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Antidiabetic activity of a triterpenoid saponin isolated from *Momordica cymbalaria* Fenzl

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Glucose uptake by isolated diaphragms of both diabetic, following streptozotocin administration, and non-diabetic animals increased in presence of an oleanane-type triterpenoid saponin isolated from the roots of *M. cymbalaria*. Insulin release was augmented by the presence of the saponin of *M. cymbalaria* (1 mg/mL) in rat insulinoma cell line (RIN-5F) pre-exposed to adrenaline (5 µM) and nifedipine (50 µM). Pancreatic histology also indicated considerable quantitative increase in β-cells (75%) when treated with the saponin. The results suggest that the saponin of *M. cymbalaria* possesses potential antidiabetic activity with respect to insulin secretion, which may be attributed to modulation of calcium channel, and β-cell rejuvenation.

Keywords: Glucose uptake, Insulin secretion, *Momordica cymbalaria*

Diabetes mellitus is becoming the third killer of mankind after cancer and cardiovascular disease due to its high prevalence, morbidity and mortality[1]. Among the currently available therapeutics, only sulfonylureas are used to stimulate the β-cells to secreate more insulin. There is a need for antidiabetic drug with multiple target and potency.

Systematic scientific investigations carried out on medicinal plants have resulted in identification of a growing number of active constituents. Many of these are important part of modern medicine. Some of the well known examples being Reserpine, Atropine, Metformin, Vincamine, Vinblastine, Vincristine, Morphine etc.

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[2]. The fruit powder and extracts of MC were previously reported to have Type 1 antidiabetic activity in experimental diabetic models[3-5]. Its various mechanisms of action have been reported in diabetic rats[6]. Antiovulatory, abortifacient, anti-implantation and cardio-protective activities have also been reported[7-9]. Fruits of MC are also reported to have antimicrobial[10] activity. The antidiabetic activity of saponins of *Momordica cymbalaria* 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 α-adrenergic blocking effect might contribute to their insulin secretion and sensitizing effects[6]. In the present study, an active phytomolecule–an oleanane-type triterpenoid saponin has been isolated and studied for antidiabetic activity.

Materials and Methods

Plant material—The fresh roots of *Momordica cymbalaria* Fenzl were collected from Gadag district of Karnataka and were identified and authenticated by the Department of Botany, Bangalore University, Bangalore, India. The roots of *M. cymbalaria* were isolated, chopped into small pieces, dried under shade at room temperature for 7 days and powdered.

Extraction and isolation—The powdered roots were Soxhlet extracted with methanol. Methanolic extract was saponified and hydrolyzed with 0.5 *N* KOH in distilled water for 1-2 h. Unsaponified fraction was then extracted with diethyl ether. Upper organic layer was extracted with diethyl ether for complete separation of sterols. Ether and 1% aqueous KOH was added and ether layer was separated. The aqueous layer was extracted twice with ether. Combined ethereal layers were washed with distilled water and checked for alkalinity with phenolphthalein

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solution. The ether was distilled off and 6 mL acetone was added and shaken. The solvent was completely removed and the residue was dried to a constant mass. Chemical tests were conducted on the unsaponifiable matter to confirm the sterols. The yield was 0.0019% of purified steroidal saponin powder. To confirm the presence of steroidal saponins, thin layer chromatography was performed using chloroform: glacial acetic acid: methanol: water (16:8:3:2) as mobile phase and spraying with anisaldehyde-sulphuric acid reagent.

Chemicals—Streptozotocin, adrenaline, nifedipine and glibenclamide were obtained from Sigma Aldrich (Bangalore, India); RPMI-1640, antibiotics solution, Na pyruvate, FBS, Kreb's-Ringer bicarbonate buffer, MTT and DMSO were obtained from Hi-Media Labs, Mumbai.

