Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas
ISSN: 0717-7917
editor.blacpma@usach.cl
Universidad de Santiago de Chile
Chile

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Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, vol. 11, núm. 6, noviembre, 2012, pp. 510-519
Universidad de Santiago de Chile
Santiago, Chile

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ISSN 0717 7917
www.blacpma.usach.cl

Artículo Original | Original Article

Influence of two hypoglycemic Cucurbitaceae (*Cucurbita ficifolia* Bouché and *Ibervillea sonorae* Greene) on ATP-sensitive potassium channels in rat aortic rings

[Influencia de dos Cucurbitaceae (Cucurbita ficifolia Bouché e Ibervillea sonorae Greene) sobre los canales de potasio sensibles a ATP en anillos aórticos de rata]

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Abstract
Cucurbita ficifolia Bouché fruit containing D-chiro-inositol and *Ibervillea sonorae* Greene root containing cucurbitane-type glycosides are used to control diabetes in Mexico. Although the hypoglycemic effect of both plants has been demonstrated and some active compounds proposed, their mechanisms are still unknown. The aim of this study was to determine if the incubation with both aqueous extracts avoids the inhibition of contraction induced by phenylephrine similarly to glibenclamide in rat aortic rings. The hypoglycemic aqueous extracts of *C. ficifolia* and *I. sonorae* were characterized for their content of either *D*-chiro inositol or cucurbitanes respectively, and then we assayed the characterized extracts *in vitro* on the diazoxide-induced relaxation of rat aortic rings precontracted with phenylephrine, using as positive control glibenclamide. *I. sonorae* extract blocked the K_{ATP} channels in a concentration-dependent manner ($p < 0.05$), whereas *C. ficifolia* extract had no effect on these channels. *I. sonorae* extract produces a hypoglycemic effect through a similar mechanism to sulphonylureas in this experimental model; however, hypoglycemic action of *C. ficifolia* extract should be explained by an independent K_{ATP} channels mechanism.

Keywords: Cucurbita ficifolia, Ibervillea sonorae, Cucurbitaceae, hypoglycemic plants, diabetes mellitus, vascular smooth muscle, K_{ATP} channels.

Resumen
Los frutos de *Cucurbita ficifolia* conteniendo D-quiro-inositol y las raíces de *Ibervillea sonorae* contteniendo glucósidos tipo cucurbitano son empleados en el control de la diabetes en México. Aunque el efecto hipoglucémico de ambas plantas ha sido demostrado y se han propuesto algunos de sus compuestos activos, aún se desconoce su mecanismo de acción. El objetivo de este estudio fue determinar si la incubación con ambos extractos acuosos evita la inhibición de la contracción inducida por fenilefrina de manera similar a la glibenclamida en anillos aórticos de rata. Los extractos acuosos hipoglucémicos de *C. ficifolia* e *I. sonorae* fueron caracterizados en su contenido de D-quiro inositol o cucurbitanos, respectivamente y entonces fueron estudios en un modelo *in vitro* en la relajación inducida por diazóxido en anillos aórticos previamente contraídos con fenilefrina, usando como control positivo glibenclamida. El extracto de *Ibervillea sonorae* bloqueó los canales K_{ATP} de manera dosis-dependiente ($p < 0.05$), mientras que *Cucurbita ficifolia* no tuvo efecto en esos canales. El extracto de *I. sonorae* produce efecto hipoglucémico a través de un mecanismo similar al de las sulfonilureas en este modelo experimental; sin embargo, la acción hipoglucémica del extracto de *C. ficifolia* puede ser explicado mediante un mecanismo independiente de los canales K_{ATP}.

Palabras Clave: Cucurbita ficifolia, Ibervillea sonorae, Cucurbitaceae, plantas hipoglucémicas, diabetes mellitus, músculo liso vascular, canales K_{ATP}

Recibido | Received: March 8, 2012.
Aceptado en versión corregida | Accepted in revised form: May 7, 2012.
Publicado en línea | Published online: November 30, 2012.
Declaración de intereses | Declaration of interests: This research was partially supported by the International Foundation for Science, Stockholm, Sweden; the Organization for the Prohibition of Chemical Weapons, The Hague, The Netherlands, through a grant to Francisco Javier Alarcon-Aguilar, PhD (Research Grant Agreement No. F/3338-2F); and by the PIFI2-PROMEP-SEP (Project P/CA-15-2006-35.53).
Este artículo puede ser citado como / This article must be cited as: Tania Rosario Banderas-Dorantes, Ruben Roman-Ramos, Alejandro Zamilpa, Rebeca García-Macedo, Margarita Díaz, Maria Guadalupe Campos, Jaime Tortoriello, Francisco Javier Alarcón-Aguilar. 2012. Influence of two hypoglycemic Cucurbitaceae (*Cucurbita ficifolia* Bouché and *Ibervillea sonorae* Greene) on ATP-sensitive potassium channels in rat aortic rings. **Bol Latinoam Caribe Plant Med Aromat** 11(6): 510 – 519.

