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Original Research Article

Insulin Secretion and Glucose Uptake Enhancement by *Mimosa pudica* and *Abutilon indicum*: Potential Antidiabetic Therapy

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ABSTRACT

Mimosa pudica)M(and *Abutilon indicum*)A(are used as an antidiabetic mixture at a ratio of 1:1)MA11(in traditional Thai medicine. However, the mechanisms by which these plants exhibit antidiabetic effects have not been clearly elucidated. In this study, RINm5F insulinoma cells were treated with 6 mM streptozotocin (STZ) for 1 h to induce cellular damage and generate an *in vitro* model of diabetes. Extracts from M and A and mixtures of M and A extracts contain several phytochemicals, including flavonoids, tannins and saponins. All the extract samples at a concentration of 1,000 µg/mL exhibited antioxidant activity without cytotoxicity to RINm5F and L6 myotube cells. Pretreatment with M or A or with a mixture of the two prevented STZ-induced RINm5F cell death in a concentration-dependent manner. The greatest recovery effect was found in MA12, followed by MA11 and MA21. In addition, the M and A extracts and the mixtures of M and A extracts (100,000 µg/mL) exhibited insulin secretory activity in RINm5F cells that was similar to that of glibenclamide. Furthermore, all the plant extracts induced glucose uptake in L6 myotube cells. The induction of glucose uptake by M, A, and mixtures of M and A was derived from the upregulation of GLUT1 and GLUT4 synthesis in L6 myotube cells. These results indicate that *M. pudica* and *A. indicum* are potential sources of antidiabetic agents. In conclusion, the antidiabetic formula of M and A at a ratio of 2:1 (MA21) has the greatest potential for therapeutic use as an antidiabetic herbal mixture.

***Keywords*:** *Abutilon indicum*, *Mimosa pudica*, Glucose uptake, Glucose transporter, Insulin, Streptozotocin.

Introduction

Diabetes mellitus (DM) is the most common chronic metabolic disease, with a global prevalence of 285 million individuals in the year 2010, and 439 million people are expected to develop diabetes mellitus by 2030.[1-2] Beta cell (*β*-cell) regeneration (neogenesis and proliferation) is necessary for the generation of competent endogenous *β*-cell masses/reserves and has a major impact on long-term protection against type 2 diabetes mellitus (T2DM).[3,4,5] The fundamental strategy for treating diabetes focuses on the prevention of *β*-cell damage and death and the promotion of *β*-cell or islet regeneration.[3,4,6,7,8] Streptozotocin (STZ) has been widely used to induce diabetes in animal models. In addition, STZ can induce *β*-cell damage in pancreatic islets through the generation of free radicals, including reactive oxygen and nitrogen species. These reactive radicals induce oxidative stress in diabetic animals and humans.[9,10] Antioxidant supplements have long been utilized to reduce oxidative stress and promote pancreatic *β*-cell proliferation and have shown some benefits in patients with T2DM. Several natural phenolic compounds, including quercetin, catechin, and ascorbic acid, have demonstrated excellent antioxidant properties.[6,11,12]

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The roles of these antioxidants in *β*-cell regeneration and their potential therapeutic benefits have led to the development of new approaches to protect and/or expand the pancreatic beta-cell mass for T2DM treatment.[13]Currently, treatment measures to treat diabetes mellitus, such as oral antidiabetic drugs (e.g., glibenclamide and metformin), remain costly, and some drugs have adverse effects, including hypoglycemia, diarrhea, liver problems, lactic acidosis, and diarrhea.[14] Many antidiabetic medicinal plant recipes have been employed for alternative therapy in traditional Thai medicine, including the polyherbal combination of *Mimosa pudica* (*M. pudica*; M) and *Abutilon indicum* Sweet (*A. indicum*; A). These herbs are typically used at a 1:1 (v/v) ratio, and herbal drinks are prepared by soaking the dry herb mixture in hot water. Previous studies in an animal model have shown that extracts of *A. indicum* leaves possess hypoglycemic, hepatoprotective, antibacterial and larvicidal properties.[15,16] The hypoglycemic activity of *A. indicum* extract is derived from the stimulation of PPAR-γ activity, the promotion of insulin secretion, the enhancement of glucose use by the body's muscles, and fat tissue consumption via the inhibition of glucose absorption. The phytochemicals of the water extract of *A. indicum* include saponins, flavonoids and glycosides.[17] These active compounds are potentially involved in hypoglycemic activity. *M. pudica* belongs to the Mimosaceae family and has been well documented for its antiseptic, antimicrobial, anticonvulsant, antiasthmatic, immune stimulating, diuretic, antidiabetic, and antioxidant activities.[18,19,20] In STZ-induced diabetic rats, *M. pudica* methanol extract significantly reduces plasma glucose levels.[21] However, the molecular mechanism of the antidiabetic effects of *M. pudica* is unknown.

An herbal mixture of *M. pudica* and *A. indicum* (MA) at a 1:1 volume ratio (v/v) significantly reduced fasting blood glucose (FBG) levels in diabetic rats ($p<0.05$). An 80% ethanolic extract at a dose of 125 mg/kg b.w. was especially effective at weeks 2, 5, 7, and 8. These doses also

substantially increased serum insulin levels and decreased alanine transaminase (ALT) and alkaline phosphatase (ALP) levels compared with those in the diabetic control group.[22] Although the herbal plants *M. pudica* and *A. indicum* have demonstrated hypoglycemic activity in many previous studies, very little is known about the possible molecular mechanisms underlying their antidiabetic effects. Therefore, the objectives of the present study were to investigate the antidiabetic effects of *M. pudica* and *A. indicum* extracts as well as their mixtures by measuring insulin secretion and cell protection in RINm5F insulinoma cells. In addition, we determined the effects of these herbs on glucose uptake and the upregulation of glucose transporters (GLUTs) in skeletal muscle L6 cells.

