Triterpenoid Saponin from *Momordica tuberosa* (Cucurbitaceae) Stimulates Insulin Secretion from Isolated Mouse Pancreatic Islets and Provides Protection from Streptozotocin and High-glucose Induced Injury

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ABSTRACT

Background: *Momordica tuberosa*, belonging to the family Cucurbitaceae, is a tropical plant that is widely cultivated in the southern states of India. **Objectives:** *Momordica tuberosa* possesses a saponin, triterpenoid in nature that has been reported to exhibit anti-hyperglycaemic activity *in vivo* and *in vitro*. In this study we intend to isolate the saponin in pure purified state, assess its anti-hyperglycaemic activity on isolated mice pancreatic islets and evaluate its protective role against streptozotocin and high-glucose induced islet injury, a central event in the pathogenesis of uncontrolled diabetes. **Methods:** Saponin purification and identification was carried out using chromatography followed by its structure elucidation IR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometry. Islets were obtained from mice employing the pancreas perfusion method with collagenase type XI. Islet viability and specificity were determined by trypan blue-DTZ double staining followed by insulin secretion and intra-islet content measurement by ELISA. STZ-induced oxidative damage was assessed by lipid peroxidation and nitric oxide assay. High glucose-induced islet cell death/viability was assessed by MTT assay. **Key findings:** Approximately 650 islets per pancreas were recovered after isolation. The islets retained normal membrane integrity, viability and glucose-stimulated insulin secretion (GSIS). The saponin stimulated secretion of insulin from the isolated islets, protected from STZ- and high glucose-induced cytotoxicity and also from oxidative stress. **Conclusion:** The saponin acted as an insulin secretagogue and protected the islets from cytotoxic and oxidative stress induced by STZ and high glucose. We thus conclude that the saponin may be considered for effective use in IDDM.

Key words: Saponin, *Momordica tuberosa*, Insulin secretagogue, Islet viability, High glucose-induced cytotoxicity.

INTRODUCTION

The islets of Langerhans of the pancreas secrete hormones that are indispensable for regulation of blood glucose and hence, a crucial emphasis of diabetes research. Isolating viable and functional islets in purified form is an intricate process and a prerequisite in carrying out further diabetes investigations. The overall classical procedure to isolate islets from rodent pancreas includes three steps: collagenase perfusion of the pancreas, digestion and purification. Numerous factors, ranging from the collagenase type, its purity and digestion protocol including time, temperature and agitation, to purification process, contribute to poor and inconsistent islet yield across laboratories.[1,2] Variations in functionality and viability of the isolated islets are also recurrent. Pancreatic perfusion with collagenase via the common bile duct post duode-

nal occlusion ensures complete separation of exocrine tissue from the islets.[3]

The use of natural products in modern medicine even though widespread in curing or preventing diseases lacks scientific evidence in most cases as to whether it is to be used as a plant or its active constituents.[4-6] Many medicinal plants have attained importance as treatments of diabetes mellitus. However, few have enticed scientific or medical scrutiny as the WHO's recommendation of treating diabetes with medicinal plants will require further scientific evaluation.[7]

One of the medicinal plants that has been widely explored for its antidiabetic potential is *Momordica charantia* (Bitter melon or bitter gourd) belonging to the family Cucurbitaceae. It is a tropical plant that is widely cultivated in Asia, India, East Africa and South America for its intensely bitter fruits that are commonly used in cooking and as natural remedy for treating diabetes.[8-10] *Momordica charantia* has been found to reduce STZ-induced apoptosis in mice pancreas, RIN5f insulinoma cells and islets. It has also found to stimulate insulin release from islets isolated from obese-hyperglycaemic mice.[11] Another species of the same genus, *Momordica cymbalaria*, has also been found to possess hypoglycaemic activity in alloxan[12] and streptozotocin-induced diabetic animals.[13] We have reported previously the presence of a triterpenoid saponin that was accountable for the hypoglycaemic activity and stimulated the release of insulin from RIN5f rat insulinoma cells. When diabetic rats were treated with the saponin it was found to induce the formation of new beta cells in the islets which were initially destroyed by STZ.[13] However, investigation on the activity of its saponin on isolated rodent islets has never been attempted.

In the present study we have carried out islet isolation from mice pancreas and explored the influence of the saponin of *Momordica cymbalaria* on the functionality, viability and oxidative status of the isolated islets maintained in various simulated diabetic conditions.