***C. ficifolia* - *Cucurbita ficifolia*; ^{13}C NMR – Carbon 13 Nuclear Magnetic Resonance Spectroscopy; DCI- *D*-chiro- inositol; GLP-1- glucagon-like peptide; HPLC – High Performance liquid chromatography; IMSSM – (Del español) Instituto Mexicano del Seguro Social, Mexico; *I. sonorae* – *Ibervillea sonorae*; K_{ATP} channels – ATP-sensitive potassium channels; KCOs – K^+ channel opener; KRB - Krebs-Ringer bicarbonate solution; STZ – streptozotocin; SU -sulfonylurea; T2D - type 2 diabetes; VSM - vascular smooth muscle.**

INTRODUCCIÓN

ATP-sensitive potassium channels (K_{ATP} channels) in pancreatic ß-cells are biological sensors of blood glucose levels, which regulate insulin secretion through modulation of membrane excitability (Akrouh *et al*., 2009). These channels are expressed in distinct muscle tissues, including skeletal, cardiac (Noma, 1983), visceral, and especially in the vascular smooth muscle (VSM), regulating distinct physiological functions (Olson and Terzic, 2010; Gribble and Reimann, 2003).

In the islets of Langerhans, when the extracellular glucose concentration is low, ß-cell metabolism is low, and the K_{ATP} channels are open. As a result, the cell membrane is hyperpolarized, causing voltage-gated Ca^{2+} channels to remain closed. Under these conditions, no insulin is released. If the extracellular glucose concentration rises, the glucose is taken up by the ß-cell and metabolized, generating ATP at the expense of MgADP, thereby closing K_{ATP} channels. The closed potassium channels cause membrane depolarization, opening of voltage-gated Ca^{2+} channels, Ca^{2+} influx and insulin secretion (Clark and Proks, 2010). The K_{ATP} channels further contribute to glucose homeostasis by controlling glucose uptake in skeletal muscle and by controlling GLP-1 (glucagon-like peptide) secretion from L-cells in the gut (Clark and Proks, 2010).

The sulfonylurea (SU) derivatives, such as glibenclamide and diazoxide, act throughout K_{ATP} channels. Particularly, glibenclamide is a K_{ATP} channel blocker and it is considered to be hypoglycemic agent (Koster *et al*., 2005; Clark and Proks, 2010), also it blocks K_{ATP} channel at extra-pancreatic sites such as liver, skeletal, heart and vascular smooth muscle (Luizi and Poza, 1997). In contrast, diazoxide is a potent K^+ channel opener (KCOs), like pinacidil and cromakalim (Miura and Miki, 2003). All these agents represent the most commonly used compounds in the search for new K_{ATP} channel blockers, using VSM as an *in vitro* model (Campos *et al*., 2009; Remedi and Koster, 2010).

In addition to antidiabetic agents, medicinal plants are also used to control diabetes mellitus in Mexico. Such is the case of the mature fruit of *Cucurbita ficifolia* Bouché (*C. ficifolia*), popularly known like "chilacayote," and the root of *Ibervillea sonorae* Greene (*I. sonorae*), popularly known like "wareque"; two Cucurbitaceae species ingested as antidiabetic remedies, both prepared as an infusion (Aguilar *et al*., 2006; Figueroa, 2009).

The phytochemical studies performed in *C. ficifolia* have showed that the fruit contains *D*-chiro-inositol (DCI), which has been proposed as one of the main active compounds in the aqueous extract (Xia and Wang, 2006a); in contrast, *I. sonorae* contains cucurbitanes and cucurbitane-type glycosides and aglycons (Achenbach *et al*., 1993). In addition, eleven monoglycerides and five fatty acids in an *Ibervillea sonorae* dichloromethane extract were proposed as the compounds that induce the anti-hyperglycemic effect (Hernandez-Galicia *et al*., 2007).