Materials and Methods

Chemicals and Reagents

Dulbecco's modified Eagle's medium (DMEM), Roswell Park Memorial Institute-1640 (RPMI-1640) medium and other cell culture products were purchased from Gibco (San Diego, CA, USA). Bovine serum albumin (BSA) and STZ were purchased from Sigma–Aldrich (Saint Louis, MO, USA). All other chemicals and reagents were of analytical grade.

Plant Materials

Dry whole *M. pudica* (M) and *A. indicum* (A) plants were purchased from Vetchapong Osod Store, Bangkok, Thailand (the plants were collected on 15 December 2020). All the plant materials used were identified by Assistant Professor Bhanubong Bongchewin, Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University. A voucher specimen (PBM 04765) was deposited at the Pharmaceutical Botany Mahidol University Herbarium.

Plant Material and Extraction

The crude extracts were obtained via separate extractions of dry *M. pudica* (M) and *A. indicum* (A) plants as well as mixtures of *M. pudica* and *A. indicum* (MA) at different ratios (weight per weight; w/w), i.e., 1:1 (MA11), 1:2 (MA12) and 2:1 (MA21). Five hundred grams of dry plant material was boiled in 5 L of distilled water (1:10 w/v) for 10 min, after which the aqueous extract was collected. The extraction was repeated twice. The combined aqueous extract was filtered, and the filtrate was lyophilized in a freeze drier, resulting in a dark brown powder (yield 3–5% w/w). The lyophilized extracts were preserved in airtight bottles in a refrigerator at 4 °C and protected from light until use.

Phytochemical Screening

Phytochemical screening of the extract was performed to identify the constituents via specific reagents, including Dragendorff's reagent, Mayer's reagent, cyanidin, ferric chloride, gelatin, gelatin salt, and saponin tests.[23]

LC–MS Analysis of M. pudica and *A. indicum Extracts*

LC–MS was employed to identify the phytochemical contents of the MA11 and MA21 extracts. The analysis utilized a Dionex Ultimate 3000 high-performance liquid chromatograph coupled with a Bruker Maxis mass spectrometer. HPLC separation was conducted using an Acclaim RSLC 120 C18 column (2.1 × 100 mm, 2.2 µm), and quantification was performed on the mass spectrometer in both positive and negative modes. The mobile phase consisted of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B), and a gradient profile was used in positive mode. For negative mode, the mobile phases were water (Solvent A) and acetonitrile (Solvent B), and the gradient profiles were applied accordingly. The flow rate was set at 0.4 mL/min. Peaks were identified on the basis of the MS/MS spectra via the METLIN and MetFrag online databases (Table 1).

Cell Culture

RINm5F rat pancreatic β cells (#CRL 11605) and L6 skeletal muscle cells (#CRL 1458) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The RINm5F cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin (100 µg/mL) and penicillin (100 U/mL) solution. L6 myoblasts were cultured in DMEM supplemented with 10% FBS and 1% P/S solution. The cells were incubated at 37 °C in a humidified incubator containing 5% CO_2.

Table 1: Phytochemical compounds of the crude extracts of the *M. pudica* and *A. indicum* mixtures

Extract	Phytochemical compound	Concentration (µg/g crude extract)
MA11	Gallic acid	1081.61
	Vanillic acid	40.32
	P-Coumaric acid	98.52
	Quercetin	180.96
MA21	Gallic acid	1325.99
	Vanillic acid	94.49
	P-Coumaric acid	120.62
	Quercetin	136.43

Cytotoxicity of the Herbal Extracts and Streptozotocin (STZ) in RINm5F Cells

The cytotoxic effects of the herbal extracts on RINm5F cells were assessed via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assays by measuring the reduction of MTT to formazan crystals by mitochondrial dehydrogenase in viable cells.[24,25] RINm5F cells were seeded into a 96-well plate at a density of 1×10^4 cells/well and incubated overnight. The cells were incubated with various concentrations of herbal extracts (1, 3, 10, 100, 300, and 1,000 µg/mL) or STZ (1, 2, 4, 6, 8, and 10 mM). After 24 h of exposure, 5 µL of the MTT stock solution (5,000 µg/mL) was added to each well and incubated at 37 °C for an additional 4 h. The medium containing MTT was removed, and the formazan crystals were dissolved in 100 µL of dimethylsulfoxide (DMSO). The absorbance was measured at 590 nm via a CLARIOstar microplate reader spectrophotometer. The degree of cytotoxicity is presented as the percentage of viable cells (% cell viability).

Cell Recovery Assay

To evaluate the effects of *M. pudica* (M), *A. indicum* (A), and a mixture of these plants (MA) at various ratios (weight per weight; w/w) on STZ-induced death in RINm5F cells, cell viability was examined via the MTT assay. The cells (1×10^4 cells/well) in the 96-well plate were exposed to STZ for 1 h, followed by treatment with various concentrations of herbal extracts, including M, A, and the mixtures (e.g., MA11, MA12, MA21) (1, 10 and 100 µg/mL) for 24 h at 37 °C. Subsequently, 5 µL of the MTT stock solution (5,000 µg/mL) was added, and the next step was the same as that in the MTT assay. [25,26] Cytotoxicity is presented as the percentage of cell viability (% cell viability).

