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EXPERIMENTAL RESEARCH

NIDDM Antidiabetic Activity Of Saponins Of *Momordica Cymbalaria* In Streptozotocin-Nicotinamide NIDDM Mice

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ABSTRACT

Objective: To evaluate the Type 2 anti-diabetic activity of saponins obtained from *Momordica Cymbalaria* in Streptozotocin–Nicotinamide Induced Type 2 diabetic mice.

Materials and Method: Type 2 diabetes in BALB/c mice was induced by a single intra-peritoneal injection of Streptozotocin (100 mg/ kg, i.p), 15 min after the intra-peritoneal administration of Nicotinamide (240 mg/ kg, i.p). Hyperglycaemia was confirmed by elevated blood glucose levels determined on day 7 after the injection. The saponin mixture was obtained from the ethanolic extract of *Momordica Cymbalaria*. Saponins of MC (SMC) 175mg/kg,p.o/30 days and Metformin350mg/kg,p.o/30 days were administered to the Type 2 diabetic mice. At the end of the treatment, their serum was analyzed for glucose, cholesterol, triglycerides and insulin; The pancreas of each mouse was studied to check whether there were any histological changes.

Result: Treatment of Type 2 diabetic mice with SMC (175mg/kg, p.o/30 days) and Metformin (350mg/kg,p.o/30 days) produced a significant fall in blood glucose ($p<0.001$), cholesterol ($p<0.001$), triglycerides ($p<0.001$) and an increase in the serum insulin level ($p<0.001$). Pancreatic islets and beta cells showed an increase in numbers.

Conclusion: Saponins of MC have significant Type 2 anti-diabetic activity and the activity may be due to increasing insulin secretion, probably by the regeneration of pancreatic beta cells.

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Introduction

Diabetes mellitus (DM) is a chronic disease caused by inherited and/ or acquired deficiency in the production of insulin by the pancreas, or by the ineffectiveness of the insulin produced [1] . Reports from the World Health Organization (WHO) indicate that diabetes mellitus is one of the major killers of our time, with people in Southeast Asia and the Western Pacific being most at risk [2].

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethno-botanical information reports about 800 plants that may possess anti-diabetic activity when being assessed using the presently available experimental techniques [3].

Momordica cymbalaria Fenzl (MC) (Cucurbitaceae) is a species found in the states of Karnataka and Andhra Pradesh, India. Its tuber is traditionally used as an abortifacient [4] and it is also used locally for the treatment of diabetes mellitus. We have reported its anti-ovulatory, abortifacient, anti-implantation and cardio-protective activities [5],[6],[7]. Fruits of MC are also reported to have antimicrobial activity [8].The fruit powder and extracts of MC were previously reported to have Type 1 anti-diabetic activity in experimental diabetic models [9],[10],[11] .Researchers have reported that SMC have anti-diabetic activity and the anti-diabetic activity may be due to reversing of the

atrophy of the pancreatic islets of β-cells, as a result of which there may be increased insulin secretion and increase in the hepatic glycogen level and these may attenuate Hyperinsulinaemia. The alpha-adrenergic blocking effect might contribute to their insulin secretion and sensitizing effects [12].

Type 2 diabetes is more prevalent than Type 1 but interestingly, the effect of *Momordica Cymbalaria* on Type 2 diabetes is not being reported. Hence, in the present study, an attempt has been made to elucidate the effect of *Momordica Cymbalaria* on Type 2 diabetes.

