 'bridging type' of necrosis.

Supplementation of *M. charantia* extract improved the architecture of islets of Langerhans, in general. Progressive improvement was appreciable from Day 15 post-treatment till the end of the study. The number, size and shape of the islets though showed a positive response, few beta cells revealed persistence of STZ effect. The morphological architecture of islets was almost comparable to that of glibenclamide treated and of normal control groups. There was an increase in the number of beta cells which appeared larger with sufficient cytoplasmic granularity.

The rats supplemented with the plant extract revealed improvement in the liver tissue architecture as observed by the decrease in vacuolations and other degenerative changes indicated. This observation was also supported by the improvement observed in the liver enzyme values which signify the health of the hepatocytes.

Discussion

Streptozotocin, a diabetogenic substance has been reported to induce diabetes in animals by its cytotoxic effect specific for β-cells of islets, accounting for hypo insulinemia. STZ has been reported to be capable of generating reactive oxygen species resulting in oxidative stress and cell death (Sarkar *et al*., 1996; Ahmed *et al*., 2000), which was also observed in the present study and substantiated well by microscopical evidence of degeneration and destruction of beta cells of islets.

Hyperglycemic state in diabetes could be due to deficiency or resistance to insulin which leads to decreased glucose transport in muscle, elevated hepatic glucose production and increased breakdown of fat (Karunanayake *et al*. 1975 and Mahdi *et al*., 2003). Hyperlipidemia is regarded as a consistent feature in experimental STZ and alloxan induced diabetes in rats as reported by Neera Singh *et al*. (1989) which could be due to deficiency of insulin that increases excessive breakdown of adipose store by stimulation of lipoprotein lipase leading to increased mobilisation of fatty acids and their accumulation in liver as triglycerides. In addition deficiency of insulin also causes diminished levels of LDL receptors leading to increased LDL cholesterol values in diabetes (Nafisa *et al*., 2007) contributing for hyperlipidemia.

ALT and AST are soluble enzymes found in the hepatocytes and released into the circulation when injury to organelles such as mitochondria occurs. STZ has been reported to induce both plasma membrane and organellar membrane damage especially that of RER and mitochondria (Laguens *et al*., 1980). The elevated serum levels of liver enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane, a factor which was well supported by the histopathology of liver in the present study (Rajesh and Latha, 2004 Muhammad *et al*., 2008).

It was observed in the present study that the mean serum glucose levels were progressively reduced on *Momordica charantia* extract treatment and were significantly lower (P<0.001) compared to those of diabetic control group. The decrease observed in glucose values was comparable to that by glibenclamide. However, the glucose values failed to reach the normal range as observed in the control group rats. This

clearly indicated that, though *Momordica charantia* was capable of declining the glucose level, the hypoglycemic effect was not total and never attained the normal range (Sarkar *et al*., 1996; Mahdi *et al*., 2003). As indicated by Welihinda *et al*. (1982), the anti hyper glycemic activity of the plant might be due to its stimulating effect on the remnant β-cell or improvement in insulin action at cellular level or it could also be due to the insulin like effect of the active principle(s) present in the extract. A marked improvement in insulin secretion in diabetic rats treated with extract of *M. charantia* fruit in the present study substantially supports the findings of the earlier workers.

The hepatoprotective effect of *Momordica charantia*observed in the present study could be attributed to the presence of flavonoids and ascorbic acid in the extract (Chaudhari *et al*., 2009) and to their free radical scavenging activity (Asli *et al*., 2007).

The results of the present study indicated that *Momordica charantia* treatment had positive effect in improving the insulin level in diabetic animals, a finding well supported by those of Ahmed *et al*. (2000) and Rao *et al*. (2001). The improved insulin level in *Momordica charantia* treatment could be due to regeneration or repair of damaged beta cells by *Momordica charantia* as reported by Chaturvedi *et al*. (2004) and Nafisa *et al*. (2007). Progressive reconstruction of normal architecture of islets from Day 15 to Day 45 post treatment in the present study also strengthens the positive effect of *Momordica charantia* on insulin levels (Mutalik *et al*. 2005, Nafisa *et al*. 2007).