Animals—Male Swiss albino mice weighing between 25-35 g were maintained in standard laboratory conditions at room temperature (25±2 °C) with 12:12 h L:D. The animals were provided with pellet chow and water *ad libitum*, except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) of Karnataka College of Pharmacy, Bangalore. Studies were performed in accordance with the CPCSEA guidelines. The oral acute toxicity study was performed using the up and down procedure (OPPTS guidelines 870.1100).

Induction of experimental diabetes—After overnight fasting, diabetes was induced in mice by ip injection of streptozotocin (SLR, Bangalore) dissolved in 0.1*M* sodium citrate buffer, *p*H 4.5 at a dose of 65 mg/kg. The animals were allowed to drink 5% (w/v) glucose solution overnight to overcome the drug-induced hypoglycemia. On the 3rd day animals with blood glucose level greater than 200 mg/dL were treated as diabetic rats and were used for the further experiments[11,12].

Experimental design

Glucose uptake by diaphragm of non-diabetic (healthy) mice—The animals were divided into the following 4 groups of 6 mice each: Gr. I: normal control (2 mL Tyrode solution with 2% glucose), Gr. II: normal control + insulin (0.25 IU/mL), Gr. III: normal control + metformin (1 mg/mL) and Gr. IV: normal control + SMC (1 mg/mL).

Glucose uptake by diaphragm of diabetic mice—The animals were divided into the following 4 groups containing 6 mice each: Gr. I: normal control (distilled water, po, once daily), Gr. II: diabetic control (streptozotocin, 65 mg/kg, ip), Gr. III: diabetic control + SMC (100 mg/kg, po for 30 days) and Gr. IV: diabetic control + metformin (50 mg/kg for 30 days).

The animals (non-diabetic and diabetic) were anesthetized using anesthetic ether and the diaphragms were dissected out quickly with minimal trauma. The diaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and were placed in small culture tubes containing 2 mL Tyrode solution with 2% glucose and incubated for 30 min at in an atmosphere of 95% O_2 and 5% CO_2 with shaking at 140 cycles per min. Following incubation, the diaphragms were weighed. The glucose content of the incubated medium was measured by glucose oxidase-peroxidase (GOD-POD) method[13]. The uptake of glucose was calculated in mg/g of moist tissue/30 min. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium.

Histopathological study of isolated pancreas—The animals were sacrificed by decapitation. The pancreas of each animal was isolated and cut into pieces, fixed in 10% formalin. Hard paraffin blocks of the pancreas was prepared, sections (5 µm thick) were obtained and fixed on slide. Eosin (acidic stain) and hematoxylin (basic stain) were used for staining the fixed tissue.

Insulin secretion *in vitro*

Cells—RIN-5F cell line was procured from National Centre for Cell Science (NCCS), Pune. The cells arrived at passage number 22. They were cultured in RPMI-1640 growth medium (4.5 g/L glucose, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 10 mM HEPES and 1 mM sodium pyruvate)-90%; fetal bovine serum 10%, and antibiotics (100 IU/mL penicillin G, 100 µg/mL streptomycin, 2.5 µg/mL amphotericin B) at 37 °C in 5% CO_2 atmosphere. Medium change was done every 3-4 days and splitting was carried out in a ratio of 1:3. All the experiments were carried out at passage number 30 to maintain uniformity of the study.

MTT assay—The assessment of cytotoxicity of SMC on RIN-5F cells was done by the reduction of 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. Cells were seeded in a 96-well plate ($0.5\text{-}10\times10^3$ cells/well), and left to attach on to the substrate overnight before being

exposed to SMC which was added to each well at a concentration of 0.25, 0.5, 1, 2, 4, and 6 mg/mL, and incubated at 37 °C for 24 h. After 24 h 100 µL MTT (5 mg/mL) solution was added to each well and the cells were incubated in the dark at 37 °C for an additional 4 h. Thereafter, the medium was removed, the formazan crystals were dissolved in 200 µL of DMSO and the absorbance was measured at 570 nm in a micro plate reader[14]. The percentage cell viability was calculated.