Measurement of Insulin Secretion

RINm5F cells were used to evaluate cellular insulin secretion.[27] The cells were seeded at a concentration of 1×10^5 cells/well in 24-well plates and incubated overnight. The cells were washed three times with Krebs–Ringer bicarbonate buffer (KRB), pH 7.4, and preincubated with KRB buffer, pH 7.4, for 40 min at 37 °C. For the insulin secretion assay, the cells were then incubated with 1 mL of KRB supplemented with 1.1 mM glucose in the absence or presence of glibenclamide (10 µg/mL), herbal extracts, or mixtures at various concentrations (1, 10, or 100 µg/mL) for 6 h. After incubation, the culture medium (supernatant) from each sample was collected, and the levels of secreted insulin were determined using an insulin high range assay kit (Cisbio Bioassays). The concentration of insulin was calculated via a standard curve as described in the manufacturer's instructions.

Glucose Uptake Assay
The glucose uptake assay was performed following published methods with slight modification.[27] In brief, L6 myotubule cells were plated in 24-well plates at a density of 2×10^4 cells/well. Once the cells reached 50% confluence, the culture medium was changed to DMEM with 2% FBS and 1% P/S (the differentiation medium). The cells were allowed to differentiate into myoblasts for 5–7 days, and the medium was changed every other day. Thereafter, L6 myoblasts were incubated at 37 °C for 24 h with insulin (100 IU/mL), metformin (10 µg/mL) and the tested herbal extracts (1, 10, and 100 µg/mL). The medium (supernatant) was subsequently collected, and the remaining glucose in the medium was measured via the peroxidase–glucose oxidase system (PGO) (Sigma–Aldrich, USA). The differentiation medium was used as the control group. Glucose uptake was calculated as the ratio of the sample to the control.

L6 Myotubule Cell Cytotoxicity
After the glucose uptake test in L6 cells, L6 cells were used to test cell cytotoxicity via the MTT assay as previously described[28] with slight modifications. Briefly, the medium was adjusted to 200 µL per well. The cells were treated with 10 µL of the MTT solution (5,000 µg/mL) and incubated at 37 °C under 5% CO_2 for 2 h. To dissolve the formazan crystals, 200 µL of DMSO was added to each well. The absorbance of each well was determined via a CLARIOstar microplate reader at 590 nm. The cytotoxicity is presented as the percentage of cell viability (% cell viability).

GLUT1 and GLUT4 mRNA Expression Analysis
L6 myotubule cells were seeded at a density of 1×10^6 cells/well in a 6-well culture plate with DMEM supplemented with 10% FBS and cultured overnight. Under serum-free conditions, the cells were treated with extracts for 6 h. Total RNA was extracted via the GeneJET RNA Purification Kit (Thermo Fisher Scientific). GLUT1 and GLUT4 mRNA expression levels were determined via real-time RT–qPCR via KAPA SYBR FAST One-step RT–qPCR kits (KAPA Biosystems) according to the manufacturer's protocol. *Gene-specific primers* for GLUT1 and GLUT4 were designed and are shown in Table 1. RT–qPCR was performed under the following conditions: reverse transcription at 42 °C for 5 min; reverse transcriptase (RT) inactivation and DNA polymerase activation at 95 °C for 2-5 min; combined annealing, extension, and data acquisition at 95 °C for 3 s and 55 °C for 30 s (40 cycles); and a final extension step at 72 °C for 1 min followed by incubation at 25 °C for 2 min. The expression of the target genes was normalized to that of GAPDH and is expressed as a fold change over that of the control (vehicle) group.[29-30]

Statistical analysis
The results are expressed as the means ± SEMs. The data were analyzed via the SPSS (IBM statistic, Ver. 21) program. Differences between the means of the experimental groups and between multiple groups were determined via ANOVA followed by least significant difference (LSD) tests. Differences with a p value < 0.05 were considered statistically significant.

Results and Discussion

Phytochemical Compounds of the Crude Extract
The qualitative phytochemical analysis of *M. pudica* and *A. indicum* mixtures at ratios of 1:1 and 2:1 was conducted via LC–MS, revealing the presence of gallic acid, vanillic acid, p-coumaric acid, and quercetin (Table 1).

Cytotoxic Effects of Herbal Extracts and STZ in RINm5F Cells
The cytotoxicity of *M. pudica* (M) and *A. indicum* (A) herbal extracts and mixtures of M and A at ratios of 1:1 (MA11), 1:2 (MA12) and 2:1 (MA21) was assessed in RINm5F cells using an MTT assay to determine the optimal concentration that is not toxic to the cells. After treatment with various concentrations of extracts and mixtures, all samples had no cytotoxic effects on RINm5F cells, as the cell viability was greater than 80% (Figure 1A). The LD_{50} values of the extracts and mixtures on RINm5F cells were estimated to be greater than 1,000 µg/mL. Therefore, the concentrations used in further experiments were 1–1000 µg/mL to ensure safety and prevent the cytotoxic effects of these extracts.
The results demonstrated that treatment with STZ induced cytotoxicity in a concentration-dependent manner, with the greatest degree of toxicity noted at a concentration of 10 mM (Figure 1B). When the cells were treated with 6 mM STZ, the percentage of viable RINm5F cells was approximately 50%. On the basis of these results, 6 mM STZ was selected as the appropriate concentration for use in subsequent experiments.

Recovery Effects of Herbal Extracts on STZ-induced Damage to RINm5F Cells
The recovery effects of the herbal extracts on STZ-induced damage to RINm5F cells were determined via the MTT assay. Incubation with 6 mM STZ significantly induced cytotoxic effects in RINm5F cells (Figure 2). Interestingly, pretreatment of RINm5F cells with various ratios of the mixtures, including MA11, MA12, and MA21, prevented STZ-induced cell death in a concentration-dependent manner (Figure 2). In addition, pretreatment with either *M. pudica* (M) or *A. indicum* (A) extracts mitigated STZ-induced cellular damage in a concentration-dependent manner (Figure 2).
Among all the mixtures, the highest cell recovery activity was found in MA12, followed by MA11 and MA21 (Figure 2). In addition, treatment with herbal extract alone at concentrations up to 1,000 µg/mL did not cause any cellular damage (Figure 2). These data indicated that *M. pudica* and *A. indicum* extracts and the mixed extracts have protective effects against STZ-induced pancreatic β-cell damage.

