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Acute hypoglycemic effect and phytochemical composition of *Ageratina petiolaris*

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ABSTRACT

Ethnopharmacological relevance: Type 2 diabetes is characterized by tissue resistance to the action of insulin combined with a relative deficiency in insulin secretion. In Mexico, medicinal plants have traditionally been used to control the disease; in this work, we investigate the hypoglycemic effect of *Ageratina petiolaris*, a plant used in Mexico for the treatment of diabetes.
Methods: The hypoglycemic effects of aqueous and methanolic extracts prepared from aerial parts of *Ageratina petiolaris* in streptozotocin-nicotinamide (STZ-NA) induced diabetic rats were assessed. An oral administration of the water extract at doses of 40 and 160 mg/kg and of the methanol extract at doses of 67 and 268 mg/kg were evaluated. Furthermore, the water extract at 160 mg/kg was evaluated under an Oral Glucose Tolerance Test.
Results: The tested extracts were able to reduce the increase in blood glucose level at three hours after administration. L-*chiro*-inositol and chlorogenic acid were isolated as important constituents of the plant, they were identified in both extracts along with other constituents.
Conclusions: The results presented here demonstrate that the main components in the aqueous extract of *Ageratina petiolaris* are chlorogenic acid and L-*chiro*-inositol, the last one with significant hypoglycemic activity, these results support the traditional use of this plant for the treatment of type 2 diabetes.
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1. Introduction

Diabetes mellitus (DM) is defined as an elevated blood glucose levels associated with absent or inadequate pancreatic insulin secretion, which may occur with or without the impairment of insulin signaling. Type 2 diabetes (T2D) is characterized by tissue resistance to insulin combined with a relative deficiency in insulin secretion. A given individual may exhibit either increased insulin resistance or increased β-cell deficiency, and these abnormalities may be mild or severe. Impaired insulin signaling also affects fat metabolism, resulting in increased free fatty acid flux, elevated triglyceride levels and reciprocally low levels of high-density lipoprotein (HDL) (Expert Committee, 2003). T2D is a polygenic disorder; the additive effects of an as-yet unknown number of genetic polymorphisms (risk factors) are required for its development, and they may not be sufficient in the absence of environmental (acquired) risk factors. The most important risk factors are those that influence insulin sensitivity: obesity (visceral), physical inactivity, high-fat/low-fiber diet, smoking, and low birth weight (Alsahli and Gerich, 2012).

The long-term complications of diabetes include retinopathy with a potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; autonomic neuropathy causing gastro-intestinal, genitourinary, and cardiovascular symptoms; and sexual dysfunction (Expert Committee, 2003).

T2D is a public health problem; according to the World Health Organization (WHO, 2015), more than 347 million people worldwide are affected. In 2010, the WHO acknowledged that this disease is a major cause of mortality in Mexico. In this country, the health services identified in 2012, 6.4 million Mexican adults with diabetes, 9.2% of adults have already been diagnosed with diabetes. The total number of adults with diabetes could be even twice that according to previous evidence on the percentage of diabetics who do not know their condition (SINAIS, 2015).

T2D is a complex disease; when diabetic patients do not feel better after a short period of conventional treatment, they seek alternative treatments. A recent field study among the Cakchiquel ethnicity reported that among the total interviewed diabetic

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population, 97% were under medical prescription, 91% used medicinal plants, and only 3% used medicinal plants exclusively (Cruz and Andrade-Cetto, 2015).

Ageratina petiolaris (Moc. & Sessé ex DC.) R. M. King & H. Rob (Asteraceae) (AP) is an endemic plant of Mexico, widely distributed in the central and south part of the country, shrub up to 2 m tall, cylindrical, with white-yellowish woody stems, opposite leaves with ovate lamina chapters arranged in corymbs, and white flowers (Rzedowski, 2001).

The plant is used traditionally in some communities in the State of Mexico for the treatment of diabetes, and it is known by its Spanish name “hierba del ángel” or its Nahuatl name “Yolochichotl”. In the state of Michoacán, it is used to treat certain stomach disorders, muscle aches, headaches, as an anticoagulant, astringent, antihistaminic, anti-rheumatic, and immunostimulant (Argueta, 1994).

