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ORIGINAL ARTICLE

Effects of the Herbal Medicines on Voltage-Dependent K^+ 2 Channels

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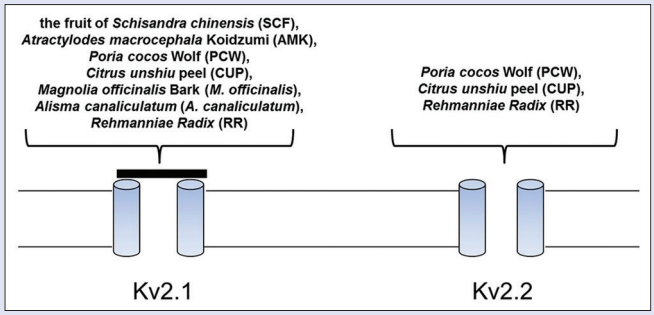
ABSTRACT

Background: Identification of selective ion channel inhibitors is necessary for understanding the physiological role of these proteins. The voltage-dependent K^+ (Kv) channels, Kv2.1 and Kv2.2, are expressed in pancreatic islets, and the development of selective Kv2.1 inhibitors that do not cross-inhibit Kv2.2 may be useful for the treatment of type 2 diabetes. **Objective:** The aim of this study was to evaluate whether herbal medicines, such as the fruit of *Schisandra chinensis* (SCF), *Atractylodes macrocephala* Koidzumi (AMK), *Poria cocos* Wolf (PCW), *Citrus unshiu* peel (CUP), *Magnolia officinalis* Bark (*M. officinalis*), *Alisma canaliculatum* (*A. canaliculatum*), *Rehmanniae* Radix (RR), and *Corni fructus* (*C. fructus*), modulate Kv2 channels and cause insulin secretion. **Materials and Methods:** We used the whole-cell patch-clamp technique to analyze the effect of these herbal medicines on Kv channels. In addition, human embryonic kidney 293 cells overexpressing Kv2.1 and Kv2.2 channels were used to confirm the role of Kv2 channels. **Results:** SCF, AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR inhibited Kv2.1 channel currents in a concentration-dependent manner (100–500 μg/mL). However, *C. fructus* had no effects on Kv2.1 channel currents. In addition, SCF, AMK, *M. officinalis*, and *A. canaliculatum* inhibited Kv2.2 channel currents in a concentration-dependent manner, but PCW, CUP, and RR had no effects on Kv2.2 channel currents. Furthermore, RR, CUP, and PCW increased insulin secretion. **Conclusion:** These findings suggested that the herbal medicines, RR, CUP, and PCW, are potential novel agents for the prevention and treatment of diabetes.

Key words: Diabetes, herbal medicine, Kv2.1, Kv2.2, voltage-dependent K^+ channels

SUMMARY

- Fruit of *Schisandra chinensis*, *Atractylodes macrocephala* Koidzumi, *Poria cocos* Wolf (PCW), *Citrus unshiu* peel (CUP), *Magnolia officinalis* Bark, *Alisma canaliculatum*, and *Rehmanniae* Radix (RR) inhibited voltage-dependent K^+ (Kv2.1) channel currents in a concentration-dependent manner
- *Corni fructus* (100–500 μg/mL) had no effects on Kv2.1 channel currents
- Administration of RR, CUP, and PCW caused an increase in insulin secretion
- PCW, CUP, and RR are potential novel agents for the prevention and treatment of diabetes.



Abbreviations used: SCF: Fruit of *Schisandra chinensis*; AMK: *Atractylodes macrocephala* Koidzumi; PCW: *Poria cocos* Wolf; CUP: *Citrus unshiu* peel; *M. officinalis*: *Magnolia officinalis* Bark; *A. canaliculatum*: *Alisma canaliculatum*; RR: *Rehmanniae* Radix; *C. fructus*: *Corni fructus*; Kv channel: Voltage-dependent K^+ channel.

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INTRODUCTION

Diabetes is a metabolic disease characterized by high blood glucose levels and type 2 diabetes is associated with both insulin resistance and insulin deficiency.[1,2] In pancreatic islet β-cells, multiple ion channels regulate the plasma membrane potential (Vm), intracellular-free Ca^{2+} concentration, and insulin secretion.[3] The β-cell defect found in type 2 diabetes mellitus is most commonly treated with the sulfonylurea class of compounds.[4,5] Sulfonylureas antagonize ATP-sensitive K^+ channels, depolarizing the membrane, opening voltage-dependent Ca^{2+} channels, and stimulating insulin secretion.[6,7] In addition, voltage-dependent outward K^+ currents have been detected in the β-cells and are believed to mediate action potential repolarization,[8-10] limiting Ca^{2+} influx and insulin secretion. Indeed, previous studies show that the general voltage-dependent K^+ (Kv) and Ca^{2+}-sensitive voltage-dependent K^+ channel antagonist, tetraethylammonium, augments membrane depolarization,[11,12] Ca^{2+} influx,[13] and insulin secretion[12,14] in a glucose-dependent manner.[7] Among K^+ channels, both members of the Kv2 channel family, Kv2.1 and Kv2.2, are expressed in the pancreatic islets across several species,[7,15-17] and Kv2.1 is highly enriched in the islet β-cells.[7,16,18] Kv2.1 regulates insulin secretion in β-cells and Kv2.2 modulates somatostatin release in δ-cells. Therefore, the development of selective Kv2.1 inhibitors without cross-inhibition of Kv2.2 will provide new agents for the treatment of type 2 diabetes.[7,18-23]

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Management of type 2 diabetes usually begins with changing diet and exercise,[24] but most patients ultimately require pharmacotherapy, such as with an oral antidiabetic drug.[25] A variety of medicinal herbal products in Chinese medicine have beneficial effects in diabetes,[26] and many of these herbs have been formulated into multi-herbal preparations for enhanced effects.[27,28] In addition, Chinese medicine formulas also have a favorable effect on obesity.[29] Obesity is a major global health problem because it is associated with an increased risk of metabolic disorders, including type 2 diabetes mellitus, hypertension, atherosclerosis, cancer, and cardiovascular disease.[30,31]

In this study, to examine whether herbal medicines (fruit of *Schisandra chinensis* [SCF], *Atractylodes macrocephala* Koidzumi [AMK], *Poria cocos* Wolf [PCW], *Citrus unshiu* peel [CUP], *Magnolia officinalis* Bark [*M. officinalis*], *Alisma canaliculatum* [*A. canaliculatum*], *Rehmanniae* Radix [RR], and *Corni fructus* [*C. fructus*]) have antidiabetic effects, we investigated their inhibitory effects on Kv2.1 channels, but not on Kv2.2 channels, and how insulin secretion was affected.

MATERIALS AND METHODS

Preparation of herbal medicines

Dried RR, CUP, and PCW were purchased from Kapdang Co. (Seoul, Korea). The samples were identified by Dr. Yun Tai Kim and voucher specimens (#NP-1090) were deposited with the research group of innovative special food, Korea Food Research Institute. Dried herbal medicines (600 g each) were extracted with 70% ethanol (6000 mL) for 2 h at 20°C. The process was repeated once and the extracts were combined and filtered through a membrane filter (0.45 μm; Millipore, Billerica, MA, USA). After removing the solvents via rotary evaporation, the remaining extracts were freeze-dried, yielding approximately 15.6%–21.5% of the dried herbal medicine weight (w/w).