Insulin assay - Grouping of cells:

The RIN-5F cells were divided into the following 9 groups: Gr. 1: normal control: untreated cells, Gr. 2: diabetic control: cells treated with streptozotocin (5 mM), Gr. 3: diabetic control + glibenclamide (0.02 mg/mL), Gr. 4: diabetic control + saponin of *M. cymbalaria* (SMC) (0.5 mg/mL), Gr. 5: diabetic control + SMC (1 mg/mL), Gr. 6: normal control + adrenaline (5 µM), Gr. 7: normal control + nifedipine (50 µM), Gr. 8: normal control + adrenaline (5 µM) + SMC (1 mg/mL) and Gr. 9: normal control + nifedipine (50 µM) + SMC (1 mg/mL).

Cells were seeded in a 96-well plate ($0.5\text{-}10\times10^3$ cells/well), cultured in RPMI-1640 growth medium containing 4.5 g/L glucose, 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 10 mM HEPES and 1 mM sodium pyruvate, fetal bovine serum (10%), and antibiotics (100 IU/mL penicillin G, 100 µg/mL streptomycin, 2.5 µg/mL amphotericin B) at 37 °C in 5% CO_2 atmosphere, and left to attach on to the substrate overnight prior to actual tests. Cells were then washed thrice with Kreb's-Ringer bicarbonate buffer and pre-incubated for 40 min at 37 °C. Cells were incubated in the absence and presence of the respective drugs at 37 °C in 5% CO_2 atmosphere for 6 h. After incubation, aliquotes of 25 µL were withdrawn from each well and assayed for insulin[15].

Insulin was assayed by sandwich ELISA using a commercially available kit (Millipore Cat. No. EZRMI-13K). ELISA plate was coated with mouse monoclonal anti-rat insulin antibodies. Biotinylated anti-insulin antibody was used for detecting insulin and mouse monoclonal anti-insulin-streptavidin-HRP conjugate was used as enzyme solution.

Results

Glucose uptake by diaphragm of non-diabetic (healthy) mice—Saponin of *M. cymbalaria* enhanced the uptake of glucose in mice diaphragm (P <0.001) when compared to control group. It was also comparable to the glucose uptake in mice diaphragm mediated by metformin (Table 1).

Glucose uptake by diabetic mice diaphragm—SMC treated diabetic mice diaphragm (Gr. III) showed significant increase (P <0.01) in glucose when compared to diabetic control mice diaphragm (Gr. II) and the results are similar to metformin-mediated action (Table 2).

Histopathological study of isolated pancreas—Pancreatic lobules in the normal control animals showed small, round, light-staining islets of Langerhans, the center of which consists of aggregates of small β-cells (75%) with basophilic granules, while the periphery comprised of large β-cells (25%) with eosinophilic granules (Fig. 1a). In the diabetic control (Fig. 1b) the number of islets was reduced in number compared to normal with a quantitative decrease in β-cells (30%) while the periphery had large β-cells (65%). This indicates

Table 1—Effect of saponin of *M. cymbalaria* on glucose uptake in non-diabetic (healthy) mice diaphragm

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

Treatment group	Diaphragm glucose uptake (mg/g diaphragm)
Group I: Normal control (2 ml Tyrode solution with 2% glucose)	2.03±0.23
Group II: Normal control + Insulin (0.25 IU/mL)	4.04±0.47*
Group III: Normal control + metformin (1 mg/mL)	7.51±0.21*
Group IV: Normal control + SMC (1 mg/mL)	6.94±0.21*

*P <0.001, when compared to control group.