MATERIALS AND METHODS

Saponin Isolation and Characterization

Tubers of *Momordica cymbalaria* were collected from Southern India and identified by the Department of Botany, Bangalore University, India. A specimen was preserved in the herbarium of the department bearing voucher No. 18122003 for reference. Each 100 g powder was subjected to extraction with 1000 mL methanol with reflux for 3 cycles of 7 hrs each, extract filtered and evaporated to dryness. Flash chromatography (CombiFlash®R$_f$, Teledyne Technologies Inc., ISCO,

USA) of extract was performed and eluted with methanol: chloroform (80:20) with a flow rate of 20 mL/min using Redisep® Silica preparative column attached to a fraction collector. Eluted saponin fraction was evaluated by HPTLC (Camag, Switzerland) with Linomat 5 applicator on 10 cm×10 cm silica gel 60-GF$_{254}$ plates (Merck). Ursolic acid (a triterpenoid) was used as a standard (U6753-100MG, Sigma). Mobile phase was chloroform: methanol::95:5 (v/v). The dried plate was derivatized by anisaldehyde-sulphuric acid spraying and heated at 105°C for 5 mins. The bands were visualized in a UV scanner (Camag TLC Scanner III) at 560 nm. The fraction was further chromatographed in reverse-phase HPLC (Agilent 1120 LC) using a C$_{18}$ column (5 µm), UV detector and eluted with methanol: chloroform (80:20) with a flow rate of 1 mL/min against ursolic acid standard (U6753-100MG, Sigma). The ^{1}H-(400 MHz) and ^{13}C-(100 MHz) NMR spectra were recorded on a Bruker-AV-400 NMR spectrometer at room temperature in MeOD and DMSO respectively with TMS acting as an internal standard. IR spectrum was obtained with a Bruker Alpha-E series FT-IR spectrophotometer with ATR. High resolution mass spectral data were obtained with an Agilent 6500 series Q-TOF mass spectrometer using electrospray ionization.

Islet Isolation, Purification and Culture

An overnight fasted Swiss albino mouse of weight not exceeding 22 g[14,15] was anaesthetized followed by cervical dislocation. Mouse islets were isolated following pancreas perfusion method post Common bile duct (CBD) occlusion.[2,3] Briefly, 3 ml ice-cold collagenase type XI (Sigma, USA) solution (1 mg/ml) in Hank's balanced salt solution (2% BSA fraction V, 20 mM HEPES, 2 mM CaCl$_2$.2H$_2$O and penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (2.5 µg/ml; GIBCO, Life technologies, NY, USA) was injected through the CBD into the pancreas. The inflated pancreas was gently dissected out and incubated in an additional 2 ml collagenase type XI solution in water bath at 37°C for 15 min with brief hand shaking for 2-3 times. A 100 µl aliquot of the digestion mixture was withdrawn at a regular interval (5 min) and observed under the microscope to monitor the digestion process.[15] The complete detachment of exocrine tissues from the islets indicates complete digestion. The tube was plunged immediately into ice to stop digestion. The HBSS was removed by centrifuging at 1000 rpm for 10 min and the pellet was resuspended in 10 ml warm RPMI (Gibco, Life Technologies, NY, USA). The digested pancreas is filtered through a sterile stainless steel mesh (500 µm pore size). Digested pancreas was obtained by centrifugation after

removing the supernatant and re-suspended gently in 10 ml of Histopaque® 1077 (Sigma, USA) at room temperature. This was then overlaid with 5 ml RPMI 1640 at room temperature extremely gently forming a clear and sharp interface between the two liquids. The tube was centrifuged at 850 x g for 20 min with brakes off. The islets were recovered by centrifugation and resuspended in fresh RPMI 1640. A final purification step was incorporated where the islets obtained were passed through a pre-wetted, inverted polypropylene 70 μm cell strainer (Cat. No. CLS431751; Corning, USA). It was re-washed with fresh medium and turned upside down over a new petri dish containing 15 ml of fresh RPMI to rinse off the captured islets. This method eliminates the exocrine cells leaving behind only islets. The islets were incubated in RPMI 1640 with 10% FBS at 37°C in 5% CO_2 in a CO_2 incubator and observed after 48 h. Healthy islets with smooth borders and no dark center were handpicked and plated freshly in RPMI 1640 complete medium.

Assessment of Specificity, Viability and Functionality of Isolated Islets

Islet viability was assessed by Trypan Blue Dye Exclusion Test and specificity was determined by Dithizone (DTZ) staining (Sigma, USA) simultaneously (Double staining). In the trypan blue dye exclusion assay islets were exposed to the membrane-impermeant dye, trypan blue (0.1% w/v) for 15 min at 37°C. For specificity, 10 μl of DTZ stock solution (10 mg/ml in DMSO) was added to islets suspended in 1 mL Krebs-Ringer Bicarbonate buffer (KRB; 115 mM NaCl, 4.7 mM KCl; 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24 mM $NaHCO_3$, 10 mM HEPES, 1 g/L BSA, 1.1 mM glucose; pH 7.4) and incubated at 37°C for 30 min. For the assessment of functionality, Glucose-stimulated insulin secretion (GSIS) assay was performed. Islets were seeded at a concentration of 50 islets per well in 12-well plates (Corning, USA), washed thrice with KRB buffer and pre-incubated for 1 h at 37°C. The islets were then maintained in glucose-free, normal glucose (5 mM) and high glucose (16.7 mM) in KRB and incubated for 1h at 37°C. After incubation, aliquotes of 10 μL were withdrawn from each well and assayed for insulin by sandwich ELISA using a commercial kit (cat. No. 10-1250-01, Mercodia, Sweden).[16]