It is among the plants listed in Mexican Traditional Medicine with hypoglycemic activity (Andrade-Cetto and Heinrich, 2005); we found the plant sold as hypoglycemic in the biggest medicinal plant market in Mexico City, “Sonora Market” where we perform ethnopharmacological field work.

Phytochemical studies of AP show that less polar extracts are composed mainly of thymol derivatives, sesquiterpene lactones, chromenes, triterpenes and *ent*-labdane derivatives (Bohlmann et al., 1977; Calderón et al., 1983; Guerrero et al., 1982).

The aim of this study was to investigate the hypoglycemic effect of water and methanolic extracts of the plant in streptozotocin-nicotinamide (STZ-NA)-induced diabetic rats and to identify the main phytochemical constituents in the plant and the tested extracts.

2. Materials and methods

2.1. Materials

Market Interviews; the Sonora market is heir to the old tradition of pre-Hispanic markets in Mexico, with the largest volume of trade in medicinal plants in the country. In five short visits in 2011 we perform direct interviews to plant sellers and type 2 diabetic patients, asking for plants used or sold to treat diabetes, we remain with the sellers the whole day waiting for people who in a spontaneous way ask for the plant, this action was repeated in 5 different “shops”. The sellers refer that the plant is brought into the market from the town of Tenancingo among others.

With the guidance of traditional sellers, samples of *AP* were collected in Tenancingo, Estado de México, in February 2012. The plant material was identified by Jose Luis Villaseñor, and a voucher specimen (MEXU-1333 471) was deposited at the National Herbarium (MEXU) of the “Instituto de Biologia, UNAM”.

2.2. Preparation of the extracts and isolation of compounds

Different water (WE) and methanol (ME) extracts of the aerial parts were prepared; (A) to study their hypoglycemic effects and (B) to investigate their phytochemical composition.

(A) For hypoglycemic assays, the water extract (WE) was prepared as an infusion, similar to the traditional used, by extraction of 20 g of the air-dried aerial parts of the plant with 500 mL of boiling water (the recommended dose by the sellers and diabetic people). The infusion was allowed to cool at room temperature, filtered and lyophilized to give 10.9 g of residue. Because alcoholic extracts sometimes extract in a better way the compounds presented in the water extract (Infusion), the methanolic extract (ME) was prepared by maceration of 20 g of the plant material in 500 mL of methanol at room temperature for 48 h, filtration and solvent elimination under vacuum; the yield of 20 g plants was 44.8 g extract.

(B) For phytochemical analysis, Methanolic extract was prepared by extracting 100 g of plant material three times with methanol (1 L) at room temperature for 48 h, filtering it and eliminating the solvent under vacuum to provide 24 g of crude extract.

Water extract was obtained as following, 1 L of boiling water was poured onto 100 g of powdered air-dried aerial parts of the plant, after 10 min the infusion was filtered, the solution allowed to cool at room temperature and lyophilized to provide 27 g of residue. The residue was *treated* with methanol (2×500 mL) to obtain a methanol soluble fraction (10 g), which was concentrated, redissolved in water and extracted with dichloromethane (DCM, 3×300 mL). The DCM and aqueous fraction afforded 50 mg and 9.850 g of residue, respectively, after removing the solvents.

The methanol residue (9.8 g) was fractionated over a Sephadex LH-20 column, using MeOH as eluent. Fifty-two eluates (50 mL each) were taken and combined into seven major fractions (f1-f7) according to thin layer chromatography (TLC) evaluation.

Fraction f4 (250 mg) was further subjected to column chromatography (CC) on octadecyl functionalized silica gel, using water as the mobile phase, and taking 50 eluates (10 mL each) that were combined into eight subfractions (f4A-f4H) based on TLC analysis. Subfraction f4F (180 mg) was purified by prep TLC on octadecyl functionalized silica gel and eluted with H_2O/MeOH 2:1 to afford compound 1 (35 mg).

Fraction f7 (500 mg) was fractionated over a Sephadex LH-20 column, using MeOH as eluent, to give 30 eluates (5 mL each) that were combined into five subfractions (f7A-f7E). From subfraction f7B compound 2 (250 mg) was obtained by spontaneous crystallization.

The DCM fraction residue (50 mg) was separated by prep TLC eluting with hexane: EtOAc (v/v 3:1) to give compounds **3** (2 mg), **4** (2 mg), **5** (5 mg) and **6** (2 mg). Compounds **3–6** were also obtained among the main constituents of the ME.