In previous pharmacological studies, the hypoglycemic effects of both *C. ficifolia* and *I. sonorae* were demonstrated in normal and alloxan-induced diabetes rodents (Roman-Ramos *et al*., 1991, 1992; Alarcon-Aguilar *et al*., 2002a, 2002b, 2005), suggesting that this activity is mediated by incremented insulin levels (Xia and Wang, 2006b). However, the mechanisms underlying this activity are yet unknown. Therefore, The aim of this study was to see If the incubation with aqueous extracts of *C. ficifolia* and *I. sonorae* beat down the inhibition of contraction induced by phenylephrine similarly to glibenclamide in rat aortic rings, which eventually may be associated with an rise in the secretion of insulin by the pancreatic ß-cells in a similar manner to hypoglycemic SU derivatives, such as glibenclamide.

MATERIAL AND METHODS

Plant material

Fresh mature fruits of *C. ficifolia* with diameters of 18−20 cm were gathered in San Bartolo el Chico, Acolman Estado de Mexico, during October, 2010. Roots of *I. sonorae* with diameters of 25-28 cm were gathered in Carbo Sonora, Mexico, during April, 2011. Botanical identification was performed by botanical experts from the Mexican Institute of Social Security, Mexico (IMSSM) Herbarium (Voucher Specimen Num. 11119 for *C. ficifolia* and 14184 for *I. sonorae*).

The seed-free endocarp of the fruit of *C. ficifolia* and the epidermis-free root of *I. sonorae* were used.

Immediately after collection, the vegetal material was dried on a flat surface in dark conditions and with constant ventilation. The seed-free endocarp of *C. ficifolia* and the epidermis-free root of *I. sonorae* were dried at room temperature and ground using a 2-mm mesh in a Model 4 Wiley electric mill.

Extract preparation

In accordance with traditional use of these plants, an aqueous extract was obtained. Ground material of *C. ficifolia* (250 g) and *I. sonorae* (500 g) was steeped in boiling water (2 L and 1 L, respectively) for 1 h and left to cool to room temperature; the supernatant was filtered and freeze-dried.

HPLC analysis of the extracts

Quantification of D-chiro-inositol in the aqueous extract of C. ficifolia

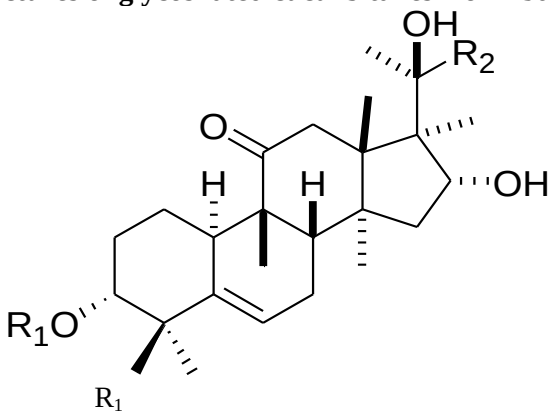
HPLC analysis of the *C. ficifolia* fruit extract was performed using a liquid chromatographic system consisting of a Waters 2695 separation module and a Waters 2697 index refractive detector (Waters; Milford, MA, USA). A LiChrospher™ NH_2 5 µm column (4 x 250 mm, 100 Å) and the mobile phase of an isocratic mixture of CH_3CN/H_2O (90:10) were used. The flow rate was maintained at 1 mL/min for 10 min. Five concentrations (100, 200, 400, 800 and 1000 µg/mL, 20 µL of each concentration) of DCI (≥ 95%) were injected using the same HPLC method.

Quantification of cucurbitane-type glycosides in the aqueous extract of I. sonorae

HPLC analysis of *I. sonorae* was performed using a chromatographic system comprising a Waters 2695 separation module and a Waters 2996 photodiode array detector. A LiChroCART-LiCrospher™ RP-18 5-µm column (4 x 125 mm, 100 Å) was used as the solid phase, and an isocratic CH_3CN/H_2O (70:30) mixture was used as the mobile phase. Five concentrations (50, 100, 200, 400 and 800 µg/mL) of each glycosylated cucurbitane, (**1**) ((22S)-3α-(*β*-D-*Glucopyranosyloxy*)-16α,20,22,25-*tetrahydroxy*-(10α)-cucurbita-5,23*t*-*dien*-11-*one)* and (**2**) 16α,20,25-*Trihydroxy*-3α-(2-O-α-L-*rhamnopyranosyl*-*β*-*D*-*glucopyranosyloxy*)-(10α)-*cucurbit*-5-*ene*-11,22-*dione*, previously isolated from *I. sonorae*, were injected (20 µL) in triplicate. Compound **1** displayed a retention time of 2.2 min, whereas that of compound **2** was of 3.7 min (Figure 1).