Effects of Herbal Extracts on Insulin Secretion in RINm5F Cells
The amount of insulin secreted into the culture medium was determined via an insulin high range kit (Cisbio). As shown in Figure 3, treatment of RINm5F cells with extracts from either M or A significantly induced insulin secretion in a concentration-dependent manner, with maximal effects at a concentration of 100 µg/mL. In addition, treatment with various ratios of the mixtures, i.e., MA11, MA12 and MA21, also increased the secretion of insulin in a concentration-dependent manner (Figure 3). The peak insulin secretion was observed in MA21, followed by MA11 and MA12. Interestingly, 100 µg/mL MA11, MA12, MA21, and A exhibited insulin secretory effects similar to those of glibenclamide (10 µg/mL) (Figure 3). Furthermore, treatment with 100 µg/mL *M. pudica* (M) extract had a more potent effect than glibenclamide (10 µg/mL) on enhancing insulin secretion from pancreatic β-cells (Figure 3). These results indicate that the mixtures of M and A exert hypoglycemic effects on the basis of their insulin secretory effects.

Effects of Herbal Extracts on Glucose Uptake in L6 Myoblast Cells
As shown in Figure 4, treatment with either insulin (1 IU/mL) or metformin (100 µg/mL) robustly increased glucose uptake compared with that of the control (vehicle) in L6 myoblasts (299.32±0.84 and 271.46±1.03, respectively). Similarly, treatment with either A, M, or the mixtures (MA11, MA12, MA21) induced glucose uptake in L6 myoblasts in a concentration-dependent manner, with maximal effects observed at a concentration of 100 µg/mL. In addition, among all the extracts and mixtures, the MA11 mixture had the lowest efficiency in stimulating glucose uptake (Figure 4). These data demonstrated that mixtures of M and A potentially have glucose-lowering effects.
As shown in Supplemental Figure 1, treatment with all the extracts and mixtures (MA11, MA12, MA21, M and A) at concentrations of 1, 10 and 100 µg/mL did not result in cytotoxic effects, with cell viability exceeding 95% (Supplemental Figure 1). These results suggest that *M. pudica* and *A. indicum* increase glucose uptake without being cytotoxic. Insulin is synthesized and secreted from β-cells and stimulates its receptor, leading to the translocation of glucose transporter (GLUT) to the cell surface and subsequently triggering glucose uptake into the cells. In addition, insulin also induces the synthesis of GLUTs in various types of cells and tissues.[31] As the herbal extracts were able to stimulate insulin secretion as well as glucose uptake into the cells (Figures 3 and 4, respectively), we next investigated the effects of the herbal extracts

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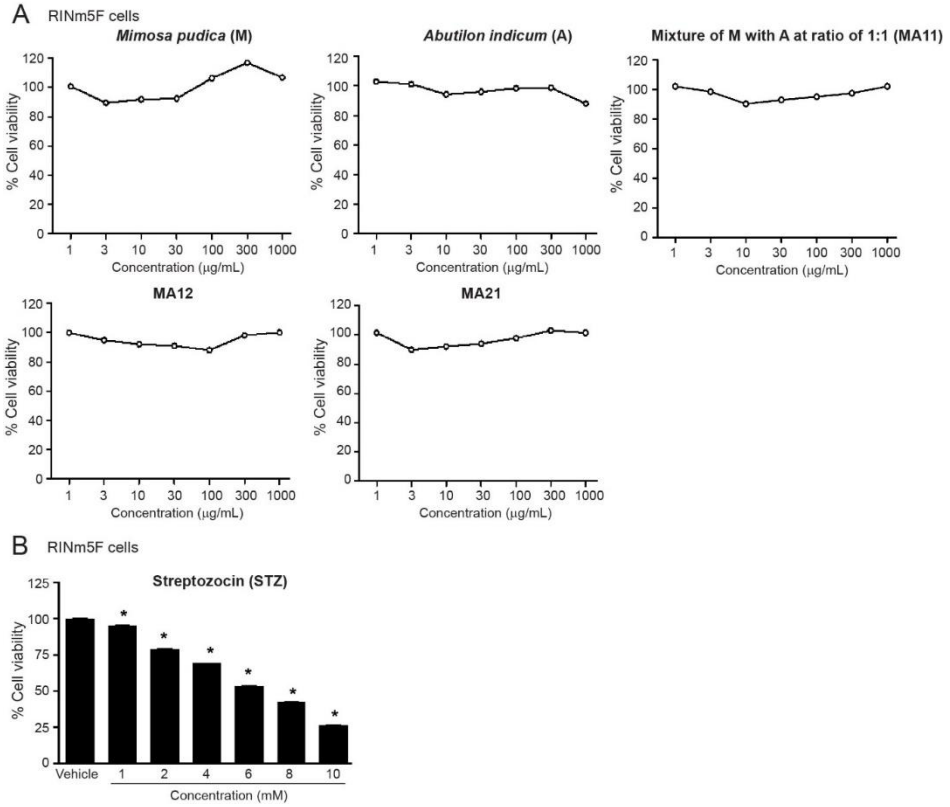

Figure 1: Cytotoxic effects of herbal extracts and STZ in RINm5F cells. RINm5F cells were treated with either herbal extracts (1–1,000 µg/mL) (A) or streptozotocin (STZ) (1–10 mM) (B) for 24 h. Cell viability was quantified as the percentage of cell viability (% cell viability) and is presented as the mean ± SEM (n=4). *, $P < 0.05$ vs. vehicle