Cell culture and transfection

Human embryonic kidney 293 cells (ATCC, Manassas, VA, USA) were maintained according to the supplier's recommendations. 293 cells were transiently transfected with cDNA encoding mKv2.1 or mKv2.2 subcloned in the pIRES-green fluorescent protein vector with the use of lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's specifications.

Electrophysiological recordings

Electrophysiological recordings were taken at room temperature (22°C–25°C) 24–48 h after transfection using the patch-clamp technique in the whole-cell configuration. Cells were collected and maintained in a 35 mm^2 Petri dish (BD Biosciences, Bedford, MA, USA) in DMEM supplemented with 10% FBS at 37°C and 20% O_2/10% CO_2. An aliquot of cells was allowed to settle in a recording chamber for 5 min before perfusion was initiated. Whole-cell patch-clamp recordings were obtained using an Axopatch 700B amplifier and pClamp v. 10.4 software, and signals were digitized at 5 kHz using Digidata 1422A (Molecular Devices, Sunnyvale, CA, USA). For whole-cell recordings, the external solution contained 135 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, 1.8 mM $CaCl_2$, and 10 mM glucose (pH was adjusted to 7.4 with NaOH). The pipette solution contained 110 mM KCl, 5 mM $MgCl_2$, 5 mM K_4BAPTA, 5 mM K_2ATP, and 10 mM HEPES (pH adjusted to 7.2 with KOH). Pipette tips were fire-polished to a resistance of 2–3 MΩ to facilitate Gigaseal formation (Narishige, Tokyo, Japan). Data were saved on a desktop computer and analyzed using Clampfit v. 10.4 (Molecular Devices), Prism v. 6.0 (GraphPad, La Jolla, CA, USA), and Origin v. 8.0 (Microcal, Northampton, MA, USA) software.

Insulin secretion experiments

Insulin secretion experiments were performed in Krebs–Ringer bicarbonate buffer containing 115 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, and 0.1% bovine serum albumin as described previously.[16] An insulin secretion experiment was performed using a mouse insulin ELISA kit from Abnova Inc. (KA3812; Taipei, Taiwan). NIT-1 cells (ATCC) from a mouse insulinoma cell line were maintained in DMEM (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C in 5% CO_2. NIT-1 cell insulin secretion experiments were performed and data were normalized to controls.

Statistical analysis

Results are expressed as mean ± standard error of the mean. *N* values refer to the number of separate cells examined. Multiple comparison testing was performed using one-way ANOVA with Bonferroni's *post hoc* comparison. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of fruit of *Schisandra chinensis*, *Atractylodes macrocephala* Koidzumi, *Poria cocos* Wolf, *Citrus unshiu* peel, *Magnolia officinalis* Bark, *Alisma canaliculatum*, *Rehmanniae* Radix, and *Corni fructus* on the voltage-gated potassium channel, Kv2.1

Under control conditions, step depolarizations from the holding potential of −60 mV elicited Kv2.1 channel currents. A representative current trace for SCF is shown in Figure 1a. SCF inhibited Kv2.1 channel currents in a concentration-dependent manner (100–500 μg/mL), and the peak and quasi steady-state currents (measured at the end of the test pulses) showed a similar degree of suppression during the voltage step pulses. This SCF-dependent inhibition was rapidly reversible. Figure 1b presents the peak current–voltage (I–V) relationships of Kv2.1 channel currents in the presence and absence of various concentrations of SCF. Figure 1c summarizes the concentration dependence of the inhibition of Kv2.1 channel currents by SCF. The results shown in Figure 1c were obtained at the end of current values at +50 mV and were normalized to the current amplitude in the absence of SCF. A nonlinear least-square fit of the logistic function to the concentration–response data yielded an apparent IC_{50} value of 226.1 μg/mL. In addition, other herbal medicines, such as AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR, also inhibited Kv2.1 channel currents in a concentration-dependent manner (100–500 μg/mL). Figures 2Aa, Ba, Ca, Da and 3Aa, Ba present the peak I–V relationships of Kv2.1 channel currents in the presence and absence of various concentrations of AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR. Figures 2Ab, Bb, Cb, Db and 3Ab, Bb summarize the concentration dependence of the inhibition of Kv2.1 channel currents by AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR. The results shown in Figures 2Ab, Bb, Cb, Db and 3Ab, Bb were obtained at the end of current values at +50 mV and were normalized to the current amplitude in the absence of AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR. A nonlinear least-square fit of the logistic function to the concentration–response data yielded an apparent IC_{50} value of 187.4 μg/mL for AMK, 221.6 μg/mL for PCW, 484.1 μg/mL for CUP, 110.0 μg/mL for *M. officinalis*, 190.1 μg/mL for *A. canaliculatum*, and 387.6 μg/mL for RR, respectively. However, *C. fructus* (100–500 μg/mL) had no effects on Kv2.1 channel currents [Figure 3C]. These results suggest that SCF, AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR inhibited Kv2.1 channel currents in a concentration-dependent manner.

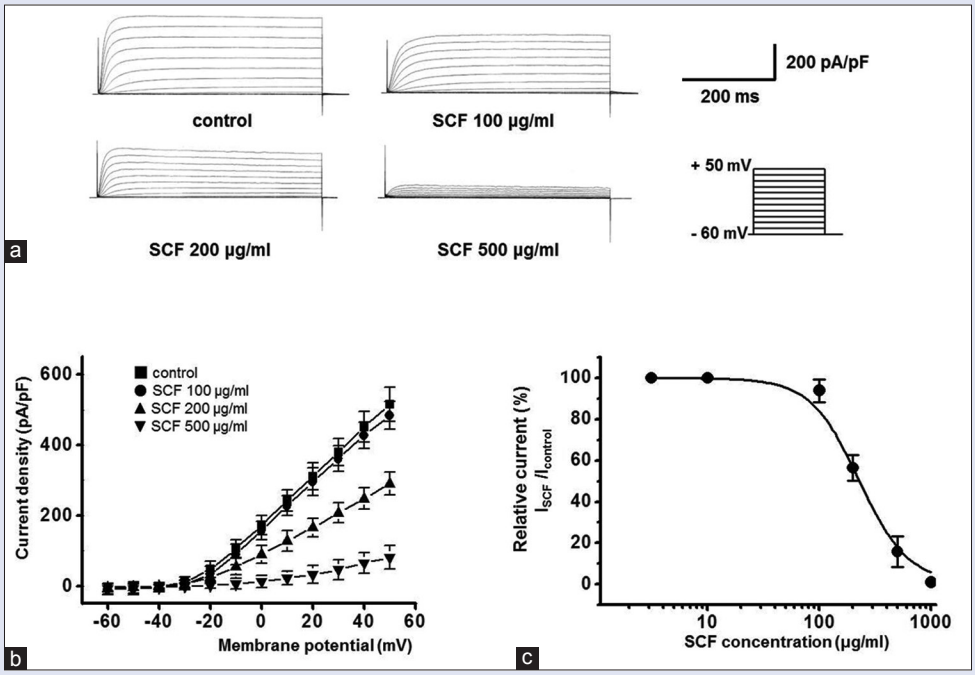

Figure 1: Effects of the fruit of SCF on Kv2.1 currents. (a) The superimposed current traces were elicited by 250-ms depolarizing pulses between −60 and +50 mV from a holding potential of −60 mV. (b) Current–voltage relationships of the peak and quasi-steady-state Kv2.1 channel currents in the presence and absence of SCF. (c) The SCF-induced inhibition of Kv2.1 channel currents was measured at the end of a 250-ms depolarizing pulse of +50 mV and normalized using the control current amplitude. SCF: *Schisandra chinensis*; Kv: Voltage-dependent K^+