A sample of ME (5 g) was fractionated over a Sephadex LH-20 column, using MeOH as eluent, to obtain 18 eluates (100 mL each) that were combined in eight major fractions (f1-f8) after TLC evaluation. Fraction f5 (950 mg) was subjected to CC on octadecyl functionalized silica gel, using MeOH as the mobile phase and a 0.5 mL min^{-1} flow rate, to obtain 50 eluates (25 mL each) that were combined into four major fractions (f1A-f1D) after TLC evaluation. Compound **2** (80 mg) crystallized spontaneously from fraction f1A. Fraction f1B (80 mg) was subjected to prep TLC on octadecyl functionalized silica gel using MeOH: H_2O (1:2) as eluent, to afford **1** (5 mg). Fraction f1C (250 mg) yielded **3** (150 mg) by prep TLC using a mixture of hexane: EtOAc (3:1) as mobile phase. Fraction f1D (500 mg) was subjected to CC on bentonite clay powder (Tonsil®), using CH_2Cl_2 as eluent, obtaining 19 eluates (5 mL each) that were combined into three fractions (f1CA-f1CC). Fraction f1CC (300 mg) was subjected to prep TLC using a mixture of hexane: EtOAc (3:1) as mobile phase to give compounds **3** (20 mg), **4** (15 mg), **5** (30 mg), **6** (10 mg), and **7** (5 mg). Compounds **3–7** and compound **8** were also obtained from the hexane extract (HE) of AP.

Melting points (uncorrected) were measured on a Fisher-Johns apparatus. Nuclear magnetic resonance (NMR) experiments were performed on a Bruker Advance III spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C and a Varian, Unity Inova spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Chemical shifts were referenced to trimethylsilane (TMS). Electron-impact mass (EIMS) spectra were recorded on an MStation JMS-700. TLC was carried out on precoated Macherey Nagel Sil G/UV_{254} plates of 1.0 mm thickness; Silica gel 230–400 mesh (Macherey-Nagel), Sephadex LH-20 (Pharmacia Biotech) and octadecyl functionalized silica gel (Sigma Aldrich) were used for column chromatography.

2.3. Animals

Eight-week-old Wistar rats weighing 200–250 g were obtained from the Bioterium of the Science School, UNAM were acclimated with free access to food and water for at least one week in an air conditioned room (25 °C with 55% humidity) on a 12 h light-dark cycle prior performing the experiments. The animals were handled according to the Health Guide for the Care and Use of Laboratory Animals (Office of Animal Care and Use, National Institute of Health, 2015).

2.4. Induction of experimental diabetes and doses calculation

Experimental diabetes was induced as described (Masiello, 1998). The rats were fasted overnight and injected intraperitoneally with 150 mg/kg nicotinamide (NA) (Sigma, N3376) 15 min before an intravenous injection of 65 mg/kg streptozotocin (STZ) in citrate buffer (Sigma, S0130).

Diabetes was identified by polydipsia and polyuria and by measuring non-fasting plasma glucose levels 48 h after injection. After seven days' animals with glucose levels between 170 and 230 mg/dL were included in the study. The administered doses were calculated considering the extraction yield (see above) and one-person weight (70 kg), the doses were adjusted to one rat (250 g weight).

2.5. Experimental groups acute effect

The animals were classified into 8 groups (1–8) each with eleven rats. Group 1 as a non-hyperglycemic control received 1.5 mL of physiological NaCl-solution (vehicle). Group 2 as a hyperglycemic control also received 1.5 mL of physiological Na Cl-solution (vehicle). Group 3 was given a standard oral hypoglycemic agent, glibenclamide (5 mg/kg bodyweight (bw)), in the same vehicle, while groups 4 and 5 received WE (40 mg/kg bw) and WE (160 mg/kg bw), groups 6 and 7 received ME (67 mg/kg bw) and ME (268 mg/kg bw), respectively. Group 8 received compound **2**, L-*chiro*-inositol (3.73 mg/kg bw). The extracts were dissolved in 1.5 mL of physiological NaCl-solution and administered orally by gastroesophageal gavage to ensure that the liquid reached the digestive tract. All groups were fed Purina Rodent Laboratory Chow 5001.