The structural identification of these compounds was performed by direct comparison of ^{13}C NMR data with those previously described in the literature (Achenbach *et al*., 1993).

Figure 1
Chemical structures of glycosilated cucurbitanes from *Ibervillea sonorae*



Animals

Male adult CD-1 strain mice (n=30) weighing 30-40 g and male Wistar rats (n = 24) weighing 270 - 300 g were given free access to water and food. All experimental animals were maintained with alternating 12-hour periods of light and dark. The handling of laboratory animals was performed in agreement with the statutes of the ICCUA (Institutional Committee for the Care and Use of the Animals) and the Official Mexican Rule (NOM-062-Z00-1999, revised 2001).

Influence of C. ficifolia and I. sonorae on glycemia and triglyceridemia of streptozotocin-induced diabetic mice

One study in streptozotocin-induced diabetic mice was designed, to verify if the gathered materials in the present investigation conserve the hypoglycemic effects that previously were reported. After an initial acclimation period (7 days), normal mice were injected with streptozotocin (STZ, 136.5 mg/kg) dissolved in a 0.1 M sodium citrate buffer solution at pH 4.5. Blood glucose levels were determined (hydrogenase method, Roche Diagnostics, Mannheim, Germany) seven days after STZ administration. In these conditions, all of the streptozotocin-treated mice developed experimental diabetes, with glycemic values between 350 - 400 mg/dL. Five groups of eight animals each were formed: Group 1, normal mice; Group 2, control diabetic mice, which received injections of isotonic saline solution (ISS) alone (4 ml/kg/day); Group 3, diabetic mice treated with pioglitazone (0.64 mg/kg, Eli Lilly Lab, USA), an insulin sensitizer that reduces hyperglycemia and hypertriglyceridemia in diabetes; Groups 4 and 5 of diabetic mice that received 250 mg/kg/day of both *C. ficifolia* extract (equivalent to 0.19 mg/kg/day of DCI) and *I. sonorae* extract (12.63 mg/kg/day and 19.78 mg/kg/day of compound **1** and compound **2,** respectively). All treatments were administered *per os* for 30 days. At the end of the treatment, blood samples for determination of glycemia were collected from the tail veins of animals with free access to food. Other blood samples were obtained from the orbital sinuses of mice anesthetized with an intra-peritoneal (ip) injection of sodium pentobarbital (25.2 mg/kg) and were collected in Eppendorf tubes to determine triglyceridemia with the Reflotron System (Roche Diagnostics, Mannheim, Germany).

Effect of Cucurbita ficifolia and Ibervillea sonorae extracts on K_{ATP} channels in rat thoracic aorta

Normal male rats were euthanized with ip injections of sodium pentobarbital (75.6 mg/kg). The thoracic aorta was isolated and excess fat and connective tissue was removed for each rat. Rings with intact endothelium, approximately 4 mm long, were cut and placed into an isolated tissue bath (5 mL) in a Krebs-Ringer bicarbonate solution (KRB) with 120 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM K_2HPO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$ and 11 mM glucose, pH 7.2-7.4 (Sigma Chemical Co, St Louis Mo, USA).

Two stainless steel hooks were inserted into the lumen of the isolated aortic rings; one was fixed, and the other was connected to a forced displacement transducer. The contractile activity was registered with transducer (tension 2 g) using Biopac equipment (Biopac System, Santa Barbara, California). The aorta rings were each washed for 15 min with KRB solution, and a mixture of 95% O_2 and 5% CO_2 was bubbled through the solution for 1 h to stabilize the response.