Table 2—Effect of SMC (100 mg/kg.po/day/30days) on glucose uptake in STZ-induced (65 mg/kg, ip/single dose) diabetic mice diaphragm

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

Treatment group	Diaphragm glucose uptake (mg/g diaphragm)
Group I: Normal control (Distilled water po once daily).	2.4±0.30
Group II: Diabetic control (Streptozotocin 65 mg/kg ip)	1.1±0.14#
Group III: Diabetic control + SMC (100 mg/kg, po/30 days)	2.21±0.24*
Group IV: Diabetic control + Metformin (50 mg/kg/30 days)	2.28±0.27*

P values: <0.001when compared with # normal control, *diabetic control group

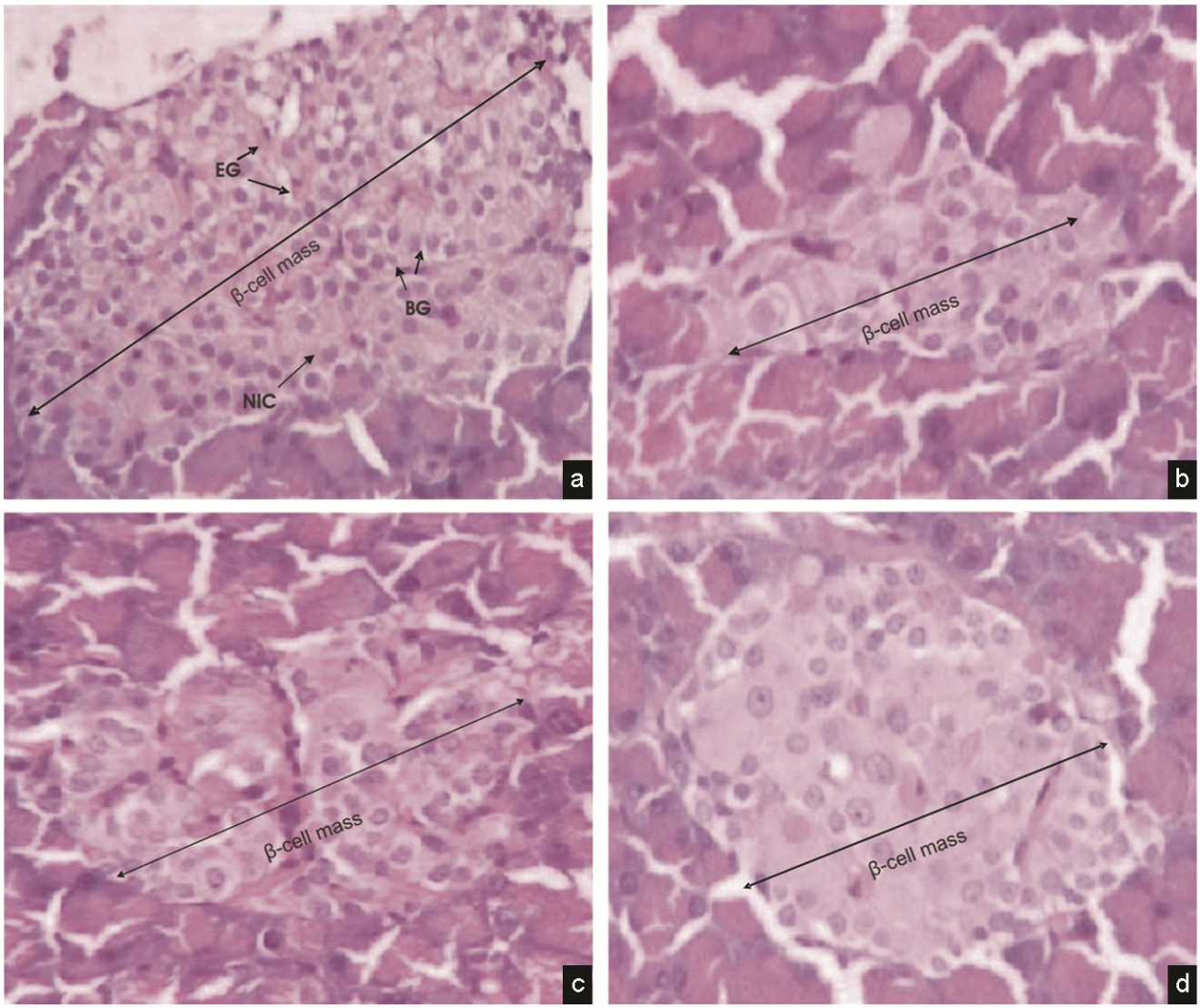

Fig. 1—Effect of saponins of *M. cymbalaria* and insulin (4U/kg/ip/day/30 days) on streptozotocin (65 mg/kg/ip/single dose) treated mice on pancreatic histology after 30 days of treatment [a: normal control shows normal Islet cells (NIC) with basophilic granules (BG) at the center and eosinophilic granules (EG) at the periphery, b: diabetic control, c: insulin treated, d: SMC treated, H&E, 100X]