2.6. Experimental groups under a OGTT

The aqueous extract similar to the traditional used infusion, was tested under a OGTT; animals were classified in other eight groups of six rats each one. Groups 1-4 received 1.5 only physiological solution; group 1 non-hyperglycemic control, group 2 hyperglycemic control, group 3 received glibenclamide (5 mg/kg bodyweight (bw)) and group 4 received WE (160 mg/kg bw).

Groups 5-8 (OGTT) received 2 g/kg of glucose dissolved in 1.5 physiological solution; group 5 non-hyperglycemic control, group 6 hyperglycemic control, Group 7 received repaglinide (1 mg/kg bodyweight (bw)) and group 8 received WE (160 mg/kg bw).

2.7. Collection of blood and determination of blood glucose

Blood samples were obtained from the tail vein according to procedures outlined in the Institutional Animal Care and Use Committee Guideline 9 (Institutional Animal Care and Use Committee, 1999). Blood was collected before the oral administration of extract or vehicle (T0) and at T60, T120 and T180 min thereafter for the acute experiment, and at times T30, T60, T90 and T120 thereafter for the OGTT. The glucose concentration was measured by an Accutrend GC ® (Cobas) by duplicate.

2.8. Statistical analysis

The data were analyzed by *two*-way ANOVA followed by a Bonferroni post hoc test, significant difference is assumed with p at least ≥ 0.05. The plasma glucose levels were expressed as the mean (S. E. M).

3. Results

3.1. Traditional use and market results

With the traditional sellers we confirm that *A. petiolaris*; is a popular specie used for the treatment of type 2 diabetes, which invariably appear in the market, it has a consistent and limited number of uses and it enjoy a high volume trade, according to Heinrich (2001), these are good criteria for plant selection on a market based study.

The interviewed patients (20) ensure that they feel better after the plant tea consumption, to prepare the infusion (here tested) the sellers recommended boiling 20 g of the aerial part of the plant in water (500 mL) and drink it over the day as the so called “agua de uso”, we confirm that the patients consume an average of 4 cups a day.

3.2. Identification of compounds

In order to identify the compounds responsible for the observed hypoglycemic effect of the plant extracts, the water and methanolic extracts were subjected to chromatographic procedures. Chromatographic separation of the WE led to the isolation and identification of compounds **1**–**6**, while the ME led to the isolation of 7 in addition to compounds 1–6, and the hexane extract afforded compounds **3**–**6** and **8**.

Secondary metabolites **1**–**8** were identified by comparison of their spectroscopic data with those previously described in the literature, including chlorogenic acid (1), L-*chiro*-inositol (**2**) (Podesva et al., 2003), 2α-*iso*-valeroyloxyeperuic acid (**3**) (Calderón et al., 1983), benzyl 2-hydroxy-6-methoxybenzoate (**4**) (Ma et al., 1991; Casu et al., 2010), benzyl 2,6-dimethoxybenzoate (**5**) (Ma et al., 1991), 3-methoxybenzyl 2,6-dimethoxybenzoate (**6**) (Rivero-Cruz et al., 2005), benzyl 2-hydroxy-3,6-dimethoxybenzoate (**7**) (Kuo et al., 2002), and 2α-tigloyloxyeperuic acid (**8**) (Bohlmann et al., 1984; Domínguez et al., 1988).

Compounds **3** and **8** have been isolated as colorless oils from *Fleischmannia viscidipes* (Bohlmann et al., 1984), and compound **3** has also been isolated before from *Ageratina petiolaris* (Calderón et al., 1983); nevertheless, their structures were assigned based on limited ^{1}H NMR, infrared (IR), and mass spectrometry (MS) data. In this paper we describe the complete assignment of ^{1}H and ^{13}C NMR data. Moreover, this is the first time that compounds **1**, **2**, **4**, **7** and **8** were isolated from AP.