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Table-1: Effect of *Momordica charantia* on serum glucose (mg/dL) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	106.00± 5.18	103.83± 3.60	106.83±4.24	107.13±3.21
Group II	428.50±6.74[a]	474.66±5.57[a]	513.66±7.09[a]	557.83±5.71[a]
Group III	436.50±4.50 [a]	363.50±6.28 [ab]	306.16±5.82[ab]	225.00±6.04 [ab]
Group IV	424.83±9.21 [a]	349.50±10.95 [ab]	293.50±10.02[ab]	241.00±8.49 [ab]

Table-2: Effect of *Momordica charantia* on serum cholesterol (mg/dL) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	43.03±1.82	42.71±1.86	42.16±1.93	41.48±1.78
Group II	74.98±4.25 [a]	91.90±5.53 [a]	105.60±5.24 [a]	119.28±4.19 [a]
Group III	74.81±1.92[a]	54.26±2.81 [ab]	45.29±3.23[b]	36.21±2.57 [b]
Group IV	76.30±1.13 [a]	65.05±1.93[abc]	56.83±2.28[abc]	54.74±1.35 [abc]

Table-3: Effect of *Momordica charantia* on serum triglyceride (mg/dL) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	98.85 ± 2.18	98.51±1.20	100.86±1.22	99.48±1.29
Group II	211.10±3.41 [a]	248.95±3.16 [a]	285.35±2.46 [a]	328.22±4.35[a]
Group III	209.17±3.01 [a]	180.67±1.58 [ab]	137.42±1.90[ab]	99.67±3.52 [b]
Group IV	207.17±2.82 [a]	173.87±2.72 [ab]	145.42±2.31[ab]	120.58±1.76[abc]

Table-4: Effect of *Momordica charantia* on serum ALT (IU/L) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	52.47 ±1.35	51.92±0.36	52.87±0.49	51.96±0.90
Group II	142.96± 7.7 [a]	206.98±1.87 [a]	249.18±2.93 [a]	345.34±1.62 [a]
Group III	135.47± 5.7 [a]	120.97±5.13 [ab]	108.90±5.95[ab]	102.80±7.01 [ab]
Group IV	139.14±3.36 [a]	108.36±3.15 [ab]	80.67±4.81[abc]	78.67 ±4.73 [abc]

Table-5: Effect of *Momordica charantia* on serum AST (IU/L) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	64.95 ± 1.08	64.86± 0.96	64.93 ± 0.99	64.68 ± 1.08
Group II	180.50± 0.74 [a]	215.03± 3.84 [a]	271.11 ± 1.97[a]	355.68 ± 4.97[a]
Group III	185.36± 1.73 [a]	152.88±3.52[ab]	123.11±6.59[ab]	114.65±7.25 [ab]
Group IV	180.72± 3.32[a]	157.41±2.60[ab]	132.45±5.75[ab]	101.72±6.13 [ab]

Table-6: Effect *Momordica charantia* on serum insulin (µU/L) in STZ induced diabetic rats

Groups	Days of post-treatment			
	3	15	30	45
Group I	56.86±1.73	56.51±1.80	56.75±1.78	41.48±1.78
Group II	16.03±0.39 [a]	12.29±0.33 [a]	11.22±0.44 [a]	119.28±4.19 [a]
Group III	25.75±1.13 [ab]	35.20±0.71 [ab]	39.16±0.73 [ab]	36.21±2.57 [b]
Group IV	19.43±0.59 [ac]	27.56±0.91 [abc]	30.96±0.72 [abc]	54.74±1.35 [abc]

Note: In all tables, values are expressed as Mean ± SE, n=10, [a] Comparison with Group -I, [b] Comparison with Group-II, [c] Comparison with Group-III, Values are statistically significant at (P≤ 0.001), Mean (± SE) values with common superscript do not have significance (P≥0.001)

Histopathology images

Plate 1 Islets of Langerhans showing normal large, round beta cells with abundant granular eosinophilic cytoplasm - H&E X 400

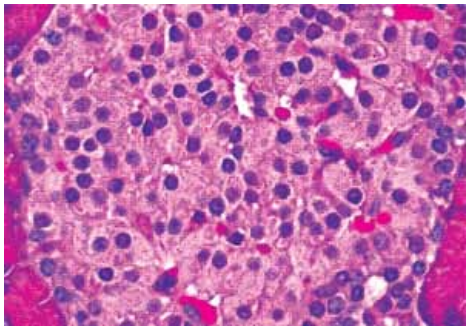

Plate 2 Islets of Langerhans on Day 45 from diabetic animal showing loss of shape and presence of vacuolated and a few necrotic cells. Proliferation of fibroblasts with elongated nucleus-H&E X 200