Effect of *Momordica cymbalaria* Saponin on Insulin Secretion from Cultured Islets

To evaluate the drug-stimulated insulin secretion, the handpicked mature islets cultured in RPMI 1640 with 11.1 mM glucose, 10% FBS and antibiotics (100 IU/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml

amphotericin B) were seeded at a concentration of 50 islets per well in 12-well plates, washed thrice with KRB buffer and treated with 5 mM streptozotocin (Sigma, USA) in PBS (pH 7.4) and saponin (1, 50 and 100 μg/ml) concentrations. Normal (5.5 mM) and high glucose-treated (16.7 mM) islets were also maintained in KRB. The islets were pre-incubated at 37°C in 5% CO_2 atmosphere for 1h in glucose-free KRB for base line correction for insulin secretion. After treatment they were further incubated for 1h and the supernatant from each well was assayed for insulin by sandwich ELISA.

Measurement of Released and Intra-Islet Insulin after Treatment with STZ and Saponin

As previous, 50 islets per well after incubation with STZ in the presence or absence of saponin (1, 50 and 100 μg/ml) were assayed for released insulin in the supernatant. For the estimation of intra-islet insulin, islets from each well were collected and washed 2-3 times with ice-cold KRB followed by lysis with RIPA buffer (Sigma, USA). The lysate was centrifuged at 10,000 rpm at 4°C and the supernatant was assayed for intra-islet insulin.

Effect of the Saponin on STZ-Induced Lipid Peroxidation and Nitric Oxide Formation

Formation of Malondialdehyde (MDA) is an index of lipid peroxidation. Malondialdehyde levels in STZ and saponin treated islets were measured by the Thiobarbituric acid (TBA) method as reported previously (Konings and Drijver).[17] The assay mixture contained - 0.5 ml cell lysate (50 islets), 1ml of 0.5 M KCl in 10 mM Tris-HCl, 0.5 ml of 30% Trichloroacetic acid (TCA) and 0.5 ml of 52 mM Thiobarbituric acid (TBA). The assay mixture was heated to 80°C for 30 min and after cooling to 0°C centrifuged at 800xg for 10 min. The absorbance of the supernatant was measured at 532nm (Grouping of cells same as previous). Levels of NMA were calculated.[18] Nitric oxide produced during STZ and saponin treatment was estimated spectrophotometrically as a formed Nitrite (NO_2). To measure the nitrite content, 100 μl of the cell lysate was incubated with 100 μl of Griess reagent (1% sulfanilamide in 0.1 mol/l HCl and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) at room temperature for 10 min. Then the absorbance was measured at 540 nm using a microplate reader. The nitrite content was calculated based on a standard curve constructed with $NaNO_2$.[19]

Effect of the Saponin on High Glucose-Induced Oxidative Stress on Isolated Islets

Islets were seeded at a concentration of 50 islets per well in 12-well plates as per the following grouping: Group I: Normal control - islets maintained in normal glucose

(5.5 mM); Group II: High glucose control - islets maintained in high glucose (31 mM); Group III, IV and V: Saponin (1, 50 and 100 μg/ml)-treated islets maintained in high glucose (31 mM). The islets were incubated for 24 h at 37°C in 5% CO_2. After incubation the islets were lysed, as previous and the lysate was used to estimate the formation of NO and MDA (Lipid peroxidation).

Cell Viability

The viability of islets after treatment, as per previous grouping and incubation, was determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.[20] Briefly, islets per well (12-well plate) were rinsed with pre-warmed 0.5 mM Ca^{2+}, Mg^{2+}-free Dulbecco's PBS (DPBS) with 0.02% EDTA (Sigma, USA) and incubated with a recombinant fungal trypsin-like cell dissociation enzyme (TrypLE Express; Gibco, Denmark) in a CO_2 incubator for 2 min. The islets were gently pipetted in and out to ensure complete dissociation yielding a single cell suspension.[21] The cells were plated on to a 96-well plate and incubated with 50 μl MTT (5 mg/ml) solution per well for 4 h at 37°C in a CO_2 incubator. After incubation 200 μl DMSO was added per well to dissolve the formazan crystals formed. The absorbance of the plate was read at 570 nm in a plate reader and the percentage viability was calculated.

Statistical Analysis

Results were expressed as mean ± SD and all statistical comparisons were made by means of one-way ANOVA test followed by Tukey's multiple comparison test (Graph Pad Prism v5.0). *P*-value < 0.05 was considered to indicate a statistically significant difference.