Materials and Methods

Drugs and chemicals

The fresh roots of *Momordica Cymbalaria*, Fenzl, were collected from Gadag district of Karnataka and were identified and authenticated by Dr.Sreenath, Department of Botany, Bangalore University, Bangalore. The roots of *Momordica Cymbalaria* were isolated, chopped into small pieces, dried under shade at room temperature for seven days and were powdered. The powder was extracted with ethyl alcohol to get a yield of 14.1 % w/w. The ethanolic extract of *Momordica Cymbalaria* was dissolved in hot distilled water and was partitioned between water saturated n-butanol and a water layer. The organic layer (n-butanolic layer) was separated and evaporated to get a residue. This n-butanolic residue was dissolved in methanol and was poured in diethyl ether (Et_2O) to obtain a flocculent precipitate. This precipitate was separated by using a filter paper and was washed with excess of Et_2O and dried to yield a crude fraction of saponins –[13].The saponin mixture was dissolved in distilled water (SMC) and was used for the study. All other chemicals and reagents used, were of analytical grade

Animals

Male BALB/c mice weighing 20-25 g, of either sex and male Swiss albino mice weighing 20 ± 5 g, were procured from NIMHANS (National Institute of Mental Health and Neuro Science), Bangalore, at least 2 weeks prior to the study. The animals were maintained under controlled standard animal house conditions with *ad libitum* access to food and water. They were fed with standard mice feed (Amrut rat and mice feed, Pranav agro industries Ltd. Sangli, India). The Institutional Animal Ethics Committee's permission was obtained before starting the experiments on the animals. The oral acute toxicity study was performed using the up and down procedure (OPPTS guidelines).

Induction of Experimental Diabetes

Streptozotocin (STZ) was dissolved in cold 50mM-citric acid buffer and Nicotinamide was dissolved in normal saline at the time of use. Type 2 diabetes mellitus was induced [14] in overnight-fasted BALB/c mice by a single intra-peritoneal injection of 100 mg/ kg of Streptozotocin, 15 min after the intra-peritoneal administration of 240 mg/ kg of Nicotinamide. Hyperglycaemia was confirmed by elevated blood glucose levels determined on day 7 after the injection. Only mice which were confirmed to have permanent Type 2 diabetes were used for the anti-diabetic study [23].

Experimental Design

A total of 24 mice (18 diabetic surviving mice, 6 control mice) were divided into four groups of six mice each.

Group 1 - Control mice,
Group 2 - Diabetic mice – Administered intra-peritoneal with Streptozotocin (100mg/kg i.p.) and Nicotinamide (240mg/kg i.p)
Group 3 - Diabetic mice administered orally with SMC 175 mg/kg, p.o/30days,
Group 4 - Diabetic mice administered orally with Metformin (350mg/kg, p.o/30days).

At the end of the experimental period on the 31st day, the mice were deprived of food overnight and blood was collected in a tube containing potassium oxalate and sodium fluoride for the estimation of plasma glucose, cholesterol, triglycerides, HDL and insulin by puncturing the retro orbital. The animals were sacrificed immediately. The pancreas of each was isolated, they were fixed in 10% formalin buffer for 24 h, dehydrated in alcohol and were then embedded

in paraffin. The paraffin blocks were sectioned at 5 mm intervals and were stained with haematoxylin-eosin for histological examinations [22].

Biochemical Procedures

Serum glucose, triglycerides, cholesterol and HDL-cholesterol were analyzed by using an *Auto span* diagnostic kit. Serum insulin was measured using The ADVIA Centaur analyzer (RIA) (Bayer diagnostics.)

HPTLC Fingerprinting

The HPTLC fingerprinting of SMC was performed. Silica gel 60F254 (Merck) was used as a stationary phase. Chloroform: Glacial acetic acid (9.5:0.5) was used as a mobile phase. The dried plate was scanned to visualize the migrated components under UV radiation at 254 nm, 336 and 540 ηm using Reprostar 3 with a digital camera (CAMAG).

Statistical Analysis

All the data were analyzed using One-Way ANOVA, followed by Tukey's multiple comparison tests. All values were reported as mean +/- SEM.

Results

Acute Toxicity Test

Mortality in the acute toxicity test of SMC was seen in the limit test at the dose of 5000 mg/kg. Mortality was not seen in the main test up to a dose of 1750 mg/kg and hence, 1/10th of 1750mg/kg (175 mg/kg) was selected for the study.