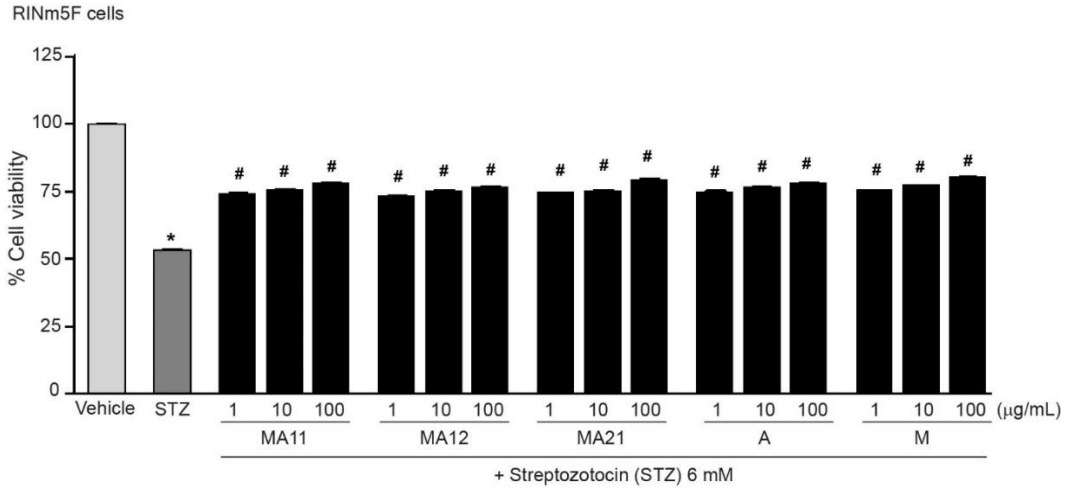

Figure 2: Recovery effects of herbal extracts on STZ-induced damage to RINm5F cells.
RINm5F cells were incubated with 6 mM streptozotocin (STZ) for 1 h and then treated with M or A extracts or a mixture (MA11, MA12, MA21) at various concentrations (1, 10 and 100 µg/mL) for 24 h. Cell viability was quantified and is expressed as the percentage of cell viability (% cell viability) and presented as the mean ± SEM (n=3). *, $P < 0.05$ vs. vehicle; #, $P < 0.05$ vs. STZ

on the synthesis of GLUTs, as determined by GLUT1 and GLUT4 mRNA expression in L6 myoblasts.

Effects of Herbal Extracts on GLUT mRNA Expression in L6 Myoblast Cells

Treatment with MA21, A and M (200 µg/mL) significantly increased GLUT1 mRNA expression, whereas MA11 and MA12 were unable to induce GLUT1 mRNA expression in L6 myotubes (Figure 5A). Furthermore, treatment of the cells with A, M, MA12, or MA21 (200 µg/mL) resulted in a significant increase in GLUT4 mRNA expression compared with that of the control (vehicle) (Figure 5B). However, treatment with MA11 had no effect on GLUT4 mRNA expression. Taken together, these results demonstrated that extracts of M and A, including the mixtures MA12 and MA21, upregulate GLUT synthesis, promoting glucose uptake into the cells. MA11 had the smallest effect on the induction of GLUT synthesis.

The antidiabetic effects of phytochemicals have been attributed to a variety of mechanisms, including glucose transporter regulation, *β*-cell

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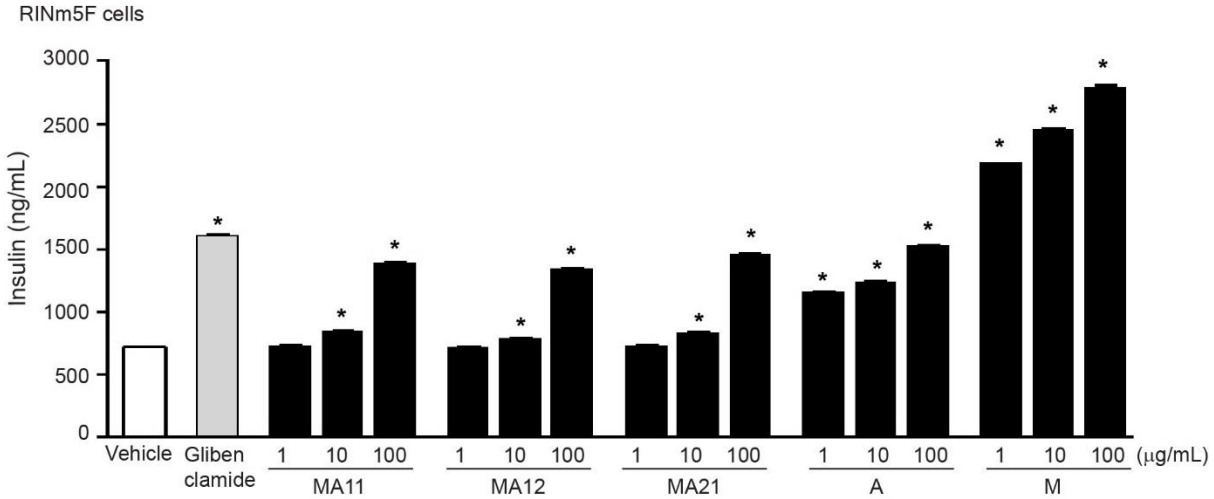

Figure 3: Effects of herbal extracts on insulin secretion in RINm5F cells. RINm5F cells were treated with M or A extracts or mixtures (MA11, MA12, MA21) at various concentrations (1, 10 and 100 μg/mL) or with glibenclamide (10 μg/mL) for 6 h. After treatment, the culture medium was collected. The amount of insulin secreted into the medium was determined and is presented as the mean ± SEM (n=3). *, $P < 0.05$ vs. vehicle