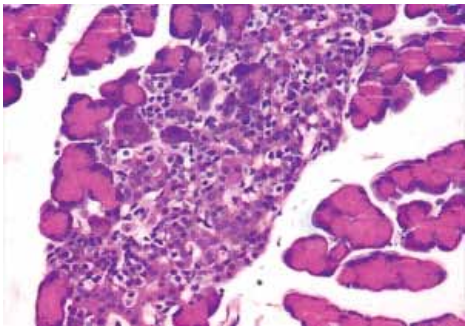

Plate 3 Pancreas from a diabetic rat treated with glibenclamide showing well formed islets with normal architecture on Day 45 of the study- H&E X 400

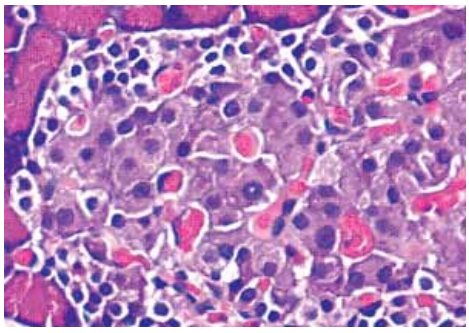

Plate 4 Pancreas from a diabetic rat treated with *Momordica charantia* showing well formed islet with compact arrangement of beta cells on Day 45 -H&E X 200

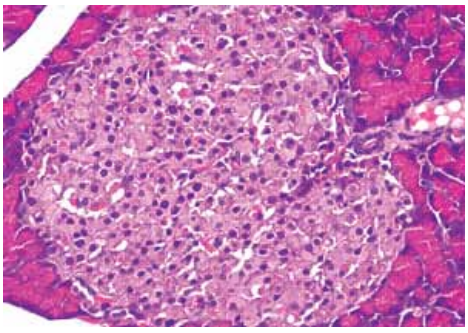

Plate 5 Section of liver from normal control rat on 45th day showing central vein and well formed hepatic cords with normal appearing hepatocytes -H&E X 100

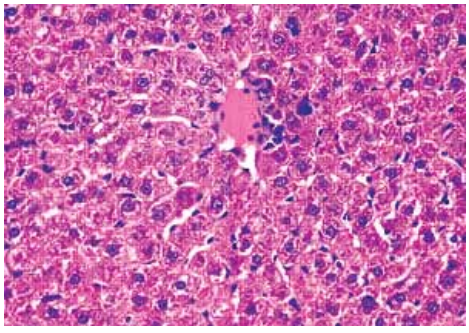

Plate 6 Section of liver from diabetic rat on Day 45 showing highly swollen hepatocyte with increased cytoplasmic granularity, vacuolations and necrosis of hepatocytes - H&E X 200

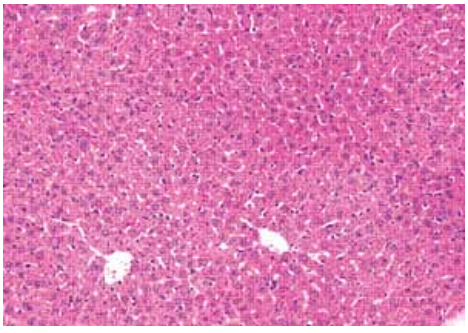

Plate 7 Section of liver from a diabetic rat treated with Glibenclamide on Day 30 of the study showing improved architecture from STZ effects - H&E X 200.

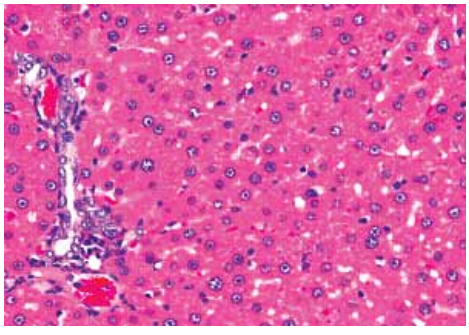

Plate 8 Section of liver on 45th day post-treatment from diabetic rat treated with *Momordica charantia* showing complete normal architecture - H&E X 200