Effects of fruit of *Schisandra chinensis*, *Atractylodes macrocephala* Koidzumi, *Poria cocos* Wolf, *Citrus unshiu* peel, *Magnolia officinalis* Bark, *Alisma canaliculatum*, *Rehmanniae Radix*, and *Corni fructus* on voltage-gated potassium channel, Kv2.2

Similar to Kv2.1 channels, we investigated the effect of these herbal medicines on Kv2.2 channels. Under control conditions, step depolarizations from the holding potential of-60 mV elicited Kv2.2 channel currents. A representative current trace of SCF is shown in Figure 4a. SCF inhibited Kv2.2 channel currents in a concentration-dependent manner (100–500 μg/mL) and the peak and quasi steady-state currents (measured at the end of the test pulses) showed a similar degree of suppression during the voltage step pulses. This SCF-dependent inhibition was rapidly reversible. Figure 4b presents the peak I–V relationships of Kv2.2 channel currents in the presence and absence of various concentrations of SCF. Figure 4c summarizes the concentration dependence of the inhibition of Kv2.2 channel currents by SCF. The results shown in Figure 4c are the end of current values at +50 mV and were normalized to the current amplitude in the absence of SCF. A nonlinear least-square fit of the logistic function to the concentration-response data yielded an apparent IC_{50} value of 103.1 μg/mL. In addition, other herbal medicines, such as AMK, *M. officinalis*, and *A. canaliculatum*, also inhibited Kv2.2 channel currents in a concentration-dependent manner (100–500 μg/mL). Figures 5Aa, Da and 6Aa present the peak I–V relationships of Kv2.2 channel currents in the presence and absence of various concentrations of AMK, *M. officinalis*, and *A. canaliculatum*. Figures 5Ab, Db and 6Ab summarize the concentration dependence of the inhibition of Kv2.1 channel currents by AMK, *M. officinalis*, and *A. canaliculatum*. The results shown in Figures 5Ab, Db and 6Ab are the end of current values at +50 mV and were normalized to the current amplitude in the absence of AMK, *M. officinalis*, and *A. canaliculatum*. A nonlinear least-square fit of the logistic function to the concentration–response data yielded apparent IC_{50} values of 42.1 μg/mL for AMK, 96.0 μg/mL for *M. officinalis*, and 161.3 μg/mL for *A. canaliculatum*. However, PCW, CUP, and RR (100–500 μg/mL) had no effect on Kv2.2 channel currents [Figures 5B, C and 6B]. These results suggested that PCW, CUP, and RR inhibited Kv2.1 channel currents in a concentration-dependent manner.

Effects of *Rehmanniae Radix*, *Citrus unshiu* peel, and *Poria cocos* Wolf on insulin secretion

Peripheral insulin resistance and defects in insulin secretion from pancreatic β-cells characterize type 2 diabetes mellitus.[1] Voltage-dependent outward K^+ currents have been detected in β-cells and are believed to mediate action potential repolarization, limiting Ca^{2+} influx and insulin secretion.[5,9-11] Therefore, we evaluated the effects of RR, CUP, and PCW on insulin secretion using the murine pancreatic β-cell line, NIT-1. RR (3.2 ± 0.3 value, $P < 0.01$), CUP (3.7 ± 0.2 value, $P < 0.01$), and PCW (1.2 ± 0.4 value) enhanced insulin secretion from NIT-1 cells [Figure 7]. These results suggested that administration of RR, CUP, and PCW caused an increase in insulin secretion.

DISCUSSION

Traditional Chinese medicine (TCM) has several thousands of years of empirical clinical practice in several different East Asian countries.[32] One of the major goals of TCM is to restore and maintain the energy and equilibrium of the human body in different systems that are closely connected. In recent years, several herbal products prescribed in TCM have demonstrated a therapeutic beneficial effect on various diseases, including metabolic syndromes, and as therapy for chronic conditions. TCM therapy for diabetes has been used in East Asian countries. Several clinical trials show that TCM can provide diabetic patients with additional benefits, such as decreasing blood glucose levels, ameliorating