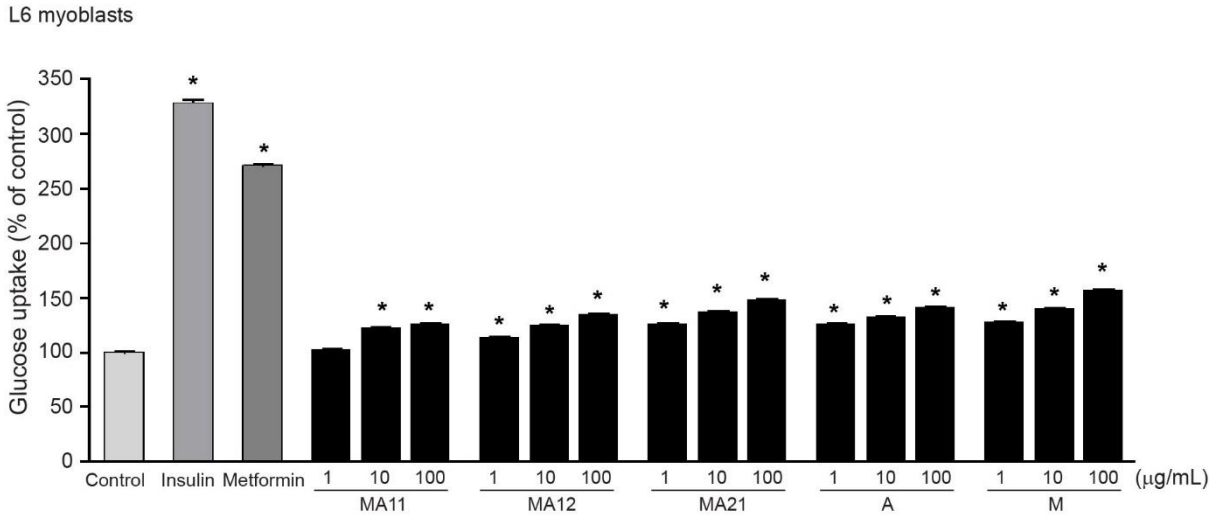

Figure 4: Effects of herbal extracts on glucose uptake in L6 myoblast cells. L6 myoblast cells were treated with M or A extracts or mixtures (MA11, MA12, MA21) at various concentrations (1, 10 and 100 μg/mL), insulin (100 IU/mL), or metformin (10 μg/mL) for 24 h. After treatment, the culture medium was collected. The remaining glucose in the medium was measured via the peroxidase–glucose oxidase system. The glucose uptake is presented as a percentage of that of the control (vehicle). The data are presented as the means ± SEMs (n=3). *, $P < 0.05$ vs. control.

regeneration, improved insulin secretion,[1,32,33,34] the inhibition of glucose absorption, decreased diffusion and inhibition of carbohydrate-metabolizing enzymes at the gut level, and glucose utilization.[33]

The extracts contained gallic acid, vanillic acid, p-coumaric acid, and quercetin, with MA21 showing relatively high concentrations of gallic, vanillic, and p-coumaric acids, indicating its strong potential as an antidiabetic agent. These findings suggest that these phytochemicals play a significant role in the extract's antidiabetic effect. Gallic acid enhances GLUT4 translocation and glucose uptake in an Akt-independent manner but is sensitive to wortmannin (an inhibitor of phosphoinositide 3-kinase [PI3K]), with further analysis indicating the involvement of the atypical protein kinase C-zeta/lambda.[35] In a rat model, gallic acid has been reported to increase PPARγ expression in the adipose tissue of high-fat diet- and streptozotocin-induced diabetic rats compared with that in untreated rats while mildly activating PPARγ in the liver and muscle; this increases insulin-dependent glucose transport in adipose tissue via GLUT4 translocation and PI3K/p-Akt pathway activation. Gallic acid docking with PPARγ demonstrated promising interactions with GLUT4, GLUT1, PI3K, and p-Akt, suggesting that it improves adipose insulin sensitivity, regulates adipogenesis, increases glucose uptake, and protects *β*-cells.[36] The combination of gallic acid and andrographolide has emerged as an antidiabetic agent due to synergistic hypoglycemic effects, improved biochemical markers, tissue normalization, enhanced GLUT4 expression, and strong molecular interactions with the adiponectin receptor, thus offering a promising alternative to current diabetes treatments with fewer side effects.[37] Vanillic acid has been reported to increase 2-deoxyglucose uptake by 3T3-L1 adipocytes and significantly increase GLUT4 translocation via the AMPK-dependent pathway. Procyanidin B1 and p-coumaric acid from highland barley synergistically improve glucose uptake and glycogen synthesis, targeting GLUT4, GSK-3*β*, and the IR*β*/IRS-1/PI3K/Akt pathway.[38] The findings of this study, along with those from previous studies,

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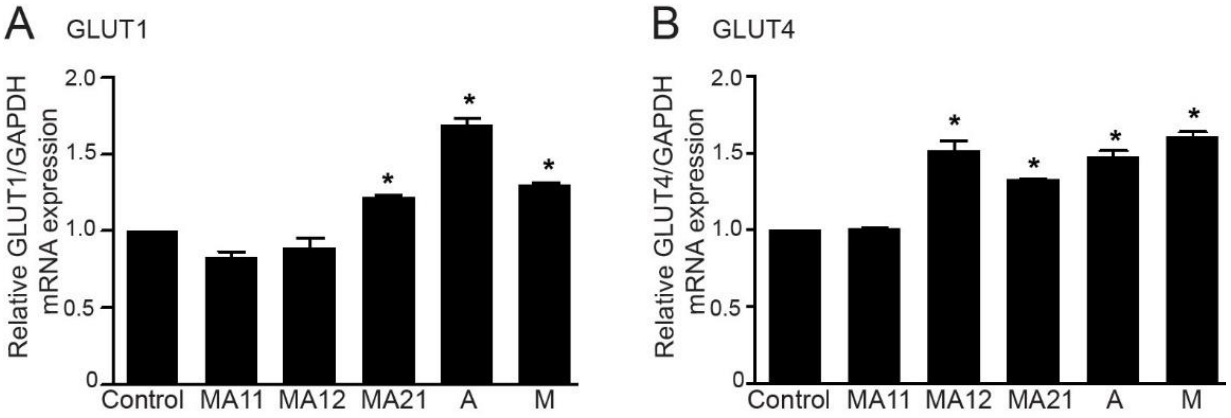