3.2.1. 2α-Iso-valeroyloxyeperuic acid (**3**)

Colorless oil; ^{1}H NMR: (400 MHz, $CDCl_3$, δ in *ppm*, *J* in Hz) data: 1.97 (dd, *J*=13.6, 4.8, 1H, H-1b), 1.93 (dd, *J*=13.6, 4.8. 1H, H-1a), 5.19 (quin, *J*=4, 1H, H-2), 1.73 (dd, *J*=14.4, 3.2, 1H, H-3b), 1.44 (dd, *J*=13.2, 7.2, 1H, H-3a), 1.18 (dd, *J*=10.8, 3.2, 1H, H-5), 1.76 (m, 1H, H-6b), 1.40 (m, 1H, H-6a), 2.40 (dt, 12.8, 2.8, 1H, H-7b), 1.97 (m, 1H, H-7a), 1.58 (dd, *J*=5.6, 4.4, 1H, H-9), 1.40 (m, 2H, H-11b, H-11a), 1.38 (m, 1H, H-12b), 1.14 (m, 1H, H-12a), 1.92 (m, 1H, H-13), 2.33 (dd, *J*=15.2, 6.0, 1H, H-14b), 2.17 (dd, *J*=15.2, 8.0, 1H, H-14a), 0.92 (d, *J*=7.2, 3H, CH_3-16), 4.86 (br s, 1H, H-17b), 4.50 (br s, H-17a), 0.97 (s, 3H, CH_3-18), 0.91 (s, 3H, CH_3-19), 0.86 (s, 3H, CH_3-20),

Table 1
Acute hypoglycemic effects of *Ageratina petiolaris* aerial parts on STZ-NA-induced diabetic rats.

Group	Glucose (mg/dl)			
	0 min	60 min	120 min	180 min
Non-hyperglycemic control.	124 ± 2	125 ± 3	129 ± 3	128 ± 1
Hyperglycemic control.	190 ± 5	185 ± 6	178 ± 4	184 ± 4
Glibenclamide (5 mg/kg)	189 ± 5	147 ± 7[a,b]	133 ± 4[a,b]	124 ± 4[a,b]
WE (40 mg/kg)	195 ± 5	189 ± 7	167 ± 5[a]	162 ± 4[a,b]
WE (160 mg/kg)	193 ± 4	187 ± 4	159 ± 5[a,b]	152 ± 5[a,b]
ME (67 mg/kg)	203 ± 8	198 ± 15	182 ± 15	181 ± 12
ME (268 mg/kg)	189 ± 5	167 ± 9[a]	148 ± 6[a,b]	145 ± 5[a,b]
L-*chiro*-inositol (3.73 mg/kg)	194 ± 4	175 ± 5	165 ± 5[a,b]	159 ± 6[a,b]

Each value is the mean ± SEM for eleven rats in each group. The data were analyzed by two-way ANOVA followed by Bonferroni post hoc test for comparison with time 0 and the hyperglycemic group.

[a] Significant difference compared with time 0 in the same group with p at least ≥ 0.05.

[b] Significant difference with the control hyperglycemic group at the same time with p at least ≥ 0.01.

2.27 (dd, J=14.4, 6.4, 1H, H-2′b), 2.08 (dd, J=14.4, 6.4, 1H, H-2′a), 1.88 (m, 1H, H-3′), 0.93 (d, J=6.0, 3 H, CH_3-4'), 0.89 (d, J=7.6, 1 H, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz): 42.1 (C-1), 70.5 (C-2), 43.8 (C-3), 33.0 (C-4), 54.3 (C-5), 24.3 (C-6), 38.3 (C-7), 148 (C-8), 57.6 (C-9), 39.1 (C-10), 21.3 (C-11), 35.9 (C-12), 30.63 (C-13), 41.9 (C-14), 179.1 (C-15), 19.5 (C-16), 107.4 (C-17), 23.7 (C-18), 33.9 (C-19), 16.3 (C-20), 173.0 (C-1′), 42.3 (C-2′), 31.9 (C-3′), 19.7 (C-4′), 11.4 (C5′); EIMS 70 eV m/z (rel. int): 304 $[M-C_5H_{10}O_2]^+$(20.1), 135 $[C_8H_{15}]^+$ (100).

3.2.2. 2α-Tigloyloxyeperuic acid (**8**)