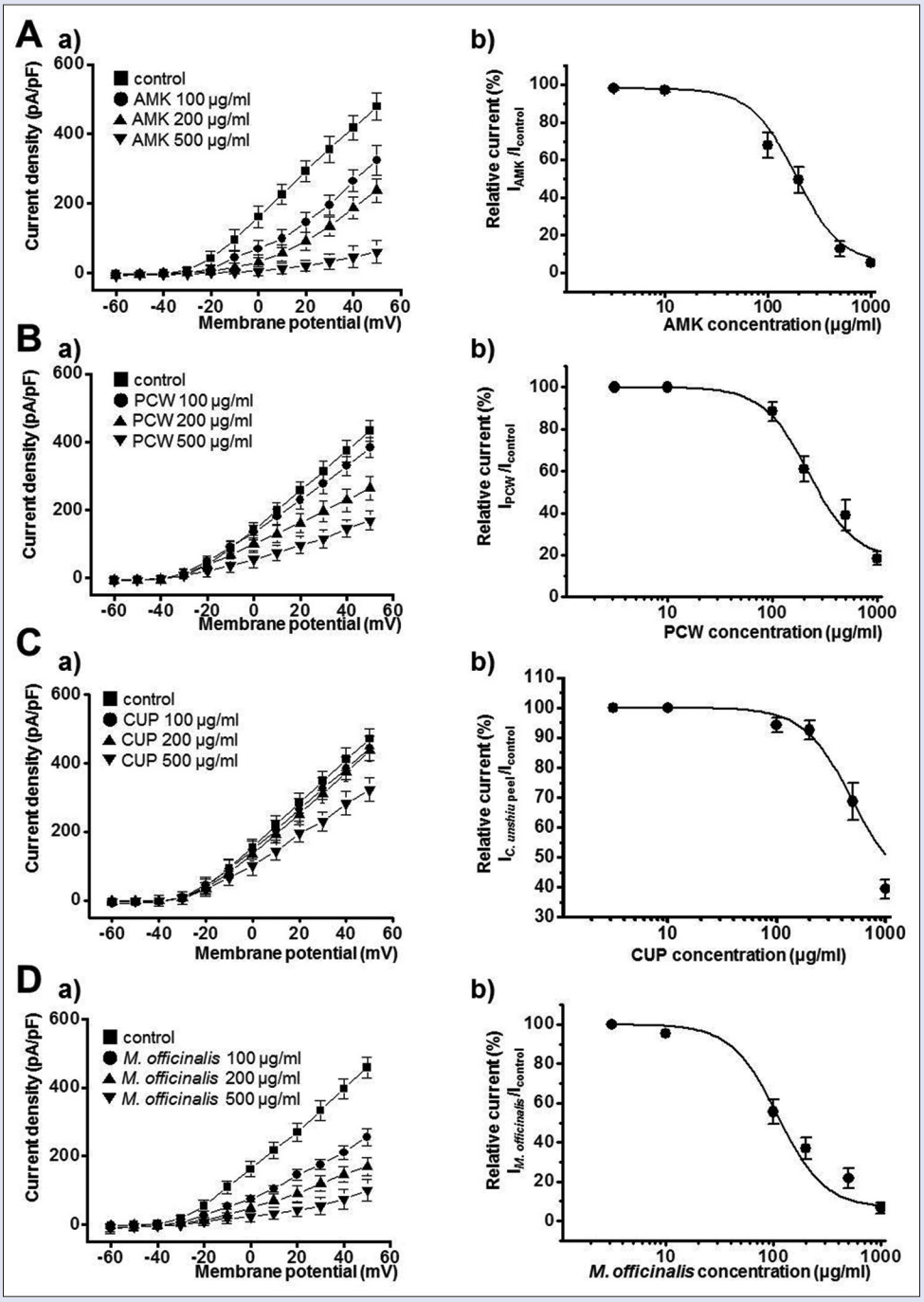

Figure 2: Effects of AMK, PCW, CUP, and *M. officinalis* on Kv2.1 currents. (Aa, Ba, Ca, and Da) Current–voltage relationships of Kv2.1 channel currents in the presence and absence of AMK, PCW, CUP and *M. officinalis* (Ab, Bb, Cb, and Db). The AMK, PCW, CUP and *M. officinalis*-induced inhibition of Kv2.1 channel currents were measured at the end of a 250-ms depolarizing pulse of +50 mV and normalized using the control current amplitude. AMK: *Atractylodes macrocephala* Koidzumi; PCW: *Poria cocos* Wolf; CUP: *Citrus unshiu* peel; *M. officinalis*: *Magnolia officinalis* Bark; Kv: Voltage-dependent K^+

insulin resistance and pancreatic islets function, weight loss, and low incidence of adverse events.[33-35] Pharmacological studies demonstrated that TCM could rehabilitate and ameliorate islet beta-cell trauma, increase insulin secretion, and strengthen the utilization of glucose in peripheral tissues.[26]

In the present study, we used eight herbal medicines, depending on whether they regulated Kv2 channels and insulin secretion. Several studies demonstrated the diverse pharmacological activities of SCF, including antioxidant,[36] antitumor,[37] anti-obesity,[38] anti-inflammatory,[39] cardioprotective,[40] hepatoprotective,[41] and gastrointestinal motility activities.[42] In addition, SCF had beneficial effects on metabolic diseases, including diabetes and obesity.[43,44] SCF inhibited preadipocyte differentiation and adipogenesis in 3T3-L1 cells, leading to decreased body weight and fat mass in high fat diet (HFD)-induced obese rats.[45] AMK is used to treat diabetes and obesity[46] and has been reported to reduce body weight and serum lipid levels in HFD-induced animal models of obesity.[47] In addition, *Atractylodes macrocephala* Koidzumi (Atractylodis Rhizoma Alba (ARA)) prevented diet-induced obesity and

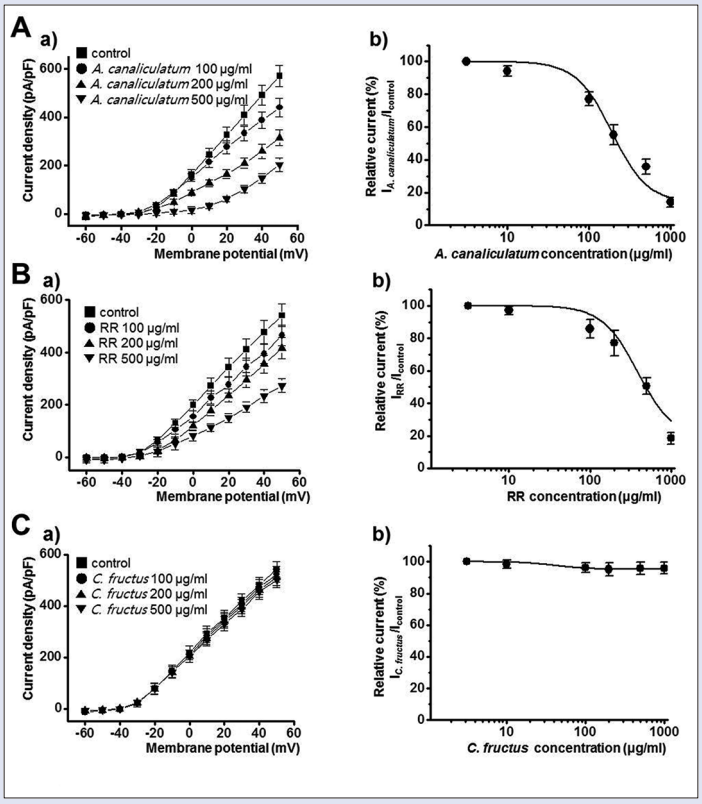

Figure 3: Effects of *A. canaliculatum*, RR, and *C. fructus* on Kv2.1 currents. (Aa, Ba, and Ca) Current–voltage relationships of Kv2.1 channel currents in the presence and absence of *A. canaliculatum*, RR, and *C. fructus* (Ab, Bb, and Cb). Average concentration dependence of Kv2.1 channel current inhibition by *A. canaliculatum* and RR. However, *C. fructus* had no effect on Kv2.1 channel currents. *A. canaliculatum*: *Alisma canaliculatum*; RR: *Rehmanniae* Radix; *C. fructus*: *Corni fructus*; Kv channel: Voltage-dependent K^+ channel

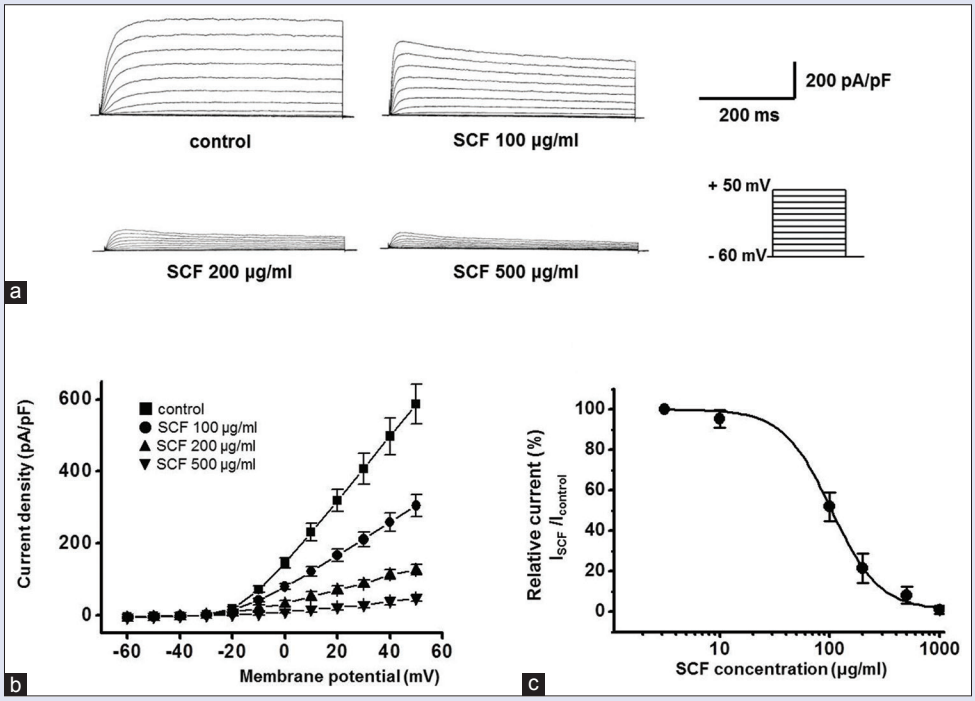

Figure 4: Effects of SCF on Kv2.2 currents. (a) The superimposed current traces were elicited by 250-ms depolarizing pulses between −60 and +50 mV from a holding potential of −60 mV in the absence and presence of SCF. (b) Current–voltage relationships of the peak and quasi-steady-state Kv2.2 channel currents in the presence and absence of SCF. (c) The SCF-induced inhibition of Kv2.2 channel currents was measured at the end of a 250-ms depolarizing pulse of +50 mV and normalized using the control current amplitude. SCF: *Schisandra chinensis*; Kv: Voltage-dependent K^+