Figure 5: Effects of herbal extracts on the expression of GLUT mRNA in L6 myoblast cells.
The cells were treated with either vehicle (control) or with M or A extracts or mixtures (MA11, MA12, MA21) (200 µg/mL) for 6 h. The relative mRNA levels of GLUT1 (A) and GLUT4 (B) were quantified and are shown as the means ± SEMs (n=3). *, P < 0.05 vs. control.

strongly suggest that the antidiabetic mechanism of MA21 extracts is related to their phytochemical composition.

The regeneration experiments revealed that the herbal extract formulations MA11, 12, and 21 as well as M and A prominently increased the number of RINm5F cells after STZ-induced damage to RINm5F insulinoma cells. On the basis of these results, we concluded that the extract had a restorative effect on insulin-secreting cells. These results are in accordance with those of a previous study showing that the original formula ratio of *M. pudica* and *A. indicum* (MA11) increased the size and number of pancreatic islets in a rat model.[22] The results of the present study suggested that the modulation of *β*-cell function in the pancreas by the active compounds found in these extracts may play an important role in protection against *β*-cell damage.

Phytochemicals such as flavonoids, alkaloids, tannins, terpenoids and glycosides have potential antioxidant activities and are generally implicated in antidiabetic activities. For example, flavonoid compounds present in *M. pudica* potentially restore damaged *β*-cells, as they show alpha-amylase inhibitory activities.[39] Furthermore, phenolic compounds and tannins inhibit the transportation of glucose by inhibiting sodium glucose cotransporter-1 (SGLUT-1) in the intestines of rats.[40] Therefore, the antioxidant and antidiabetic activities of the extracts from M and A are potentially derived from the presence of several phytochemicals, such as flavonoids, phenolic compounds, tannins, and saponins, in the crude extracts.

The extracts and mixtures, including MA11, MA12, MA21, M and A, exhibited insulin secretory effects in a concentration-dependent manner. Compared with the MA11 and MA12 mixtures, the MA21 mixture elicited greater insulin secretory effects. These results suggest that the mixed herbal extract at a ratio of 2:1 may be more potent than the original extract at a ratio of 1:1 for stimulating insulin secretion. All the herbal extracts and mixtures demonstrated antidiabetic properties, which increased insulin secretion and thereby controlled glucose homeostasis. However, the mixed extracts stimulated insulin secretion in a manner similar to that of the *A. indicum* extract, although to a lesser extent than that of the *M. pudica* extract (Figure 5). Thus, no synergistic effect of these two herbal extracts on the stimulation of insulin secretion was observed.

Another key target of antidiabetics is the stimulation of glucose uptake by muscle and adipose tissues. The extracts of the mixtures of M and A (MA) and antihyperglycemic drugs (insulin and metformin) reduced glucose uptake in L6 myoblasts. However, insulin (1 IU/mL) and metformin (100 µg/mL) had more potent effects than did all ratios M and A extracts (100 µg/mL). The reduction in glucose uptake may be due to the insulin secretory effects of the extracts. The production and secretion of insulin can decrease and restore plasma glucose levels to normal levels.[30] There is a significant and prolonged elevation in plasma glucose levels in individuals with diabetes, and this condition might be derived from damage to pancreatic *β*-cells. Thus, the effects of extracts from the mixture of M and A on the stimulation of insulin secretion as well as glucose uptake could help protect *β*-cells and restore their functions in diabetic patients.