RESULTS

Saponin Isolation and Characterization

The Chromatogram Obtained after Produced Identical Pattern

HPTLC of the saponin fraction (Figure 1A) revealed its near identical retention factor (R_f = 0.69) to that of the ursolic acid standard (R_f = 0.68) (Figure 1B and 1C). A single spot with identical R_f as that of the standard indicates isolation of the saponin in pure form [Lane 1 = ursolic acid (L1); Lane 2 = saponin fraction (L2)]. The reverse-phase HPLC chromatograms of ursolic acid and saponin are shown in Figure 2A and 2B. The retention time (R_t) of ursolic acid is 4.723 min and the isolated saponin is 4.727 min. The retention pattern of the saponin matches with that of the standard which indicates its similarity with the standard (Ursolic acid). The physical and spectroscopic properties of the compound

were the following: buff crystals; FT-IR (Zn-Se, cm^{-1}): 3374.93 (OH), 1732.90 (C=O ester), 1713.67 (C=O ketone), 1246.93 and 1081.43 (C-O ester); EI-mass value at m/z M+: 468; ^{1}H and ^{13}C NMR assignments (Table 1). By comparing the obtained spectroscopic data with published data,[22] this compound is identified as an oleanane-type triterpenoid saponin - 3,12-dioxo-8-nor-oleane-9(11)-ene-28-oic acid methyl ester (Figure 3).

Specificity, Viability and Functionality of Isolated Islets

The islets isolated were of high purity (Free of contaminating exocrine cells) and long term viability (up to 72 h). Approximately 650 healthy islets per pancreas were recovered after 48 h incubation with smooth rounded surface (Figure 4) and were employed in further experiments. Islets stained with dithizone appeared reddish-brown (Figure 5A) confirming their identity. In double staining with trypan blue and dithizone simultaneously healthy islets stained reddish-brown with dithizone while the dead and membrane-compromised exocrine cells stained blue with trypan blue in the same view field (Figure 5B). The islets responded normally in Glucose-stimulated insulin secretion (GSIS) assay secreting 103.9 and 134.9 μIU/mL insulin when challenged with 5.5 (Normal) and 16.7 (high) mM glucose against islets maintained without added glucose (Glucose-free control) secreting 21.68 μIU/mL insulin (Figure 6). The results, thus, indicate the functional wellbeing of the isolated islets.

Effect of Saponin on Insulin Secretion from Cultured Islets

The saponin of *Momordica cymbalaria* acted as an insulin secretagogue stimulating its secretion from the islets. Treatment with STZ reduced insulin secretion when compared to normal control as STZ destroys the β-cells irreversibly. The incubation of islets with 1, 50 and 100 μg/ml of saponin increased insulin secretion in a dose dependent manner and significantly when compared with STZ control (*p*<0.05) and glucose controls (5.5 and 16.7 mM) respectively (Figure 7).

### Intra-Islet and Released Insulin Levels after Treatment with STZ and Saponin

The release of insulin from the islets was suppressed (12 folds) significantly as compared to normal glucose control (*p*<0.05) upon treatment with STZ. Islets treated simultaneously with STZ and the saponin (1, 50 and 100 μg/ml) did not exhibit any reduction in insulin secretion while, on the contrary, had a stimulatory effect on it increasing insulin levels by more than 10 and 12 folds respectively (Figure 8). The results indicate that

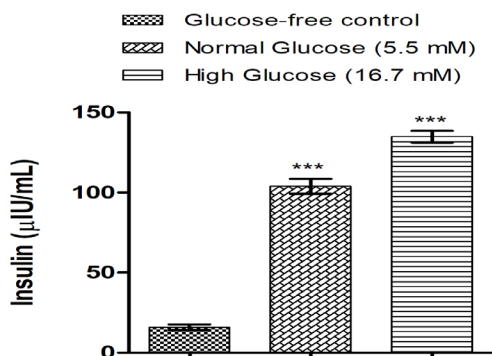| Table 1: ¹H and ¹³C NMR chemical shifts (δ, ppm) of saponin in MeOD at 298 K with TMS as internal standard.		
Position	δ C	δ H
1	30.097	1.072
2	28.052	1.612
3	176.553	-
4	35.859	-
5	48.830	0.802
6	22.112	1.271
7	35.684	1.702
8	35.480	1.091
9	140.882	-
10	38.564	-
11	144.790	4.824
12	178.017	-
13	39.081	1.769
14	42.175	-
15	28.642	1.900
16	24.519	2.035
17	44.898	-
18	49.229	3.300
19	34.036	1.670
20	30.194	-
21	31.248	1.312
22	31.708	2.820
23	28.940	0.889 (s)
24	17.559	0.914 (s)
25	14.572	1.197 (s)
26	18.485	1.220 (s)
27	20.895	0.770 (s)
28	22.476	1.134 (s)
29	173.523	-
30	66.069	3.957