Effect On Type 2 Diabetes

Type 2 diabetic rats treated with SMC (175mg/kg, p.o/day/30days) showed a significant ($p<0.001$) decrease in serum glucose, cholesterol and triglyceride levels, whereas there was a significant ($p<0.001$) increase in the serum insulin level. Type 2 diabetic rats treated with Metformin (350mg/kg, p.o/day/30days) also showed a significant decrease in serum glucose, cholesterol and triglyceride levels and a significant increase in serum insulin levels [Table/Fig 1].

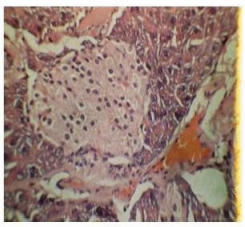
(Table/Fig 1) Effect of administration of SMC (175 mg/kg, p.o, /30days) on serum glucose, triglycerides, total cholesterol, HDL, and serum insulin in NIDDM mice:

Treatment	Serum glucose (mg/dl)	Serum TGS (mg/dl)	Total Cholesterol (mg/dl)	Serum HDL (mg/dl)	Serum Insulin (mU/l)
Group 1 Control (Dist water)	85. 09 ±1.85	67 ±1.18	65.61 ±1.14	39.6 ±1.53	14.98 ±0.29
Group II Diabetic control STZ(100mg/kg i.p.) and NA (240mg/kg i.p)	163.41 ±2.12ϮϮϮ	237.96 ±2.61ϮϮϮ	114.20 ±2.02ϮϮϮ	32.19 ±1.16ϮϮϮ	7.16 ±0.13ϮϮϮ
Group III SMC (175mg/kg,p.o/30days)	107.46 ±2.51***	96.29 ±2.09***	103.59 ±1.46***	36.18 ±1.08***	11.61 ±0.18***
Group IV Metformin (350 mg/kg,p.o/30days)	90.46 ±1.14***	79.65 ±0.72***	90.43 ±1.21***	37.94 ±0.41**	12.126 ±0.31***

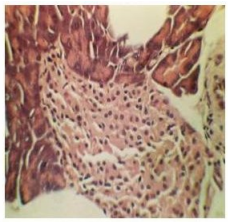
Values expressed as mean ±SEM for six animals
ϮϮϮP<0.001when compared to normal control group.
***P<0.001, **P<0.01when compared to diabetic control group.

Histopathology

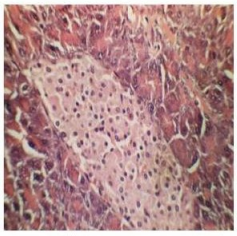
Histopathology studies in the NIDDM control group showed reduction in the number of pancreatic islets as well as in the number of beta cells. The islets were irregularly shaped, relatively small and atrophic. Most of the beta cells were destroyed and even if present, they were destroyed partially [Table/Fig 2]. Most cells of the islets were small and degranulated, with scanty cytoplasm. Insulin producing beta cells were drastically decreased, whereas glucagon producing alpha cells were predominantly present. Severe vacuolation and degranulation were present in the beta cells of a maximum number of islets. The treatment group (SMC) showed an increase in the number of pancreatic islets and in the number of beta cells in the pancreas. Beta cells were seen in clusters.



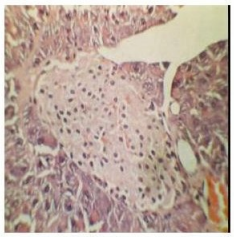
(Table/Fig 2 A) Light microscope sections of pancreas of normal control mice



(Table/Fig 2 B) Light microscope sections of NIDDM mice



(Table/Fig 2 C)Light microscope sections of pancreas pancreas of SMC (175 mg/kg) treated NIDDM mice

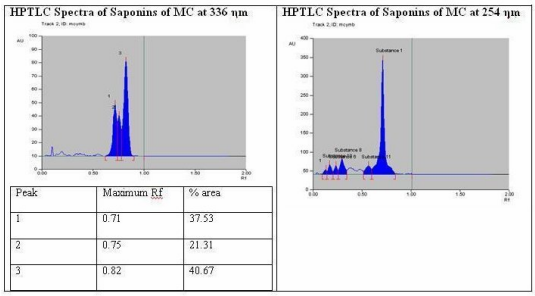


(Table/Fig 2 D) Light microscope sections of of Metformin (350 mg/kg) treated NIDDM mice