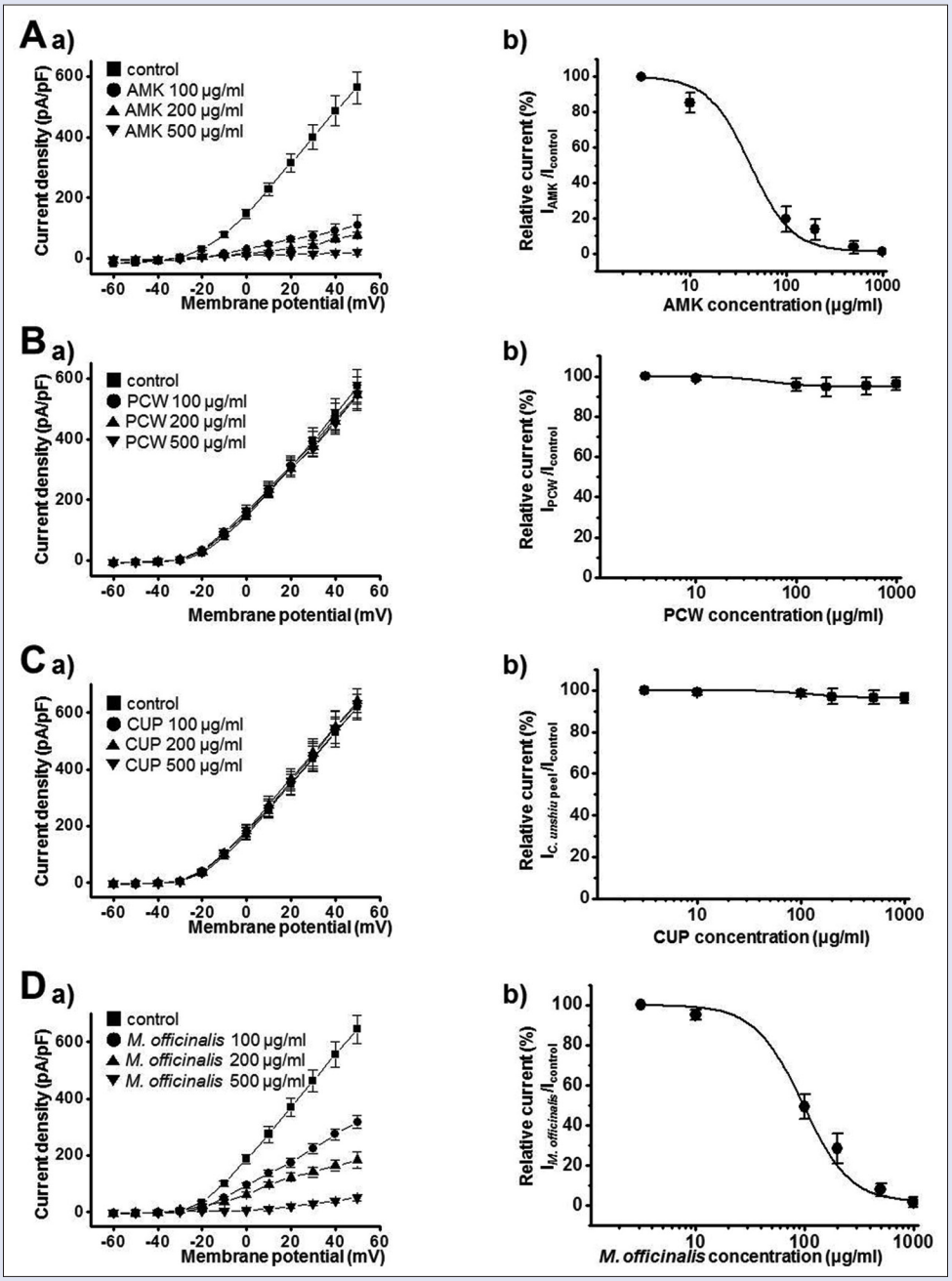

Figure 5: Effects of AMK, PCW, CUP, and *M. officinalis* on Kv2.2 currents. (Aa, Ba, Ca, and Da) Current–voltage relationships of Kv2.2 channel currents in the presence and absence of AMK, PCW, CUP and *M. officinalis* (Ab, Bb, Cb, and Db). Average concentration dependence of Kv2.2 channel current inhibition by AMK and *M. officinalis*. However, PCW and CUP had no effect on Kv2.2 channel currents. AMK: *Atractylodes macrocephala* Koidzumi; PCW: *Poria cocos* Wolf; CUP: *Citrus unshiu* peel; *M. officinalis*: *Magnolia officinalis* Bark; Kv: Voltage-dependent K^+

glucose intolerance in C5BL/6 mice.[48] PCW has been used in China for the treatment of many types of diseases with signs of a deficiency of *Yin* in the kidneys and it improved insulin resistance in diabetes mellitus, in part, by regulating the canonical PI3K/Akt signaling pathway in the liver.[49] Mice supplemented with CUP extract also displayed a significant decrease in body weight gain and body fat mass,[50] and hepatic steatosis and hypertriglyceridemia were ameliorated via the inhibition of gene expression and increased activation of lipogenic enzymes and Fatty acid (FA) oxidation in the liver.[50] Therefore, CUP had a beneficial effect on the metabolic syndrome.[51] *M. officinalis* has been widely used as a traditional herbal remedy for various disorders such as obesity, hyperglycemia, hyperlipidemia, and diabetic complications, including cardiovascular, hepatic, and renal complications.[52] *A. canaliculatum*, a member of the plant family *Alismataceae*, has hepatoprotective,[53] antitumor,[54] and antibacterial effects.[55] RR is a perennial herb that belongs to the *Scrophulariaceae* family and has been used as an important ingredient in a variety of oriental medicines.[56] Recently, many studies have evaluated its effects on antioxidants,[57,58] the autonomic nervous system,[59] and the human body.[60,61] In addition, RR is effective for treating patients with various inflammatory and metabolic diseases, such as high blood pressure and diabetes.[62] *C. fructus* is an important crude herb used in TCM, and it is one of the 25 plant-based drugs most