The rings were repeatedly washed with KRB solution to assess the viability of aortic tissues; these tissues were depolarized with a solution of 60 mM KRB-K60, which was identical in composition to the KRB except that the KRB-K solution contained 64.7 mM NaCl and 60 mM KCl instead of 120 mM NaCl and 4.7 mM KCl. To determine the adequate concentrations of *C. ficifolia* and *I. sonorae* extracts for these experiments, the rings were washed for 15 min to recover the basal values and were then incubated for 30 min with different concentrations of the extracts (1 - 300 µg/mL). Finally, the rings were depolarized with KRB-K60 until the depolarization reached a plateau. The concentrations of the extracts were considered innocuous when the tissue response was similar before and after the incubation; these concentrations were used in the study.

Phenylephrine at a concentration of 3.2×10^{-7} M in EtOH/DMSO, 2.5:1 was added to new aortic rings in the bath to induce a sustained contraction; cumulative doses of diazoxide were added (1, 1.8, 3.2, 5.6 and 10×10^{-5}) to inhibit phenylephrine-induced contraction until a plateau had been reached. These rings were incubated for 30 min with the selected concentrations of the extracts of *C. ficifolia* or *I. sonorae* (1, 3, 10 and 30 µg/mL, using a different ring

for each concentration) or with glibenclamide (1 x 10^{-5} M) in ethanol/dimethylsulfoxide (EtOH/DMSO) 2.5:1 or the vehicle (EtOH/DMSO). After the incubation, the phenylephrine-diazoxide curve was repeated to compare the effects of aqueous extracts of *C. ficifolia* or *I. sonorae* against the effects of the standard drug (glibenclamide). Final concentrations of ethanol and DMSO in the bath did not exceed of 0.25 % to 0.1 %, respectively, and proved to be innocuous for the tissues.

Data analysis

Results were expressed as the mean ± standard deviation (SD). Differences among groups were established by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer Multiple Comparison *post-hoc* test. Values of $p < 0.05$ were considered statistically significants. All statistics were computed using the NCSS 2000 software.

The relaxation effect of diazoxide was expressed as a percentage of the contraction induced by 3.2 x 10^{-7} M phenylephrine (mean ± SD). When the tension of the tissue returned to basal values, the relaxation was considered to reach 100%. The R_{max} and IC_{50} values for each concentration-response curve were calculated using a non-linear regression analysis with GraphPad Prism v4.0 for Windows (GraphPad Prism Software, San Diego, CA, USA).

RESULTS

The yields of aqueos extracts were 24.1% (*C. ficifolia)* and 37.4% (*I. sonorae).*

Quantification of D-chiro-inositol and cucurbitane-type glycosides by HPLC

The standard compound, DCI, displayed a retention time of 8.6 min with a linear calibration curve (R^2 = 0.99). The injection of the *C. ficifolia* extract (20 µL, 2 mg/mL), exhibited data that corresponded to a DCI concentration of 0.76 mg/g of extract.

The standard curve was linear (R^2 = 0.99), and the *Ibervillea sonorae* extract (20 µL, 4 mg/mL) was injected in triplicate using the described chromatographic quantitative method. One gram of the *I. sonorae* aqueous extract yielded 50.5 mg of compound **1** and 79.1 mg of compound **2**.

Effects of the aqueous plant extracts on blood glucose and triglyceride levels in streptozotocin-induced diabetic mice

The changes in blood glucose and triglyceride levels in normal and control diabetic (STZ control) groups are shown in Table 1. The glucose and triglyceride levels remained unchanged in the normal group. Both values were significantly higher in the STZ control. Pioglitazone significantly diminished the glucose and triglyceride levels compared with STZ control ($p < 0.05$). The administration of *C. ficifolia* (0.19 mg/g of DCI) or *I. sonorae* (12.63 mg/g of compound **1** and 19.78 mg/g of compound **2**) significantly diminished glucose and triglyceride levels against the STZ control group ($p < 0.05$).

Table 1
Effect of *Cucurbita ficifolia* and *Ibervillea sonorae* extracts on blood glucose levels and triglycerides in streptozotocin (STZ)-induced mice

	Glucose (mg/dL)		Triglycerides (mg/dL)	
	Day 0	Day 30	Day 0	Day 30
Normal	105.16 ± 16.49	110.67 ± 21.41	80.51 ± 20.5	83.78 ± 25.71
Control (STZ)	368.21 ± 15.70	372.80 ± 21.85[a]	270.04 ± 7.88	274.14 ± 6.44[a]
Pioglitazone	357.45 ± 11.24	156.25 ± 16.80[b]	262.31 ± 9.20	70.00 ± 10.00[b]
C. ficifolia	372.22 ± 12.10	148.33 ± 25.18[b]	271.23 ± 5.73	97.81 ± 17.87[b]
I.sonorae	365.28 ± 13.79	197.80 ± 22.79[b]	265.47 ± 8.24	159.66 ± 26.49[b]