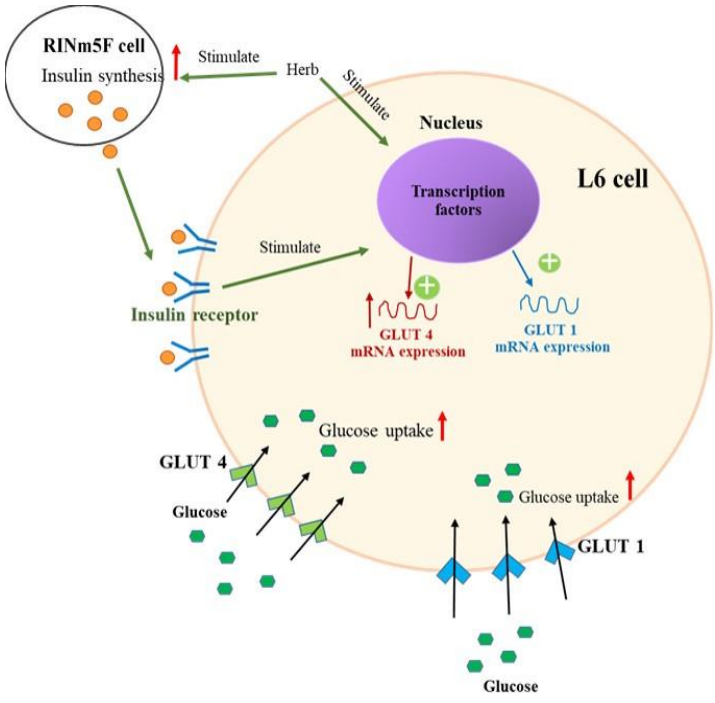
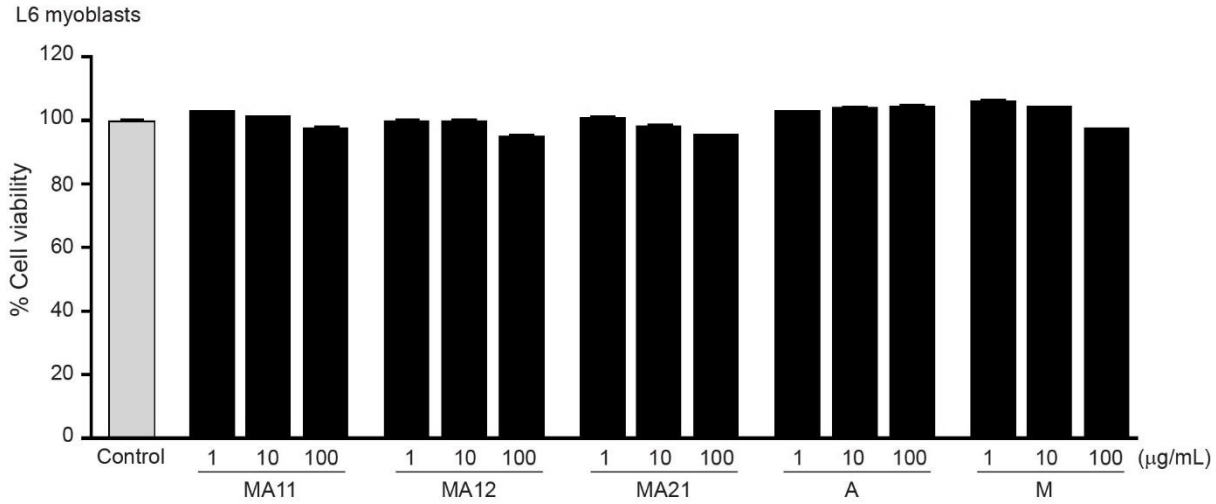


Figure 6: Schematic diagram representing the molecular mechanism of the antidiabetic effects of *M. pudica* and *A. indicum*. The extracts from *M. pudica* (M) and *A. indicum* (A) as well as mixtures of M and A (MA) exhibited antidiabetic effects by stimulating insulin secretion and protecting against cellular damage in RINm5F cells. In addition, all the herbal extracts induced the upregulation of GLUT1 and GLUT4, resulting in increased glucose uptake in L6 myoblasts.

According to the findings of this study, all the crude extracts, including MA11, MA12, MA21, M, and A, significantly improved glucose uptake in L6 myoblasts in a concentration-dependent manner. The maximum glucose uptake activity was observed in the M extract, followed by A, MA21, MA12, and MA11. Compared with the original MA11 mixture, the modified mixed extracts MA21 and MA12 exhibited greater glucose uptake activity. Notably, the mixed extracts promoted glucose uptake in a manner similar to that of the *A. indicum* extract, although to a lesser extent than that of the *M. pudica* extract. These results are similar to its insulin-specific activity. Thus, the better activities of M might be attributed to the presence of alkaloids in the extract. The alkaloids present in M potentially contain key substances involved in the activation of glucose uptake at high levels. Mimosine is an alkaloid found in *Mimosa*. However, further identification of phytochemicals corresponding to the stimulation of insulin secretion as well as glucose uptake is warranted.

There are at least three mechanisms by which insulin may modulate the function of insulin-regulated GLUT4 in skeletal muscle and adipose tissue.[41] First, insulin promotes GLUT4 translocation to the plasma membrane.[42] Second, insulin may increase the intrinsic transport activity of preexisting GLUT4 proteins to the cell surface either by directly modifying GLUT4 or by interacting with other regulatory molecules.[41] In addition, insulin induces the upregulation of GLUT expression via increased biosynthesis, decreased degradation or both.[41] The present study demonstrated that the modified mixed extracts MA12 and MA21 were more effective than the original MA11 at increasing GLUT4 synthesis. The M, A, and MA21 herbal extracts significantly increased GLUT1 mRNA expression in L6 cells. In terms of mixed extract activity, we discovered that modified mixed extract A had peak activity, followed by M and MA21. These results suggested that the modified mixed extract of both MA12 and MA21 stimulated GLUT 1 and GLUT 4 mRNA expression more effectively than the original MA11. The extract also stimulated glucose uptake through numerous different mechanisms, such as the upregulation of GLUT1 and GLUT4 synthesis and the induction of GLUT translocation to the plasma membrane, resulting in an increase in glucose uptake into cells in response to insulin stimulation.[41,42] Many phytochemicals with different antidiabetic effects are present in herbal extracts from *M. pudica* and *A. indicum*. Therefore, further studies to determine the activity-guided isolation of bioactive compounds are warranted.



Supplemental Figure 1: Cytotoxic effects of herbal extracts on L6 myoblast cells.
L6 myoblast cells were treated with either vehicle (control) or with M or A extracts or mixtures (MA11, MA12, MA21) at various concentrations (1, 10 and 100 μg/mL) for 24 h. Cell viability was quantified and is expressed as the percentage of cell viability (% cell viability) and presented as the mean ± SEM (n=3).

Conclusion

The herbal extracts from *M. pudica* and *A. indicum*, including mixtures of the two, have antidiabetic effects via cytoprotective mechanisms that involve the stimulation of insulin secretion. Moreover, these herbal extracts induced glucose uptake into the cells by increasing GLUT1 and GLUT4 synthesis. Among all the herbal extracts, the mixture of M and A at a ratio of 2:1 (MA21) presented the greatest antidiabetic effects and could be used in a potential antidiabetic herbal recipe.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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