Colorless oil; ^{1}H NMR: (400 MHz, $CDCl_3$, δ in *ppm*, J in Hz) data: 2.06 (m, 1H, H-1b), 1.42 (m, 1H, H-1a), 5.23 (quin, J=3.6, 1H, H-2), 1.78 (m, 1H, H-3b), 1.48 (dd, J=15.2, 4.0, 1H, H-3a), 1.20 (m, 1H, H-5), 1.76 (m, 1H, H-6b), 1.39 (m, 1H, H-6a), 2.40 (m, 1H, H-7b), 1.97 (m, 1H, H-7a), 1.58 (m, 1H, H-9), 1.40 (m, 2H, H11b, H-11a), 1.37 (m, 1H, H-12b), 1.14 (m, 1H, H-12a), 1.91 (m, 1H, H-13), 2.31 (dd, J=15.2, 6.0, 2H, H-14b), 2.17 (dd, J=14.0, 6.0, 1H, H-14a), 0.97 (d, J=6.4, 3H, H-16), 4.86 (br s 1H, H-17b), 4.50 (br s, H-17a), 0.99 (s, 3H, CH_3-18), 0.92 (s, 3 H, CH_3-19), 0.88 (s, 3H, CH_3-20), 6.82 (qq, J=7.2, 1.6, 1H, H-3′), 1.78 (dd, J=6.8, 1.2, 1H, H-4′), 1.83 (q, J=1.2, 1H, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz): 42.1 (C-1), 70.9 (C-2), 43.9 (C-3), 32.1 (C-4), 54.6 (C-5), 24.3 (C-6), 38.3 (C-7), 148.0 (C-8), 57.6 (C-9), 39.0 (C-10), 21.3 (C-11), 35.9 (C-12), 30.9 (C-13), 41.7 (C-14), 177.5 (C-15), 19.7 (C16), 107.4 (C-17), 23.6 (C-18), 33.9 (C-19), 16.2 (C-20), 167.9 (C-1′), 129.2 (C-2′), 137.0 (C-3′), 14.6 (C-4′), 12.3 (C5′); EIMS 70 eV m/z (rel. int): 304 $[M-C_5H_{10}O_2]^+$ (31.1), 135 $[C_8H_{15}]^+$ (100).

3.3. Activity in diabetic rats

STZ administration at the dosage of 65 mg/kg bw after intraperitoneal administration of NA to normal rats significantly ($P < 0.001$) elevated their blood glucose levels compared with rats injected with citrate buffer alone as reported (Masiello et al. 1998).

In the hyperglycemic rats, both extracts as well as L-*chiro*-inositol showed significant hypoglycemic effects, whereas the positive control glibenclamide showed effects from 60 through 180 min ($p < 0.001$) compared to the control group and their own time zero (T0) (Table 1).

The WE at the dose of 40 mg/kg bw (traditional infusion) showed activity at 120 min with significant reduction ($p < 0.01$), which remained significant until 180 min compared with time 0. At this dose the WE treated rats also had significant hypoglycemia when compared with the hyperglycemic group after 180 min ($p < 0.001$). At 160 mg/kg, rats treated with WE bw **(**= traditional infusion considering 4 cups per day) had hypoglycemia from 60 min with $p < 0.05$ and with $p < 0.001$ at 120 and 180 min compared with time 0. At these doses, the extract showed a significant hypoglycemic effect from 120 min ($p < 0.05$) until 180 min ($p < 0.001$) compared with the hyperglycemic group. The maximum activity of the WE was observed at the higher dose after 180 min of treatment.

The ME exhibited effects only at the higher dose after 60 min $p < 0.05$ with $p > 0.001$ at 120 and 180 min compared with time 0 and the control group.

L-*chiro*-inositol **2** was active at the dose of 3.73 mg/kg bw with $p < 0.01$ from 120 min until 180 min compared with the hyperglycemic group. The compound led to a significant decrease in plasma glucose level compared with time 0 from 60 min to 180 min with a progressive increase in significance from $p < 0.05$ (60 min), $p < 0.01$ (120 min) to $p < 0.001$ (180 min).

3.4. Activity in diabetic rats under a OGTT

In the two hours' acute experiment, without the glucose load the positive control group and the experimental group shown a similar behavior than in the previous experiment; at time 60 the plant shown statistical significance vs the hyperglycemic group (Table 2). Under the OGTT, the hyperglycemic group shown elevated glucose levels since time 30 as compared with their own time 0, these levels of glucose were observed until 120 min, the positive group did not show a difference against their own time 0; this indicates that repaglinide can suppress the glucose peak under an OGTT, while the WE did not show any difference against time 0 (Table 3).

4. Discussion

In Mexico, diabetic patients who live in urban areas use medicinal plants to control the disease. These species are obtained mainly in fresh forms from markets, such as the Sonora market in México, CD. MX. Among the plants sold for the treatment of type

Table 2
Effect of *Ageratina petiolaris* water extract on blood glucose levels on STZ-NA-induced diabetic rats.