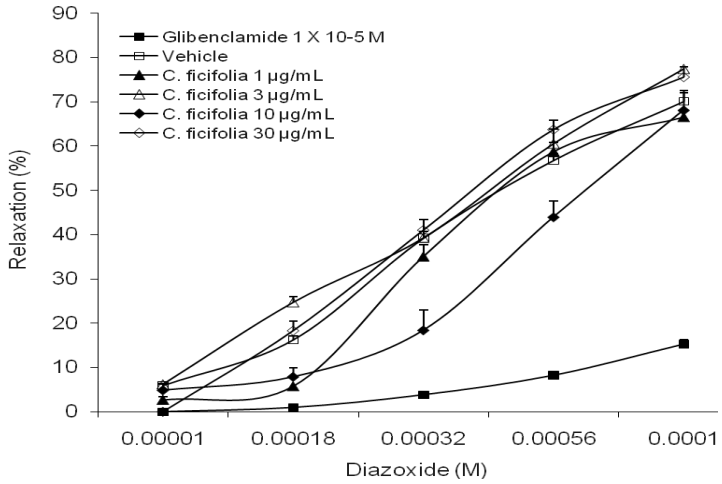
Data represent mean ± standard deviation (n = 8). [a]$p < 0.05$ vs Normal group; [b]$p < 0.05$ vs Control group.
Normal group: Healthy mice
Control group: Diabetic mice by streptozotocin.

Effects of the plant extracts on diazoxide-induced relaxation in rat thoracic aorta

Figures 2 and 3 show that glibenclamide produced a relaxation of 18.32% of the phenylephrine-precontracted aortic rings, whereas incubation with the vehicle alone induced a relaxation of 74.41%, as shown in Table 2. The response maximum (R_{max}) of glibenclamide-treated group was significantly lower than that of the vehicle-treated group ($p < 0.05$).

Figure 2

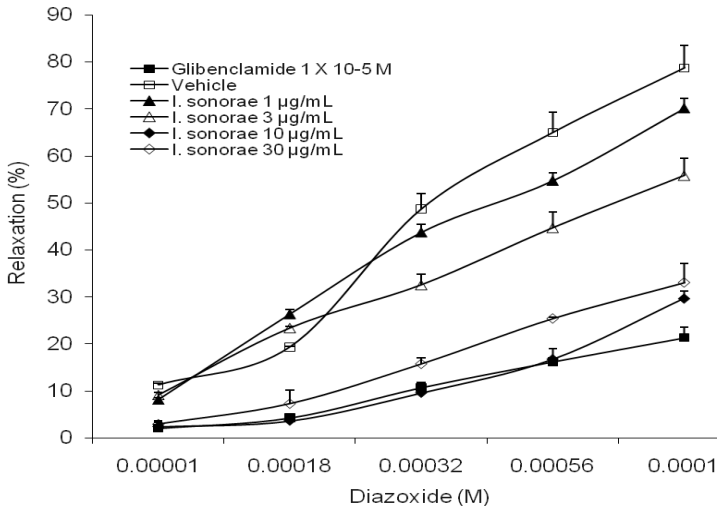
Effect of *Cucurbita ficifolia* on the diazoxide-induced relaxation of aortic rings pre-contracted with phenylephrine (3.2 x 10^{-7} M).



Data are mean of six experiments; vertical bars represent S.D.

Figure 3

Effect of *Ibervillea sonorae* on the diazoxide-induced relaxation of aortic rings pre-contracted with phenylephrine (3.2 x 10^{-7} M)



Data are mean of six experiments; vertical bars represent S.D.

The effects of *C. ficifolia* are shown in Figure 2. The different concentrations of the *C. ficifolia* aqueous extract did not induce relaxation of the pre-contracted tissues ($p > 0.05$), without changes in the R_{max} and IC_{50} in relation to the vehicle (Table 2).

In contrast, the *I. sonorae* aqueous extract (10 and 30 µg/mL) inhibited the diazoxide-induced relaxation in thoracic aorta rings (29.71% and 33.12%, respectively). This effect was similar to the effect of glibenclamide (18.32%), as they exhibited similar R_{max} and IC_{50} values (Figure 3 and Table 2).