degeneration of β-cells. With insulin treatment (Fig. 1c) a moderate increase in the β-cells (50%) was observed while with SMC treatment (Fig. 1d) the increase was quite considerable (75%).

Insulin secretion in vitro—Exposure of β cells to SMC in a dose related manner of 0.5 and 1 mg/mL showed significant (P <0.001) insulin release activity as compared to STZ control (Table 3). Treatment with SMC at 0.5 and 1 mg/mL concentrations showed significant (P <0.001) increase in insulin release when compared to normal control. Insulin release was significantly inhibited when the RIN-5F cells were incubated with adrenaline (5 mM), and nifedipine (50 mM) respectively. Treatment with SMC at 0.5 and 1 mg/mL concentrations to adrenalin treated group showed significant increase in insulin secretion (P <0.001, <0.01 respectively). Also treatment with SMC at 0.5 and 1 mg/mL concentrations to nifedipine treated group showed significant increase in insulin secretion (P <0.001; Table 4). The percentage viability of RIN-5F cells after treatment with SMC as assayed by MTT assay was found to be 52% with 2 mg SMC (Table 4).

Discussion

Hypoglycemic herbs increase insulin secretion, enhance glucose uptake by adipose or muscle tissues

Table 3—Effect of nifedipine (50 μM) and adrenaline (5μM) on SMC (0.5 and 1 mg/mL) induced insulin release

[Values are mean±SE from 6 repetitions in each group]

Treatment group	Insulin release (μIU/mL)
Group 1: Normal control	5.58±0.02
Group 2: Cells treated with Streptozotocin (5 mM) - serves as Diabetic control.	1.09±0.06#
Group 3: Diabetic control + Glibenclamide (0.02 mg/mL).	7.18±0.03*
Group 4: Diabetic control + SMC (0.5 mg/mL).	4.74±0.05*
Group 5: Diabetic control + SMC (1 mg/mL).	6.90±0.06#
Group 6: Normal control + Adrenaline (5 μM)	2.14±0.05#
Group 7: Normal control + Nifedipine (50 μM)	1.10±0.06#
Group 8: Normal control + Adrenaline (5 μM) + SMC (1 mg/mL)	3.04±0.06*
Group 9: Normal control + Nifedipine (50 μM) + SMC (1 mg/mL)	4.10±0.02$

P values : <0.001when compared with #normal control (Gr. 1), *diabetic control group (Gr. 2), $nifedipine group (Gr. 7)

Table 4—Evaluation of cytotoxicity of SMC by MTT Assay.

[Values are mean±SE from 6 repetitions in each group]

Treatment	Dose (mg/mL)	Cell viability (%)
Control	0	100
SMC	6	1.5±0.57*
	4	25±0.01*
	2	51±0.042*
	1	99.1±0.021
	0.5	99.76±0.03
	0.25	99.82±0.09