Group	Glucose (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Non-hyperglycemic control.	122 ± 1.4	124 ± 2.1	118 ± 3	116 ± 4.1	118 ± 4.3
Hyperglycemic control.	299 ± 11.6	301 ± 9.4	296 ± 12.1	293 ± 8.1	284 ± 12.8
Glibenclamide (5 mg/kg)	308 ± 8.4	291 ± 11.8	252 ± 14.4[b,a]	199 ± 12.9[b,a]	166 ± 12.4[b,a]
WE (160 mg/kg)	304 ± 7.9	297 ± 13.3	265 ± 11.5[a]	247 ± 9.8[b,a]	234 ± 11.8[b,a]

Each value is the mean ± SEM for six rats in each group. The data were analyzed by *t*-student test for comparison with time 0 and the hyperglycemic group.

[a] Significant difference compared with time 0 in the same group.

[b] Significant difference with the control hyperglycemic group at the same time with $p \leq 0.05$.

Table 3
Effect of *Ageratina petiolaris* water extract on blood glucose levels under an oral glucose tolerance test on STZ-NA-induced diabetic rats.

Group	Glucose (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Non-hyperglycemic control.	123 ± 2.8	171 ± 6.8	159 ± 10.4a	142 ± 11	125 ± 6
Hyperglycemic control.	297 ± 8.7	485 ± 27.6[a]	469 ± 34.9[a]	403 ± 25.4[a]	394 ± 17.1[a]
Repaglinide (1 mg/kg)	300 ± 11	352 ± 14.0[b,a]	319 ± 17.8[b]	274 ± 21.5[b]	253 ± 24.1[b]
WE (160 mg/kg)	303 ± 14.2	436 ± 26.4[a]	408 ± 20.1[a]	369 ± 16[a]	324 ± 14.7[b]

Each value is the mean ± SEM for six rats in each group. The data were analyzed by *t*-student test for comparison with time 0 and the hyperglycemic group.

[a] Significant difference compared with time 0 in the same group.

[b] Significant difference with the control hyperglycemic group at the same time with $p \le 0.05$.

2 diabetes *Ageratina petiolaris* is important. It has a consistent pattern of uses and a limited number of different therapeutic claims. At the same time it enjoys a high-volume trade. Consequently based on these ethnobotanical data, the study of the species' phytochemistry and pharmacology seems of considerable merit.

Here we demonstrate that, in the rat model used, the administration of STZ after the injection of NA led to a significant increase in plasma glucose level compared with the non-diabetic control group, glibenclamide at 5 mg/kg decreases these levels. Therefore, we confirm that the STZ-NA-induced diabetic rat is a convenient model to test the effects of medicinal plants. Under a GTT, repaglinide at doses of 1 mg/kg was able to suppress the glucose peak; this means that it is a suitable control drug.

Both the WE and ME of AP produced hypoglycemic effects in rats. However, the water extract showed better activity than the methanolic extract. It is remarkable that the extract works in an acute way without a glucose load, and this must be connected with the mechanism of action.

Chlorogenic acid is a compound with demonstrated capacity to block hepatic glucose output, and it was identified as the main constituent of the WE of *Cecropia obtusifolia* and *C. peltata*, two plants traditionally used by the Mexican diabetic population to treat T2D (Andrade-Cetto and Cárdenas, 2010). Moreover, the hypoglycemic activity of the common naturally-occurring D-chiro-inositol has been well documented as an active constituent in Cucurbita ficifolia and Momordica charantia, plants also used against T2D (Xia and Wang, 2007, 2006). On the other hand, the biological properties of the rarely occurring enantiomeric isomer L-*chiro*-inositol have been not investigated. It was proved that L-chiro-inositol shows hypoglycemic effect as D-*chiro*-inositol (Xia and Wang, 2007, 2006) and very likely is involved in the hypoglycemic effect of the plant.

The phytochemical results presented here indicated that the methanolic extract contains lesser concentrations of chlorogenic acid (1) and chiro-inositol, two plant metabolites of proven hypoglycemic activity (Andrade-Cetto and Wiedenfeld, 2001; Xia and Wang, 2006), and this may be the reason why lower doses of the extract did not produce a hypoglycemic effect (Fig. 1).