the deleterious effect of STZ on the β-cells was somehow neutralized by the saponin. The intra-islet insulin content of islets treated with STZ was found to be considerably low as compared to the normal control group which can be attributed to the fact that the β-cell mass was destroyed by streptozotocin. Intra-islet insulin content of STZ + saponin treated groups was significantly
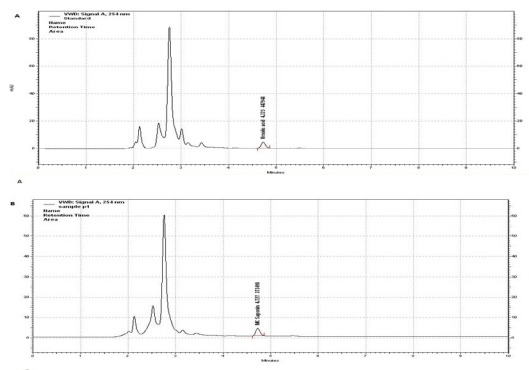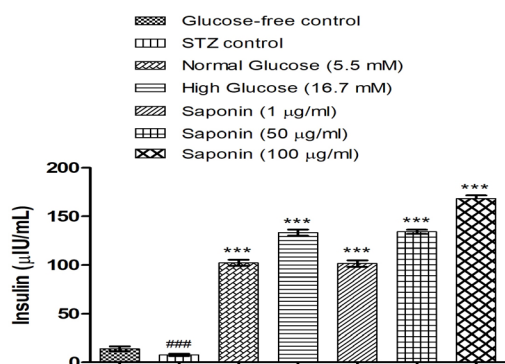

Figure 2: Reverse-phase HPLC chromatograms of ursolic acid standard, R$_t$ 4.723 min (A) and isolated saponin from *Momordica cymbalaria*, R$_t$ 4.727 min (B).
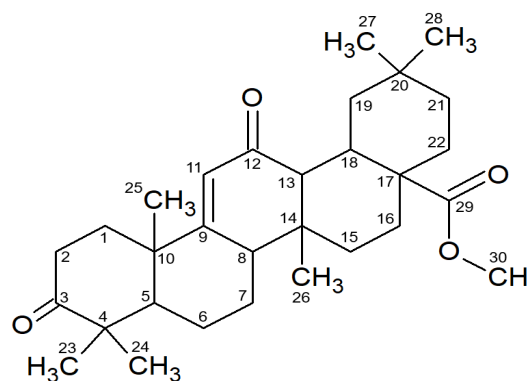
Figure 3: Chemical Structure of the Saponin Isolated from *Momordica cymbalaria*.
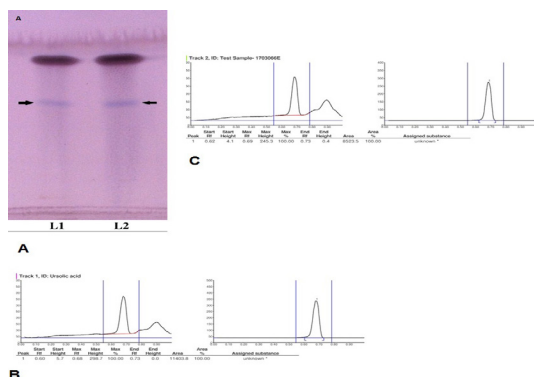
Figure 1: High Performance Thin Layer Chromatography (HPTLC) of saponin fraction collected through flash chromatography (Lane 2) against ursolic acid standard (Lane 1) (A), densitometric scans of ursolic acid standard (B) and saponin (C) at 560 nm.
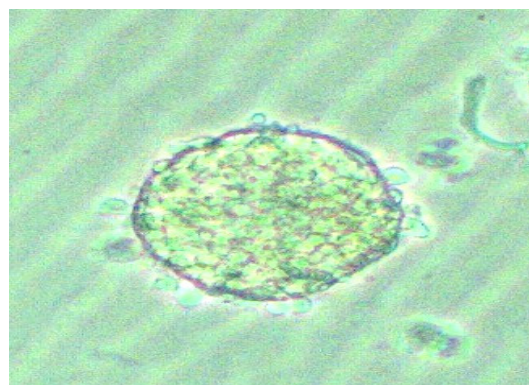
Figure 4: A healthy islet recovered after cell straining, density gradient centrifugation and handpicking. A smooth and uniform boundary without central necrosis (dark centre) after 48 h is evident (magnification ×20).
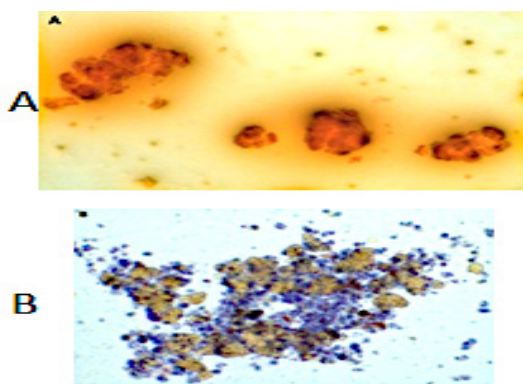
Figure 5: Specificity and viability of isolated islets. (A) Islets stained with dithizone (DTZ) appear reddish brown, (B) Islets double stained with DTZ and trypan blue where the viable islets appear reddish-brown with DTZ while the contaminating, dead exocrine cells stained blue with trypan blue (magnification ×20).
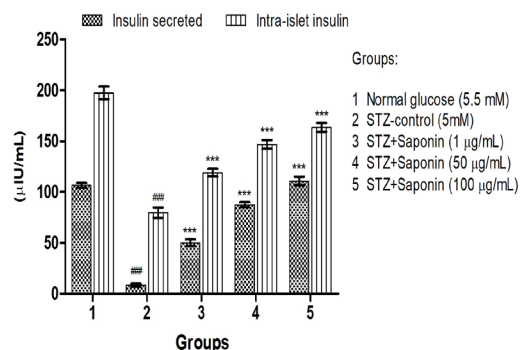