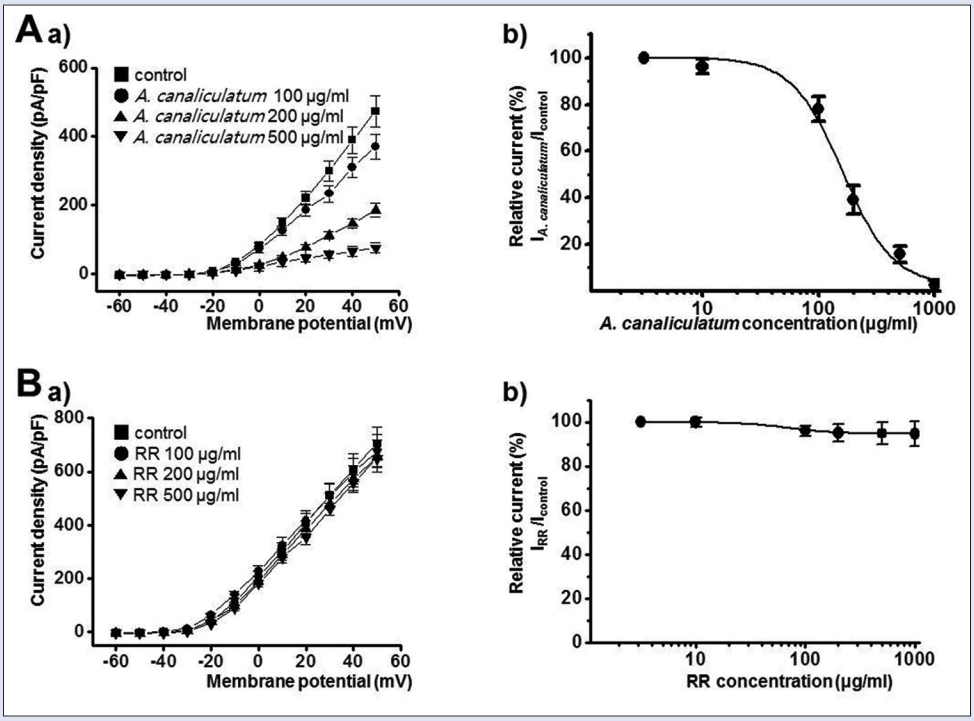

Figure 6: Effects of *A. canaliculatum* and RR on Kv2.2 currents. (Aa, Ba and Ca) Current–voltage relationships of Kv2.2 channel currents in the presence and absence of *A. canaliculatum* and RR (Ab, Bb, and Cb). Average concentration dependence of Kv2.2 channel current inhibition by *A. canaliculatum* and RR. However, RR had no effect on Kv2.2 channel currents. *A. canaliculatum*: *Alisma canaliculatum*; RR: *Rehmanniae* Radix; Kv channel: Voltage-dependent K^+ channel

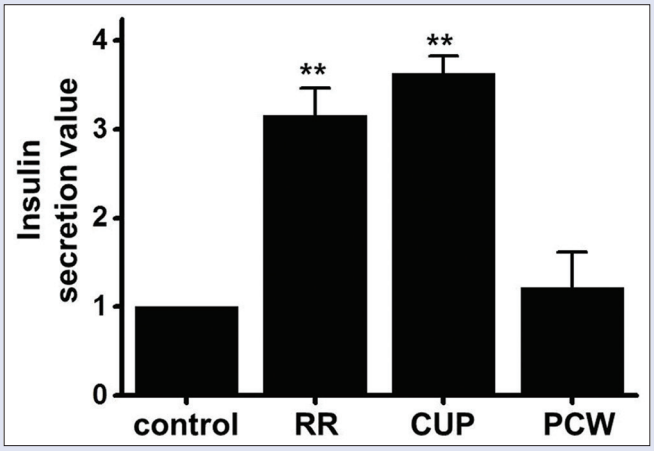

Figure 7: RR, CUP, and PCW enhance insulin secretion in pancreatic β-cells. RR, CUP, and PCW enhanced insulin secretion from NIT-1 cells compared with controls ($n = 6$). $^{**}P < 0.01$ compared with control. CUP: *Citrus unshiu* peel; RR: *Rehmanniae Radix*; PCW: *Poria cocos* Wolf

frequently used in China, Japan, and Korea. It is known to exhibit several biological activities, including hypoglycemic activity, and to improve liver and kidney function.[63] In addition, *C. fructus* has beneficial effects on diabetes and diabetic complications.[64]

Kv channels open in response to membrane depolarization and are present in many cell types. In excitable cells, Kv channels serve as the primary mechanism of repolarization of action potentials, whereas in nonexcitable cells, Kv channels control the cell resting potential.[21] The Kv2 channel family consists of two members, Kv2.1 and Kv2.2. Kv2.1 is prominently expressed in the brain, notably in the pyramidal neurons of the hippocampus and cortex, where it regulates excitability.[65] Kv2.1 also regulates insulin secretion from the pancreatic β-cells.[20] Kv2.2 is expressed in the brain,[66] smooth muscle,[67] and somatostatin secreting δ-cells of the pancreatic islet.[16,68] Development of selective Kv2.1 inhibitors without cross-inhibition of Kv2.2 might provide new avenues for the treatment of type 2 diabetes mellitus.[7,18-23]

In the present study, SCF, AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR inhibited Kv2.1 channel currents in a concentration-dependent manner (100–500 μg/mL) [Figures 2Aa, Ba, Ca, Da and 3Aa, Ba]. However, *C. fructus* had no effect on Kv2.1 channel currents [Figure 3C]. In addition, among SCF, AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR, only PCW, CUP, and RR had no effect on Kv2.2 channel currents [Figures 5B, C and 6B]. Therefore, we concluded that only PCW, CUP, and RR played key roles in insulin secretion. In addition, we evaluated the enhancement of insulin secretion after administration of PCW, CUP, and RR [Figure 7]. However, we do not know the active components of herbal medicines. Therefore, in the future, we will determine the efficacious components to clarify the precise mechanisms of these herbal medicines in diabetes mellitus.