Table 2
Relaxation response of rat aortic rings precontracted with phenylephrine to diazoxide in the presence of *C. ficifolia* and *I. sonorae*

Concentration	Treatment	IC_{50}	R_{max}
1 µg/mL	*C. ficifolia*	2.90 ± 0.85	77.46 ± 1.73
	I. sonorae	3.32 ± 0.54	70.27 ± 2.01
3 µg/mL	*C. ficifolia*	2.70 ± 0.96	75.55 ± 1.96
	I. sonorae	2.51 ± 0.04	55.84 ± 3.73
10 µg/mL	*C. ficifolia*	3.20 ± 0.44	66.52 ± 4.45
	I. sonorae	6.81 ± 0.6[a]	33.10 ± 1.59[a]
30 µg/mL	*C. ficifolia*	3.00 ± 0.22	68.12 ± 2.34
	I. sonorae	5.73 ± 0.74[a]	29.71 ± 4.07[a]
(10^{-5}M)	Glibenclamide	8.15 ± 3.86[a]	18.32 ± 1.58[a]
2.5:1	Vehicle	2.80 ± 0.77	74.41 ± 3.36

Data represent mean ± standard error (n=6 for *C. ficifolia* and *I. sonorae*; n=12 for glibenclamide and Vehicle groups).
IC_{50}, expressed like concentration ($1x10^{-5}$M).
R_{max}, expressed like percentage.
[a] $p < 0.05$ vs Vehicle.

DISCUSSION

In previous studies, it was shown that administration of *C. ficifolia* and *I. sonorae* caused significant reductions in both glucose and triglyceride levels in alloxan-induced diabetic rodents. The median lethal dose in normal mice (LD_{50}, *per os*) calculated for *C. ficifolia* was 3,689 mg/kg (Alarcon-Aguilar *et al*., 2002b), whereas for *I. sonorae* the median LD_{50} was 2,158 g (Alarcon-Aguilar *et al*., 2002a). In our study, a dose of 250 mg/kg of *C. ficifolia* and *I. sonorae* extracts significantly reduced glucose and triglyceride levels in streptozotocin-induced diabetic mice. After 30 days of treatment with these extracts, no mice exhibited any side effects such as diarrhea, decreased motor activity or significant weight changes. No macroscopic alterations in the liver, heart, lungs or kidneys were observed in any group. These results confirm the reproducibility of the hypoglycemic effects of both Cucurbitaceae in distinct diabetes models. In fact, the model of diabetes induced by administration of STZ is considered a best model to generate a diabetes most stable and lower severe that with alloxan (McNeill, 1999).

Therefore, our investigation represents the first report of the hypoglycemic effect of *I. sonorae* in this model.

The principal contribution of this study was investigate a possible mechanism of action of *C. ficifolia* and *I. sonorae* aqueous extracts as blockers of the K_{ATP} channels *in vitro*, using activators and

inhibitors of K_{ATP} channels in VSM, such as diazoxide and glibenclamide, respectively.

The results showed that the cucurbitane-characterized extracts from *I. sonorae* inhibited the relaxation of phenylephrine-precontracted thoracic aorta induced by diazoxide. The aqueous extract of the *Ibervillea sonorae* (10 µg corresponding to $5x10^{-4}$ M and $7 x 10^{-4}$ M, and 30 µg corresponding to $1.5 x 10^{-3}$ M and $2.37x10^{-3}$ M of compounds **1** and **2**, respectively) produced a similar response to that observed with glibenclamide in rat thoracic aorta, which was included as a positive control in the *in vitro* study. Like glibenclamide, which is a non-selective K_{ATP} channels blocker *I. sonorae* might act as a modulator of the insulin secretion by pancreatic β-cells; nevertheless, additional studies are needed to confirm this hypothesis. *I. sonorae* aqueous extract blocks the K_{ATP} channels only at concentrations of 30 and 10 µg/mL. A lower percentage of inhibition (29.71%) was observed at 10 µg/mL, an effect that may be explained by the presence of other compounds in the extract, such as alkaloids, glycosides, flavones, steroidal saponins (charantins), sterols, phenolics, cucurbitacin triterpenes and other tetracyclic triterpenes, as well as insulin-like peptides reported in Cucurbitaceae (Marles and Farnsworth, 1995). Interactions between these compounds on these K_{ATP} channels or other receptors could dampen the effect of *I. sonorae*.