Figure 8: Measurement of insulin from islets treated with STZ and saponin (1, 50 and 100 µg/ml). Insulin released from the islets and estimated in cell culture supernatant and intra-islet insulin content measured by lysing the islets after incubation. Values are expressed as Mean ± SEM (*n*=6). ###*p*<0.05 compared with the normal control, ***p*<0.05 compared with the STZ control.

Figure 6: Glucose-stimulated insulin secretion (GSIS) from the islets. The islets responded to glucose stimulation (5.5 and 16.7 mM) by secreting insulin in a dose dependent fashion. Values are expressed as Mean ± SEM (*n*=4). ***p*<0.05 compared with the glucose-free control.

Effect on STZ-Induced Lipid Peroxidation and Nitric Oxide Formation
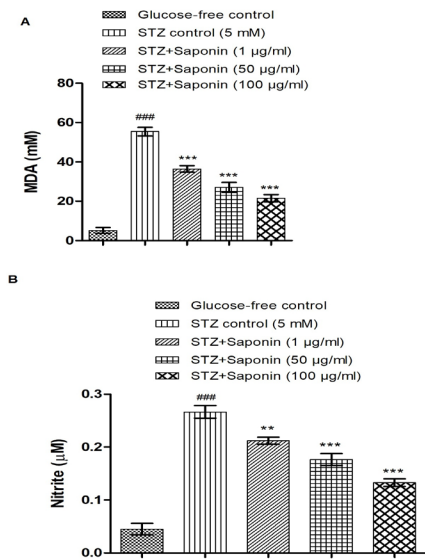
Islets exposed to 5 mM STZ for 1 h at 37°C exhibited high levels of lipid peroxidation (MDA levels) as compared to the untreated islets. However, islets exposed concomitantly to STZ and saponin (1, 50 and 100 µg/ml) exhibited reduction in MDA levels by more than 1.5, 2 and 2.5 folds (Figure 9A). A significant Nitrite (NO_2) formation (a Stable oxidized product of NO) was evident in islets treated with STZ as compared to untreated islets (Figure 9B). However, upon treatment with the saponin, nitrite levels were lowered by 1.25, 1.5 and 2 folds.

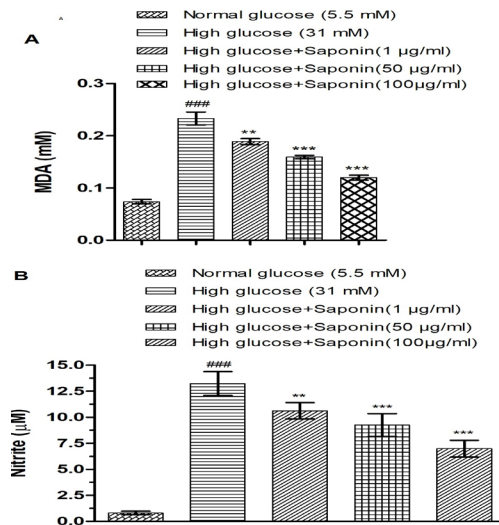## Effect in High Glucose-Induced Oxidative Stress on Isolated Islets

The formation of MDA and nitrite were found to be increased significantly in the high glucose (31 mM) controls as compared to the normal glucose (5.5 mM) islet (p<0.05) thereby exerting ample oxidative stress on the islets (Figure 10A and 10B). However, with saponin treatment, MDA and nitrite formation was reduced considerably indicating its protective effect in high glucose-induced oxidative stress.
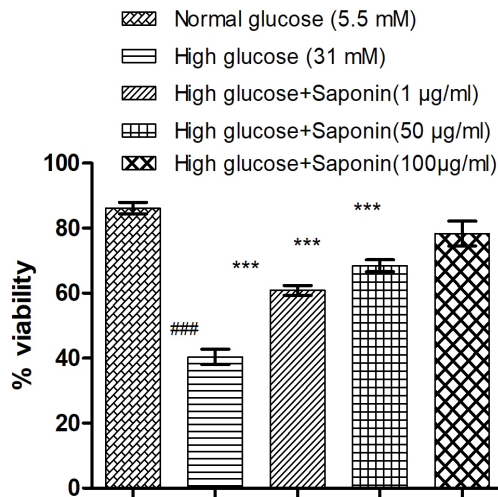
Islet Viability