CONCLUSION

The present study shows that SCF, AMK, PCW, CUP, *M. officinalis*, *A. canaliculatum*, and RR inhibit Kv2.1 channel currents in a concentration-dependent manner. Among these, only PCW, CUP, and RR have no effects on Kv2.2 channel currents. In addition, PCW, CUP, and RR cause an increase in insulin secretion. Based on these results, we conclude that PCW, CUP, and RR are potential novel agents for the prevention and treatment of diabetes.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nat Med 2017;23:804-14.
2. Lee YS, Jun HS. Glucagon-like peptide-1 receptor agonist and glucagon increase glucose-stimulated insulin secretion in beta cells via distinct adenylyl cyclases. Int J Med Sci 2018;15:603-9.
3. Velasco M, Díaz-García CM, Larqué C, Hiriart M. Modulation of ionic channels and insulin secretion by drugs and hormones in pancreatic beta cells. Mol Pharmacol 2016;90:341-57.
4. Boyd AE 3rd. The role of ion channels in insulin secretion. J Cell Biochem 1992;48:235-41.
5. Ashcroft FM, Gribble FM. ATP-sensitive K+ channels and insulin secretion: Their role in health and disease. Diabetologia 1999;42:903-19.
6. Dunne MJ, Cosgrove KE, Shepherd RM, Ämmälä C. Potassium channels, sulphonylurea receptors and control of insulin release. Trends Endocrinol Metab 1999;10:146-52.
7. MacDonald PE, Sewing S, Wang J, Joseph JW, Smukler SR, Sakellaropoulos G, *et al.* Inhibition of Kv2.1 voltage-dependent K+ channels in pancreatic beta-cells enhances glucose-dependent insulin secretion. J Biol Chem 2002;277:44938-45.
8. Dukes ID, Philipson LH. K+ channels: Generating excitement in pancreatic beta-cells. Diabetes 1996;45:845-53.
9. Philipson LH. Beta-cell ion channels: Keys to endodermal excitability. Horm Metab Res 1999;31:455-61.
10. Su J, Yu H, Lenka N, Hescheler J, Ullrich S. The expression and regulation of depolarization-activated K+ channels in the insulin-secreting cell line INS-1. Pflugers Arch 2001;442:49-56.
11. Atwater I, Ribalet B, Rojas E. Mouse pancreatic beta-cells: Tetraethylammonium blockage of the potassium permeability increase induced by depolarization. J Physiol 1979;288:561-74.
12. Henquin JC, Meissner HP, Preissler M. 9-aminoacridine- and tetraethylammonium-induced reduction of the potassium permeability in pancreatic B-cells. Effects on insulin release and electrical properties. Biochim Biophys Acta 1979;587:579-92.
13. Philipson LH, Rosenberg MP, Kuznetsov A, Lancaster ME, Worley JF 3rd, Roe MW, *et al.* Delayed rectifier K+ channel overexpression in transgenic islets and beta-cells associated with impaired glucose responsiveness. J Biol Chem 1994;269:27787-90.
14. Henquin JC. Tetraethylammonium potentiation of insulin release and inhibition of rubidium efflux in pancreatic islets. Biochem Biophys Res Commun 1977;77:551-6.
15. MacDonald PE, Wheeler MB. Voltage-dependent K(+) channels in pancreatic beta cells: Role, regulation and potential as therapeutic targets. Diabetologia 2003;46:1046-62.
16. Yan L, Figueroa DJ, Austin CP, Liu Y, Bugianesi RM, Slaughter RS, *et al.* Expression of voltage-gated potassium channels in human and rhesus pancreatic islets. Diabetes 2004;53:597-607.
17. Herrington J, Sanchez M, Wunderler D, Yan L, Bugianesi RM, Dick IE, *et al.* Biophysical and pharmacological properties of the voltage-gated potassium current of human pancreatic beta-cells. J Physiol 2005;567:159-75.
18. Li XN, Herrington J, Petrov A, Ge L, Eiermann G, Xiong Y, *et al.* The role of voltage-gated potassium channels Kv2.1 and Kv2.2 in the regulation of insulin and somatostatin release from pancreatic islets. J Pharmacol Exp Ther 2013;344:407-16.
19. Tamarina NA, Kuznetsov A, Fridlyand LE, Philipson LH. Delayed-rectifier (KV2.1) regulation of pancreatic beta-cell calcium responses to glucose: Inhibitor specificity and modeling. Am J Physiol Endocrinol Metab 2005;289:E578-85.
20. Jacobson DA, Kuznetsov A, Lopez JP, Kash S, Ammälä CE, Philipson LH, *et al.* Kv2.1 ablation alters glucose-induced islet electrical activity, enhancing insulin secretion. Cell Metab 2007;6:229-35.
21. Herrington J, Solly K, Ratliff KS, Li N, Zhou YP, Howard A, *et al.* Identification of novel and selective Kv2 channel inhibitors. Mol Pharmacol 2011;80:959-64.
22. MacDonald PE, Ha XF, Wang J, Smukler SR, Sun AM, Gaisano HY, *et al.* Members of the Kv1 and Kv2 voltage-dependent K(+) channel families regulate insulin secretion. Mol Endocrinol 2001;15:1423-35.
23. Dai XQ, Manning Fox JE, Chikvashvili D, Casimir M, Plummer G, Hajmrle C, *et al.* The voltage-dependent potassium channel subunit Kv2.1 regulates insulin secretion from rodent and human islets independently of its electrical function. Diabetologia 2012;55:1709-20.
24. Colberg SR, Zarrabi L, Bennington L, Nakave A, Thomas Somma C, Swain DP, *et al.* Postprandial walking is better for lowering the glycemic effect of dinner than pre-dinner exercise in type 2 diabetic individuals. J Am Med Dir Assoc 2009;10:394-7.
25. Chan JY, Leung PC, Che CT, Fung KP. Protective effects of an herbal formulation of radix astragali, radix codonopsis and cortex lycii on streptozotocin-induced apoptosis in pancreatic beta-cells: An implication for its treatment of diabetes mellitus. Phytother Res 2008;22:190-6.
26. Yin J, Zhang H, Ye J. Traditional Chinese medicine in treatment of metabolic syndrome. Endocr Metab Immune Disord Drug Targets 2008;8:99-111.
27. Shetty YC, Godbharle S, Brahma S, Salgaonkar S, Rege NN. Evaluation of oral multi-herbal preparation of *Dashmoolarishta* on mice model of osteoarthritis. J Basic Clin Physiol Pharmacol 2017;28:583-91.
28. Yeo J, Kang YM, Cho SI, Jung MH. Effects of a multi-herbal extract on type 2 diabetes. Chin Med 2011;6:10.
29. Xiangwen G, Jinwen Z, Qinhe Y, Haizhen Y, Yupei Z, Yizhen L. Application of evidence-based medicine and systems biology mediated by translational medicine in TCM study. J Int Transl Med 2013;4:219-25.
30. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond) 2008;32:1431-7.
31. Jang MK, Yun YR, Kim JH, Park MH, Jung MH. Gomisin N inhibits adipogenesis and prevents high-fat diet-induced obesity. Sci Rep 2017;7:40345.
32. Jang HJ, Um JY, Ko H, Chung WS. Looking for new herbal treatments for metabolic syndrome. Evid Based Complement Alternat Med 2016;2016:7198490.
33. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet 2005;365:1415-28.
34. Jang S, Jang BH, Ko Y, Sasaki Y, Park JS, Hwang EH, *et al.* Herbal medicines for treating metabolic syndrome: A systematic review of randomized controlled trials. Evid Based Complement Alternat Med 2016;2016:5936402.
35. Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol 2004;92:1-21.
36. Choi YW, Takamatsu S, Khan SI, Srinivas PV, Ferreira D, Zhao J, *et al.* Schisandrene, a dibenzocyclooctadiene lignan from *Schisandra chinensis*: Structure-antioxidant activity relationships of dibenzocyclooctadiene lignans. J Nat Prod 2006;69:356-9.
37. Min HY, Park EJ, Hong JY, Kang YJ, Kim SJ, Chung HJ, *et al.* Antiproliferative effects of dibenzocyclooctadiene lignans isolated from *Schisandra chinensis* in human cancer cells. Bioorg Med Chem Lett 2008;18:523-6.
38. Park HJ, Kim HJ, Kim SR, Choi MS, Jung UJ. Omija fruit ethanol extract improves adiposity and related metabolic disturbances in mice fed a high-fat diet. J Nutr Biochem 2017;41:137-41.
39. Choi MS, Kwon KJ, Jeon SJ, Go HS, Kim KC, Ryu JR, *et al. Schizandra chinensis* alkaloids inhibit lipopolysaccharide-induced inflammatory responses in BV2 microglial cells. J Biomol Tech 2009;17:47-56.
40. Chun JN, Cho M, So I, Jeon JH. The protective effects of *Schisandra chinensis* fruit extract and its lignans against cardiovascular disease: A review of the molecular mechanisms. Fitoterapia 2014;97:224-33.
41. Park HJ, Lee SJ, Song Y, Jang SH, Ko YG, Kang SN, *et al. Schisandra chinensis* prevents alcohol-induced fatty liver disease in rats. J Med Food 2014;17:103-10.
42. Ahn TS, Kim DG, Hong NR, Park HS, Kim H, Ha KT, *et al.* Effects of *Schisandra chinensis* extract on gastrointestinal motility in mice. J Ethnopharmacol 2015;169:163-9.
43. Niu J, Xu G, Jiang S, Li H, Yuan G. *In vitro* antioxidant activities and anti-diabetic effect of a polysaccharide from *Schisandra sphenanthera* in rats with type 2 diabetes. Int J Biol Macromol 2017;94:154-60.
44. Panossian A, Wikman G. Pharmacology of *Schisandra chinensis* bail: An overview of Russian research and uses in medicine. J Ethnopharmacol 2008;118:183-212.
45. Park HJ, Cho JY, Kim MK, Koh PO, Cho KW, Kim CH, *et al.* Anti-obesity effect of *Schisandra chinensis* in 3T3-L1 cells and high fat diet-induced obese rats. Food Chem 2012;134:227-34.
46. Huang Y, Wang L, Wang S, Cai F, Zheng G, Lu A, *et al.* Treatment principles of obesity with Chinese herbal medicine: Literature analysis by text mining. Engineering 2013;5:7-11.
47. Kim CK, Kim M, Oh SD, Lee SM, Sun B, Choi GS, *et al.* Effects of *Atractylodes macrocephala* Koidzumi rhizome on 3T3-L1 adipogenesis and an animal model of obesity. J Ethnopharmacol 2011;137:396-402.