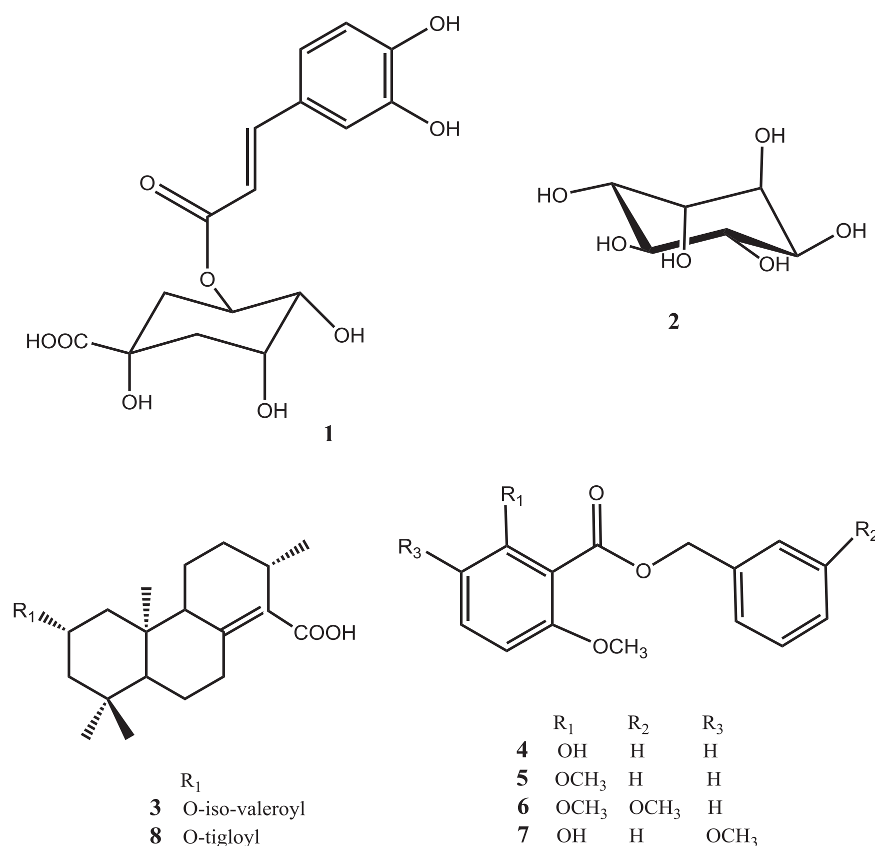

Fig. 1. Structures of compounds **1–8** from *Ageratina petiolaris*.

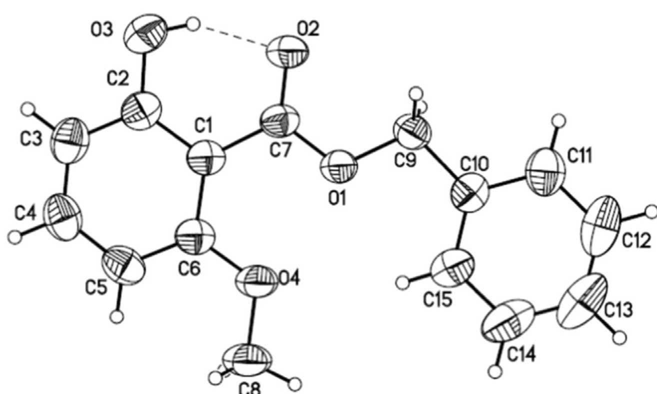

Fig. 2. X-ray crystal structure of benzyl 2-hydroxy-6-methoxybenzoate (**4**).

Besides, benzyl benzoates (**4**–**7**) obtained from *A. petiolaris* have been previously isolated from other species, other biological activities like; (**4**) anti-tumor (Zin et al., 2005), (**5**) antinociceptive (Palacios- Espinosa et al., 2008) and (**7**) antifungal (Sartorelli et al., 2012), have been previously evaluated. Although compound **4** has been isolated previously, its crystal structure is determined for the first time in this work (Fig. 2).

Although the extracts tested here show a hypoglycemic effect, this effect is slight and is not connected to a glucose load. Because traditionally the plant infusion is continuously consumed as "agua de uso" this sustained "slight" effect could improve the glycemic control in diabetic patients, further studies are needed to clarify the mechanism of the hypoglycemic effect.

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