The viability of islets exposed to high glucose (31 mM) was evaluated by the reduction of MTT to purple formazan. The viability of islets was found to be significantly less in the group exposed to high glucose (31 mM) as compared to the normal glucose control (p<0.05). However, saponin treatment was found to reverse this glucose toxicity in islet groups III, IV and V. A dose dependent increment in cell viability was observed indi-

Figure 7: Insulin secretion from islets under the stimulation of glucose (5.5 and 16.7 mM) and saponin of *Momordica cymbalaria* (1, 50 and 100 µg/ml). The saponin stimulated insulin secretion from the islets in a dose dependent manner. Values are expressed as Mean ± SEM (*n*=6). ###*p*<0.05 compared with the normal control, ***p*<0.05 compared with the STZ control.

found to be augmented as compared to the STZ group (p<0.05).

Figure 9: Effect of saponin on STZ-induced (A) lipid peroxidation and (B) NO production in isolated islets. An overall reduction in lipid peroxidation and NO formation was observed after saponin treatment. Values are expressed as Mean ± SEM (*n*=6). ###*p*<0.05 compared with the glucose-free control, ***p<0.05, **p<0.5 compared with the STZ control.

Figure 10: Effect of saponin on high glucose-induced (A) lipid peroxidation and (B) NO formation in isolated islets. A protection from the oxidative stress on islets induced by high glucose (31 mM) is evident upon saponin treatment. Values are expressed as Mean ± SEM (*n*=6). ###*p*<0.05 compared with the normal glucose control, ***p<0.05, **p<0.5 compared with the high glucose control.

Figure 11: Effect of saponin on viability of islets exposed to high glucose. Values are expressed as Mean ± SEM (*n*=6). ###*p*<0.05 compared with the normal glucose control, ***p<0.05 compared with the high glucose control.

cating the protective activity of the saponin in a simulated diabetic condition (Figure 11).

DISCUSSION

The ability to isolate islets from the pancreas enables investigators to use them in a number of downstream applications. In the present study we have attempted islet isolation from mouse pancreas through pancreatic perfusion and distention with Collagenase Type XI via the Common bile duct (CBD) after duodenal clamping yielding healthy islets. Perfusing the pancreas allows collagenase to access the islets using anatomical structures and stationary digestion reduces mechanical damage to the islets.[23-25] Purifying islets from the acinar tissue is of paramount importance as it secretes various digestive enzymes which is achieved though density gradient centrifugation.[26] Besides density gradient centrifugation we have introduced filtration steps through 500 μm mesh as well as 70 μm polypropylene cell strainer to completely eliminate the remnant contaminating exocrine cells.[27] Islets recovered after handpicking were round with smooth and uniform boundary and without central necrosis after 48 h demonstrating decent specificity and viability through dithizone and trypan blue staining. The islets were also found to respond appropriately to GSIS assay, thereby qualifying for the functionality, specificity and viability assessments.

Medicinal plants have always gained ethnopharmacological importance in the management and treatment of diabetes. The traditional knowledge of such plants has been substantiated with modern molecular-level experiments where the exact cellular level implications of these phytoconstituents have been accurately elucidated. *Momordica cymbalaria* has been found to possess hypoglycaemic activity in alloxan and streptozotocin-induced diabetic animals.[12,13] We have shown in this study that the saponin isolated is an oleanane-type triterpenoid and oleanolic acid has already been reported to

possess anti-hyperglycemic activity in alloxan-induced diabetic mice.[28] In an earlier study we have reported that a triterpenoid saponin of it possesses insulin secretagogue activity and induced β-cell neogenesis in the islets of rats that were initially destroyed by STZ treatment.[13] However, such studies on isolated pancreatic islets were never attempted which, for the first time, is reported by us. We have shown that the saponin has stimulated the secretion of insulin from islets significantly when compared to STZ treated islets which is in absolute agreement with the GSIS activity of soybean isoflavone genistein.[29,30] We further show that simultaneous exposure of islets to STZ and the saponin (1, 50 and 100 μg/mL) augmented insulin secretion by ten and twelve folds unlike the STZ treated islets. Similar results were obtained when islets treated concomitantly with STZ and bitter gourd fruit juice.[31] The intra-islet insulin content was found to increase gradually in a dose-dependent fashion as opposed to the STZ control. This is suggestive of a hypothesis that the beta cell mass remained unaffected by STZ in the presence of the saponin. Although it is known that the cytotoxicity produced by STZ depends on DNA alkylation, evidence indicate that free radicals also play an essential role in DNA damage.[32-34] Streptozotocin is a Nitric oxide (NO) donor and was found to bring about the destruction of pancreatic islet cells contributing to STZ-induced DNA damage.[35,36] Nitric oxide, once generated, initiate peroxidation of polyunsaturated lipids that are components of biological membranes thereby destroying them.[37] Nitric oxide has also been found to mediate cytokine-induced inhibition of insulin secretion by human pancreatic islets.[38] We have explored the detrimental effect of STZ on islets exposed simultaneously to the saponin. Our study revealed that saponin treatment suppressed the formation of Malondialdehyde (MDA), an index of lipid peroxidation and nitric oxide as opposed to the STZ treated islets.