**P* <0.001

and inhibit glucose absorption from intestine and glucose production from liver[16]. Insulin lowers the concentration of glucose in blood by stimulating the uptake and metabolism of glucose by muscle. An anti-hyperglycemic compound GII isolated from the water extract of seeds of fenugreek (*T. foenum-graceum*) reduced blood glucose in subdiabetic, moderately and severely diabetic rabbits at a dose of 50 mg/kg in glucose tolerance test (GTT)[17]. Also it decreased the elevated serum lipids TC, TG, (LDL+VLDL) and increased the decreased HDL, decreased the elevated liver and heart total lipids (TC and TG), increased the decreased liver and muscle glycogen, increased the decreased hexokinase, glucokinase, pyruvate kinase, malic enzyme, glucose-6-phosphate dehydrogenase, superoxide dismutase and glutathione peroxidase, decreased the increased glucose-6-phosphatase, sorbitol dehydrogenase and aldose reductase[18]. Crude powder, ethanolic extract and n-butanol and hexane fraction of *Pterocarpus marsupium* Roxb. showed marked decline in blood glucose level on STZ-induced diabetic rats, and four out of five phenolic C-glycosides isolated from n-butanol fraction of the ethanolic extract enhanced glucose uptake by skeletal muscle cells (C2C12) in a dose dependent manner[19]. Three orally active hypoglycemic compounds Kakara Ib (400 mg/kg), IIIa1 (100 mg/kg) and IIIb1 (300 mg/kg) isolated from the unripe fruits of *Momordica charantia* Linn (bitter gourd) improved glucose tolerance in subdiabetic (alloxan recovered) rabbits in acute (single dose) and subacute (10 days) studies[20]. Metformin and other biguanides have reported increase in the insulin-stimulated glucose uptake[21]. The enhanced glucose utilization by the hemidiaphragm suggests that SMC may have direct insulin like activity which enhances the peripheral utilization of glucose and have extra pancreatic effect[22]. *Helicteres isora* at 200 mg/mL dose showed glucose uptake activity and found to be active comparable with insulin and metformin[23].

Insulin secretion *in vitro* RIN-5F cell line was used to evaluate insulin secretion. Sulfonylureas function by stimulating insulin secretion. The net effect was increased responsiveness of β-cells (insulin secreting cells located in the pancreas) to both glucose and non-glucose secretagogues, resulting in more insulin being released. Korean red ginseng significantly stimulated insulin release from isolated rat pancreatic islets[24]. Bitter melon increased the mass of β-cells in the pancreas and insulin production[25,26]. In the present study there was significant increase in the release of insulin under the influence of SMC (1 mg/mL) when compared to the diabetic control. The mass of β-cells in the pancreas also significantly increased under the influence of SMC. In order to characterize the

insulinotropic activity, insulin antagonists were used to elucidate the mechanism of SMC evoked insulin secretion. One such inhibitor of insulin release is a catecholamine, adrenaline, which binds to α-adrenergic receptors and interferes with cAMP system or to alter calcium handling by the β-cells[27]. Postsynaptic α2-adrenergic receptors (α2-ARs) are present on pancreatic cells[28] and their selective stimulation inhibits glucose-induced insulin release[29]. In the cells of pancreas, inhibition of α2-ARs increases insulin secretion[30]. *In vitro* studies with yohimbine showed that the insulin secretion from the pancreatic islets increased significantly suggesting that when the α2-ARs are blocked, it enhances islet cell proliferation and insulin secretion[31]. Earlier studies reported that *M. cymbalaria* has α-adrenergic antagonizing activity[9]. In the present study insulin secretion decreased significantly in the presence of adrenaline whereas reversed in presence of adrenaline and SMC. Hence α-adrenergic antagonizing activity is suggested to be responsible for insulin secreting property of SMC. The voltage-dependent calcium channel blocker nifidepine significantly reduced insulin release providing evidence that Ca^{2+} plays a key role in mediating the effect. Consistent with this view, insulin secretory effects of SMC were abolished in absence of extracellular Ca^{2+}. Incorporation of SMC in the culture immediately provoked insulin secretion. Similar effect is reported for *Asparagus racemosus*[32] and *Ocium sanctum*[33].

These data support the hypothesis that the insulinotropic effect of SMC is due, at least in part, to modulation of calcium handling by the β-cells. Hence, SMC may exert stimulatory effect on insulin secretion from β cells via physiological pathways. Experimental data suggest that the saponin of *M. cymbalaria* possesses potential antidiabetic activity as it lowers blood glucose level and have shown to improve beta cell density. Stimulation of insulin secretion from RIN-5F cells was also observed in a dose dependent manner. In the presence of insulin antagonist (adrenaline) and calcium channel blocker (nifedipine), the saponin stimulated insulin secretion was significantly inhibited. These observations lead to the inference that the insulinotropic effect of saponin may be attributed to modulation of calcium handling by the β-cells.