48. Song MY, Lim SK, Wang JH, Kim H. The root of *Atractylodes macrocephala* koidzumi prevents obesity and glucose intolerance and increases energy metabolism in mice. Int J Mol Sci 2018;19. pii: E278.
49. Dai B, Wu Q, Zeng C, Zhang J, Cao L, Xiao Z, *et al*. The effect of Liuwei Dihuang decoction on PI3K/Akt signaling pathway in liver of type 2 diabetes mellitus (T2DM) rats with insulin resistance. J Ethnopharmacol 2016;192:382-9.
50. Park HJ, Jung UJ, Cho SJ, Jung HK, Shim S, Choi MS. *Citrus unshiu* peel extract ameliorates hyperglycemia and hepatic steatosis by altering inflammation and hepatic glucose- and lipid-regulating enzymes in db/db mice. J Nutr Biochem 2013;24:419-27.
51. Alam MA, Subhan N, Rahman MM, Uddin SJ, Reza HM, Sarker SD, *et al*. Effect of *Citrus* flavonoids, naringin and naringenin, on metabolic syndrome and their mechanisms of action. Adv Nutr 2014;5:404-17.
52. Zhao X, Li F, Sun W, Gao L, Kim KS, Kim KT, *et al*. Extracts of *Magnolia* species-induced prevention of diabetic complications: A brief review. Int J Mol Sci 2016;17. pii: E1629.
53. Hong X, Tang H, Wu L, Li L. Protective effects of the *Alisma orientalis* extract on the experimental nonalcoholic fatty liver disease. J Pharm Pharmacol 2006;58:1391-8.
54. Huang YT, Huang DM, Chueh SC, Teng CM, Guh JH. Alisol B acetate, a triterpene from *Alismatis rhizoma*, induces bax nuclear translocation and apoptosis in human hormone-resistant prostate cancer PC-3 cells. Cancer Lett 2006;231:270-8.
55. Mikamo H, Kawazoe K, Izumi K, Sato Y, Tamaya T. Effects of crude herbal ingredients on intrauterine infection in a rat model. Curr Ther Res 1998;59:122-7.
56. Chae BS, Yang JH. Regulatory effect of fresh *Rehmanniae radix* extract on the *in vitro* production of proinflammatory cytokines in pristane-induced lupus mice. Nat Prod Sci 2007;13:322-7.
57. Sui Z, Li L, Liu B, Gu T, Zhao Z, Liu C, *et al*. Optimum conditions for radix *Rehmanniae* polysaccharides by RSM and its antioxidant and immunity activity in UVB mice. Carbohydr Polym 2013;92:283-8.
58. Kang KH, Lee KH, Yoon HM, Jang KJ, Song C, Kim CH. *Rehmannia glutinosa* pharmacopuncture solution regulates functional activation, fcεRI expression, and signaling events in mast cells. J Pharmacopuncture 2012;15:32-41.
59. Yook T, Yu J, Lee H, Song B, Kim L, Roh J, *et al*. Comparing the effects of distilled *Rehmannia glutinosa*, wild ginseng and astragali radix pharmacopuncture with heart rate variability (HRV): A randomized, sham-controlled and double-blind clinical trial. J Acupunct Meridian Stud 2009;2:239-47.
60. Choi JH, Chung MJ, Oh DH. Classification of sasang constitutional body types using immunostimulatory activities of constitution-specific herbal extracts in human primary immune cells. J Med Food 2012;15:824-34.
61. Lee B, Shim I, Lee H, Hahm DH. *Rehmannia glutinosa* ameliorates scopolamine-induced learning and memory impairment in rats. J Microbiol Biotechnol 2011;21:874-83.
62. Kim SH, Yook TH, Kim JU. *Rehmanniae radix*, an effective treatment for patients with various inflammatory and metabolic diseases: Results from a review of Korean publications. J Pharmacopuncture 2017;20:81-8.
63. Vareed SK, Reddy MK, Schutzki RE, Nair MG. Anthocyanins in *Cornus alternifolia*, *Cornus controversa*, *Cornus kousa* and *Cornus florida* fruits with health benefits. Life Sci 2006;78:777-84.
64. Yokozawa T, Kang KS, Park CH, Noh JS, Yamabe N, Shibahara N, *et al*. Bioactive constituents of *Corni fructus*: The therapeutic use of morroniside, loganin, and 7-O-galloyl-D-sedoheptulose as renoprotective agents in type 2 diabetes. Drug Discov Ther 2010;4:223-34.
65. Misonou H, Mohapatra DP, Trimmer JS. Kv2.1: A voltage-gated K+ channel critical to dynamic control of neuronal excitability. Neurotoxicology 2005;26:743-52.
66. Hwang PM, Glatt CE, Bredt DS, Yellen G, Snyder SH. A novel K+ channel with unique localizations in mammalian brain: Molecular cloning and characterization. Neuron 1992;8:473-81.
67. Schmalz F, Kinsella J, Koh SD, Vogalis F, Schneider A, Flynn ER, *et al*. Molecular identification of a component of delayed rectifier current in gastrointestinal smooth muscles. Am J Physiol 1998;274:G901-11.
68. Wolf-Goldberg T, Michaelevski I, Sheu L, Gaisano HY, Chikvashvili D, Lotan I. Target soluble N-ethylmaleimide-sensitive factor attachment protein receptors (t-SNAREs) differently regulate activation and inactivation gating of Kv2.2 and Kv2.1: Implications on pancreatic islet cell Kv channels. Mol Pharmacol 2006;70:818-28.