In contrast, the aqueous extract of *C. ficifolia* was characterized to determine its DCI content, and it did not show any influence on K_{ATP} channels in rat thoracic aorta. This lack of effect can be attributed to the conformation of the subunits of the K_{ATP} channel: the pore-forming Kir subunit and the regulatory subunit of the sulfonylurea receptor SUR. Kir 6.1 and SUR 2B constitute the VSM-type K_{ATP} channel, whereas pancreatic ß-cells have Kir 6.2 and SUR 1 (Nagashima *et al*., 2004). However, both glibenclamide and *I. sonorae* have afinity by both conformations. In fact, it has been reported that all the SU derivatives have an unselective activity on these K_{ATP} channels, excepting tolbutamide that is selective uniquely to pancreatic ß-cells K_{ATP} channels.

On the other hand, DCI is found in *C. ficifolia* fruit (Xia and Wang, 2006b), and when it is ingested, it may increase the activity of insulin. DCI is an important mediator of insulin's effects; it has classically been found in plants, insects and, in smaller relative quantities in animal tissues, including the human placenta, human urine, uremic serum and blood. DCI has been identified with myo-inositol in the glycosyl phosphatidyl inositol lipid (GPI) fraction from H35 hepatoma rat cells. A pattern of increased myo-inositol and decreased DCI excretion in urine and blood has been observed in patients with type 2 diabetes (T2D), suggesting that T2D patients do not produce enough DCI and therefore exhibit a decrease in insulin sensitivity (Larner, 2002). As observed by Larner (2002), doses of DCI (1-10 mg/kg) decrease hyperglycemia (50%), preventing glucosamine-induced peripheral insulin resistance but do not affect hepatic glucose output; its mechanism is explained by initial incorporation into the glycosyl phosphatidyl inositol lipid precursor store and its subsequent release by phospholipases regulated by insulin (Larner, 2002). In addition, it has been demonstrated that GLP-1, a hormone released into the circulation from the gut after meal ingestion, might modulate K_{ATP} channel activity through a mechanism independent of insulin secretion. The proposed mechanisms include protein kinase-dependent and kinase-independent mechanisms, which may be modulated by incretins (McClenaghan *et al*., 2006). Thus, the hypoglycemic activity of *C. ficifolia* could be explained by this mechanism, with no effects on VSM.

Cucurbitanes and cucurbitane-type glycosides are found most frequently in the *Cucurbitaceae* family and are currently recognized for their anti-cancer activities and their wide range of *in vitro* and *in vivo* pharmacological effects, such as purgative, anti-inflammatory, anti-fertility, hepatoprotective, anti-microbial, anthelmintic and antihyperglycemic effects (Chen *et al*., 2005; Paduch *et al*., 2007). It has been shown that stevioside and aglucon steviol potentiate insulin secretion from isolated mouse islets in a dose- and glucose-dependent way.

In this research, only concentrations between 10 and 30 µg/mL were used because only this range of concentrations produced the maximum inhibition of relaxation in thoracic aorta rings and there was no statistically significant difference between 10 and 30 µg/mL of *I. sonorae*.

CONCLUSION

This study provides additional support to the popular use of preparation of *C. ficifolia* and *I. sonorae* to treat diabetes mellitus in traditional Mexican medicine. This study also diminishes the possibility that the effect on insulin levels reported for *C. ficifolia* (Xia and Wang, 2006b) is mediated through the K_{ATP} channels and suggests that the hypoglycemic effect of *I. sonorae* is mediated directly through these channels. It is necessary to continue studying the extract of *I. sonorae* in isolated pancreatic β-cells to investigate

whether blocking is achieved through the SUR or Kir subunit of K_{ATP} channels.

ACKNOWLEDGMENTS

This research was partially supported by the International Foundation for Science, Stockholm, Sweden; the Organization for the Prohibition of Chemical Weapons, The Hague, The Netherlands, through a grant to Francisco Javier Alarcon-Aguilar, PhD (Research Grant Agreement No. F/3338-2F); and by the PIFI2-PROMEP-SEP (Project P/CA-15-2006-35.53). We are grateful to Deyanira Ojeda Ramirez, PhD, and Atilano Gutierrez Carrillo, PhD, for spectroscopic determinations. We are grateful to The Gonzalo Rio Arronte and IMSS Foundations.

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