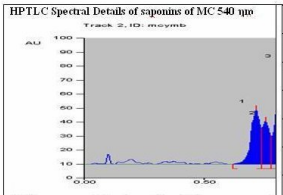
Discussion

Streptozotocin (STZ) is a widely used chemical inducer for Type 1 diabetes [15]. STZ has been shown to produce free radicals in the body, which specifically cut DNA chains in the pancreatic beta cells, resulting in disorder of the function of the pancreatic beta cells and at a later phase, destruction of the beta cells by necrosis [16]. Nicotinamide dinucleotide(NA) [17] causes activation of the poly ADP ribose synthase to repair the damaged DNA [18] and STZ/NA. The Type 2 diabetic model possesses characteristics quite similar to the Type 2 non-obese diabetes, which constitutes a majority of East Asian diabetic cases [Table/ Fig 3].

(Table/Fig 3) HPTLC spectra of SMC at 336, 254 and 540 nm



Peak	Maximum Rf	% area
1	0.71	37.53
2	0.75	21.31
3	0.82	40.67



Peak	Maximum Rf	% area
1	0.71	37.53
2	0.75	21.31
3	0.82	40.67

Peak	Maximum Rf	% area
1	0.17	4.34
2	0.23	4.76
3	0.29	10.28
4	0.38	5.91
5	0.45	3.42
6	0.55	6.01
7	0.75	65.21

Fruits of MC and its extracts have been shown to have Type 1 anti-diabetic activity[9],[10],[11]. The present study showed that the anti-diabetic activity of SMC may be due to reversing of the atrophy of the pancreatic islets of the beta-cells, as a result of which there may be increase in insulin secretion, increase in the hepatic glycogen level and attenuation of Hyperinsulinemia. The alpha-adrenergic blocking effect might contribute to insulin secretion and sensitizing properties [12] .

Hypoglycaemic phytochemicals in *Momordica Charantia* include a mixture of steroidal saponins known as charantins which are insulin-like peptides [19]. The anti-diabetic activity in the present study is also attributed to Saponins. SMC significantly lowered the serum glucose levels and increased serum insulin levels in Type 2 diabetic mice. Histopathology of the pancreas was done to study the effect of SMC on beta cells. The SMC treated group showed an increase in the number of pancreatic islets and beta cells in the pancreas. This indicated that SMC was regenerating beta cells. The regeneration of the beta cells of the STZ-destructed islets is probably due to the fact that the pancreas contains stable (Quiescent) cells which have the capacity of regeneration.

Therefore, the surviving cells can proliferate to replace the lost cells. *Gynmnema Sylvestre* also increases insulin secretion, probably by the regeneration of pancreatic beta cells [20]. Many other plants are being reported to regenerate atrophied pancreatic islets, restore the secretion of insulin, and thus correct hyperglycaemia.

Diabetes is associated with hypercholesterolaemia and hypertriglyceridaemia. STZ-diabetes showed increased plasma levels of cholesterol, triglycerides, free fatty acids and phospholipids [21]. Insulin deficiency may be responsible for dyslipidaemia. Insulin has an inhibitory action on HMG-COA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol rich LDL particles. The mechanisms responsible for the development of hypertriglyceridaemia in uncontrolled diabetes in humans are due to a number of metabolic abnormalities that occur sequentially. SMC significantly lowered serum cholesterol and triglyceride levels. As reported earlier [12], this effect may be due to increased insulin secretion and the inhibition of the HMG CoA enzyme.

Hence, Saponins of MC in the present study have shown significant Type 2 anti-diabetic activity and the activity may be due to increased insulin secretion, probably by the regeneration of the pancreatic beta cells.

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