Finally, we assessed the effect of high glucose on isolated islets cultured in the presence of the saponin. It is well-known that high glucose induces oxidative stress on islets leading to widespread effects like protein carbonylation,[39] ROS formation,[40] lipid peroxidation,[36] DNA damage[41] and apoptosis.[42] In agreement to this, we found that islets exposed to high glucose showed increased formation of MDA and nitric oxide which, on the contrary, were reduced in islets treated with the saponin. It was also revealed that viability of islets in high glucose was reduced by almost two folds. High glucose has been found to induce apoptosis in pancreatic islets by overexpressing the proapoptotic genes Bad, Bid and Bik.[43] However, the viability of islets treated with the saponin remain unaffected, as opposed to the high glucose group, as almost 70% islets were found viable. In a different *in vivo* study, we have reported that the pancreatic islet mass had regenerated in the saponin-treated STZ diabetic rats.[13] This *in vivo* finding correlates precisely with the present data obtained from the experiments on isolated islets. The results indicate that the saponin could protect the islets from the deleterious consequences of high glucose, thus proving our hypothesis.

CONCLUSION

The saponin of *Momordica cymbalaria* stimulated insulin secretion from the islets, provided protection from STZ-induced cytotoxicity and oxidative stress and also from that of experimental high glucose-induced toxicity that mimics the pathological state of diabetes. While future work is required to study the influence of the saponin on the expression of different pro- and anti-apoptotic proteins to get an insight into how exactly the cytoprotection is imparted, the present results raised the possibility of exploring the saponin in the diseased state of diabetes, after meticulous clinical trials.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this work.

ABBREVIATIONS

DTZ: Diethylthiocrabazone; **STZ:** Streptozotocin; **ELISA:** Enzyme-linked Immunosorbent assay; **IDDM:** Insulin-dependent diabetes mellitus; **HPLC:** High Performance Liquid Chromatography; **HPTLC:** High Performance Thin Layer Chromatography.
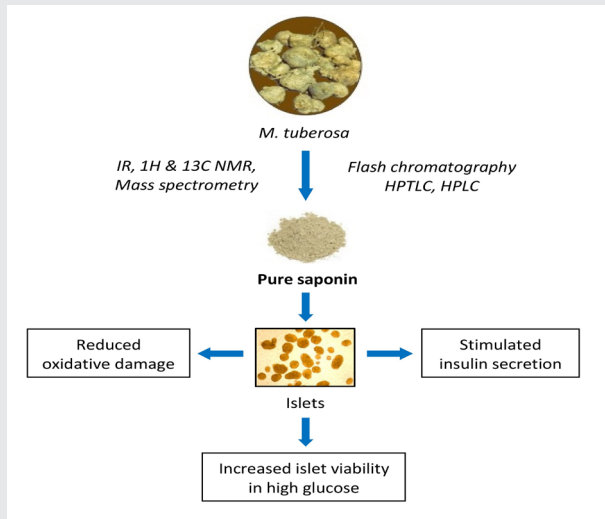
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PICTORIAL ABSTRACT

M. tuberosa

IR, 1H & 13C NMR,
Mass spectrometry

Flash chromatography
HPTLC, HPLC

Pure saponin

Reduced oxidative damage

Islets

Stimulated insulin secretion

Increased islet viability in high glucose

SUMMARY

- The present study was aimed at investigating the insulin secreting and islet protecting potentials of saponin of Momordica tuberosa.
- The pharmacologically active phytoconstituent, the saponins, were isolated through flash chromatography and characterized by HPTLC, HPLC, IR, NMR (1H and 13C) and mass spectrometry.
- The saponin was found to stimulate insulin secretion from the islets in the presence or absence of STZ treatment. A similar trend was observed with the intra-islet insulin secretion.
- Oxidative damage due to STZ and high glucose (31 mM) is indicative of the elevated formation of malondialdehyde (MDA) and nitric oxide (NO). The saponin was found to reduce the levels of both (MDA and NO) in islets exposed to STZ and high glucose.
- The viability of islets in high glucose was assessed by MTT assay. It was found that the saponin increased viability of islets cultured in high glucose.
- To conclude, the saponin of Momordica tuberosa stimulated insulin secretion from the cultured islets and protected them from oxidative damage.