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References

1 Li W L, Zheng H C, Bukuru J & De Kimpe N, Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus, *J Ethnopharmacol*, 92 (2004) 1.

2 Nadkarni A K, Nadkarni K M, Cucurbitaceae, in *Indian materia medica* (Popular Book Depot, Bombay) 1982, 755.

3 Kameswararao B, Kesavulu M M & Apparao C, Evaluation of antidiabetic effect of *Momordica cymbalaria* fruit in alloxan-diabetic rats, *Fitoterpia*, 74 (2003) 7.

4 Kameswararao B, Kesavulu M M & Apparao C, Antihyperglycemic activity of *Momordica cymbalaria* in alloxan diabetic rats, *J Ethnopharmacol,* 78 (2001) 67.

5 Kameswararao B, Kesavulu M M, Giri R & Apparao C, Antidiabetic and hypolipidemic effect of *Momordica cymbalaria* Hook fruit powder in alloxan-diabetic rats, *J Ethnopharmacol* 67 (1999) 103.

6 Raju K & Balaraman R, Antidiabetic mechanisms of saponins of *Momordica cymbalaria*, *Phcog Mag,* 4 (2008) 160.

7 Koneri R, Balaraman R & Saraswati C D, Antiovulatory and abortifacient potential of the ethanolic extract of roots of *Momordica cymbalaria* Fenzl in rats, *Indian J Pharmacol,* 38 (2006) 111.

8 Koneri R, Saraswati C D, Balaraman R & Ajeesha E A, Anti-implantation activity of the ethanolic root extract of *Momordica cymbalaria* Fenzl in rats, *Indian J Pharmacol,* 9 (2007) 90.

9 Koneri R, Balaraman R, Hariprasad, Vinoth Kumar M & Ali A, Cardioprotective effect of *Momordica cymbalaria* Fenzl in rats with isoproterenol-induced myocardial injury, *J Clin Diagnos Res*, 2 (2008) 699.

10 Swamy V B M & Jayaveera K N, Antimicrobial properties of *Momordica cymbalaria* Hook, *Pharmacologyonline*, 3 (2007) 505.

11 Sharada L D & Somshekhar S K, Isolation and characterization of phytoconstituents from *Chlorophytum borivilianum, Pharmacognosy Res*, 2 (2010) 343.

12 Kade I & Barbosa N, Aqueous extracts of *Sphagneticola trilobata* attenuates streptozotocin-induced hyperglycemia in rat models by modulating oxidative stress parameters, *Biol Med*, 2 (2010) 1.

13 Okine L & Nyarkb N. The antidiabetic activity of the herbal preparation ADD-199 in mice: a comparative study with two oral hypoglycaemic drugs. *J Ethanopharmacol*, 97 (2005) 37.

14 Freshney Ian R, Cytotoxicity. *Culture of animal cells: A manual of basic technique*, fifth edition (John Wiley & Sons, Inc.) 2005, 359.

15 Hamdan N, Stephen J & Ashcrot, Pharmacological chracterisation of the antihyperglycemic properties of *Tinospora crispa* extract, *J Ethanopharmacol*, 62 (1998) 7.

16 Hongxiang H, George T, & Liang V W G, Hypoglycemic herbs and their action mechanisms, *Chin Med*, 4 (2009) 11.

17 Moorthy R, Prabhu K M & Murthy P S, Anti-hyperglycemic compound (GII) from fenugreek (*Trigonella foenum-graecum* Linn.) seeds, its purification and effect in diabetes mellitus, *Indian J Exp Biol*, 48 (2010) 1111.

18 Puri D, Prabhu K M, Dev G, Agarwal S & Murthy P S, Mechanism of antidiabetic action of compound GII purified from Fenugreek (*Trigonella foenum graecum*) seeds, *Indian J Clin Biochem*, 26 (2011) 335.

19 Mishra A, Srivastava R, Srivastava S P, Gautam S, Tamrakar A K, Maurya R & Srivastava A K, Antidiabetic activity of heart wood of *Pterocarpus marsupium* Roxb. and analysis of phytoconstituents, *Indian J Exp Biol*, 51 (2013) 363.

20 S. Pugazhenthi S & Murthy P S, Purification of three orally active hypoglycemic compounds Kakara Ib, IIIa1 and IIIb1 from the unripe fruits of *Momordica charantia* Linn (Bitter gourd), *Indian J Clin Biochem*, 11 (1996), 115.

21 Gilman A G, Hardman J G, & Limbard L E, Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. *The pharmacological basis of therapeutics* 10th ed. (McGraw-Hill Medical Publishing Division) 2001, 1679.

22 Chattopadhyay R R, Sarkar S K, Ganguly S, Banerjee R N, & Basu T K, Effect of extract of leaves of *Vinca rosea* Linn on glucose utilization and glycogen deposition by isolated rat hemidiaphragm, *Indian J Physiol Pharmacol*, 36 (1992) 137.

23 Chakrabarti R, Vikramadithyan R K, Mullangi R, Sharma V M, Jagadheshan H, Rao Y N, Sairam P & Rajagopalan R, Antidiabetic and hypolipidemic activity of *Helicteres isora* in animal models, *J Ethnopharmacol*, 81 (2002) 343.

24 Kim K & Kim H Y, Korean red ginseng stimulates insulin release from isolated rat pancreatic islets, *J Ethnopharmacol*, 120 (2008) 190.

25 Shetty A K, Kumar G S, Sambaiah K & Salimath P V, Effect of bitter gourd (*Momordica charantia*) on glycaemic status in streptozotocin induced diabetic rats, *Plant Foods Hum Nutr*, 60 (2005) 109.

26 Chao C Y & Huang C, Bitter gourd (*Momordica charantia*) extract activates peroxisome proliferator-activated receptors and upregulates the expression of the acyl coA oxidase gene in H4IIEC3 hepatoma cells, *J Biomed Sci*, 10 (2003) 782.

27 Schwartz D D, Activation of alpha-2 adrenergic receptors inhibits norepinephrine release by a pertussis toxin-insensitive pathway independent of changes in cytosolic calcium in cultured rat sympathetic neurons, *J Pharmacol Exper Therp*, 282 (1997) 248.

28 Ruffolo R R Jr, Nichols A J, Stadel J M & Hieble J P, Pharmacologic and therapeutic applications of alpha 2-adrenoceptor subtypes, *Annu Rev Pharmacol Toxicol*, 33 (1993) 243.

29 Kurose T, Seino Y, Nishi S, Tsuji K, Taminato T, Tsuda K & Imura H, Mechanism of sympathetic neural regulation of insulin, somatostatin, and glucagon secretion, *Am J Physiol*, 258 (1990) 220.

30 Hsu W H, Yohimbine increases plasma insulin concentrations and reverses xylazine-induced hypoinsulinemia in dogs, *Am J Vet Res*, 49 (1988) 242.

31 Naghadeh M M, Moghadam F H & Ibrahimi H, Effects of yohimbine on plasma levels of leptin in normal and streptozotocin induced diabetic rats, *Acta Medica Iranica*, 44 (2006) 77.

32 Hannan J M, Marenah L, Ali L, Rokeya B, Flatt P R & Abdel-Wahab Y H, Insulin secretory actions of extracts of *Asparagus racemosus* root in perfused pancreas, isolated islets and clonal pancreatic beta cells, *J Endocrinol*, 192 (2007) 159.

33 Hannan J M, Marenah L, Ali L, Rokeya B, Flatt P R & Abdel-Wahab Y H, *Ocimum sanctum* leaf extracts stimulate insulin secretion from perfused pancreas, isolated islets and clonal pancreatic beta-cells, *J Endocrinol*, 189 (2006) 127.