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ORIGINAL ARTICLE

Antihyperglycemic Activity of the Leaves from *Annona diversifolia* Safford. and Farnesol on Normal and Alloxan-Induced Diabetic Mice*

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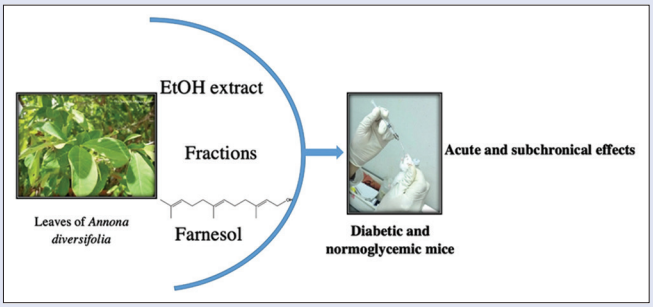
ABSTRACT

Background: Diabetes mellitus (DM) is a chronic disease characterized by high blood glucose levels resulting from insulin resistance or inadequate insulin secretion. In the world, DM is one of the most frequent non-contagious diseases that affect more than 371 million people. **Objective:** This study aimed to evaluate the antihyperglycemic properties of the ethanol extract, subsequent fractions, and farnesol obtained from the leaves of *Annona diversifolia* on alloxan-induced diabetic and normal mice. **Materials and Methods:** Bioassay-guided fractionation of the ethanol extract of the leaves of *A. diversifolia* (EEL*Ad*) was performed on alloxan-induced Type 2 diabetic and normoglycemic (NM) mice. Oral glucose tolerance test (OGTT), oral sucrose tolerance test (OSTT), and oral lactose tolerance test (OLTT) were performed in fast NM mice (FNM). **Results:** The EEL*Ad*, $CHCl_3$ fraction, and farnesol induced a significant reduction of postprandial hyperglycemia in acute and subchronic tests using AITD mice. When EEL*Ad*, $CHCl_3$ fraction, and farnesol were tested on NM in subchronic assays, these did not affect glycemic levels. In the case of acute test on NM, only $CHCl_3$ fraction induced a hypoglycemic effect at 2 h after the treatment. OLTT and OSTT showed that the EEL*Ad*, $CHCl_3$ fraction, and farnesol induced a significant reduction of hyperglycemia levels in FNM at 2 h after a lactose or sucrose load comparable to acarbose. In the case of OGTT was observed a significant reduction of hyperglycemia levels in FNM mice at 2 h after a glucose load comparable to canagliflozin. **Conclusion:** The EEL*Ad* and farnesol induced a significant reduction of postprandial hyperglycemia on AITD mice in acute and subchronic assays. Our results suggest that the control of postprandial hyperglycemia may be mediated by the regulation of absorption of glucose and inhibition of disaccharide digestion such as sucrose and lactose. Finally, the results explained the use of *A. diversifolia* in Mexican traditional medicine as an antihyperglycemic agent.

Key words: *Annona diversifolia*, *Annonaceae*, antihyperglycemic activity, diabetes mellitus, farnesol, sesquiterpene

SUMMARY

- The ethanol extract of the leaves from *Annona diversifolia*, subsequent fraction, and farnesol were studied on alloxan-induced Type 2 diabetic and normal mice. The results suggest that farnesol was responsible in part of the antihyperglycemic effect of *A. diversifolia*.



Abbreviations used: EEL*Ad*: Ethanol extract of leaves from *A. diversifolia*; AITD: Alloxan-induced Type 2 diabetic mice; DM: Diabetes mellitus; OGTT: Oral glucose tolerance test; OSTT: Oral sucrose tolerance test; OLTT: Oral lactose tolerance test.

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INTRODUCTION

Diabetes mellitus (DM) is a chronic disease characterized by high blood glucose levels resulting from insulin resistance or inadequate insulin secretion.[1-3] In the world, DM is one of the most frequent non-contagious diseases that affect more than 371 million people and in 2012 caused the death of 1.5 million people.[4-6] In México, DM is the first cause of mortality in women and the second in men with a rate that is increasing for both sexes, with a prevalence in women of 7.8% and of 7.2% in men.[3,5,7]

Oral hypoglycemic drugs are used for the treatment of DM, such as sulfonylureas, meglitinides, DPP4-inhibitors, glucagon-like peptide-1, α-glucosidase inhibitors, sodium glucose transporter 2 (SGLT2) inhibitors, biguanides, and thiazolidinediones.[8-11] However, DM and its secondary complications remain a major problem in the world

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population, and most of these have undesirable side effects. Therefore, there is a need of new antidiabetic drugs devoid of side effects, to improve the quality of life of the patient. In this sense, medicinal plants constitute an important source of new compounds with potential therapeutic effects.[12]

Mexico is recognized as a country with high biological diversity, containing the 10% of the world's total flora, occupying the fourth place with 18,000 species, of which 4000 (20%) are attributed medicinal characteristics; however, only the 5% have scientific studies that demonstrate their use for the treatment of diseases.[13] Among these, some species of *Annonaceae* family are used as antihyperglycemic agents for the treatment of DM, such as *Annona muricata*, *Annona cherimola, Annona squamosa*, *Annona glabra,* and *Annona diversifolia.*[14-19] In the case of *A. diversifolia* is known in Mexico as "ilama and papause," the fruits of this plant are used as food. In Mexican traditional medicine the leaves of *A. diversifolia* are used as anticonvulsant, analgesic, and anti-inflammatory; at present, it is used to control hyperglycemia in people with DM.[20-23] *In vitro* and *in vivo* pharmacological studies have shown that an aqueous extract of *A. diversifolia* possesses flavonoids with α-glucosidase inhibitor properties and antihyperglycemic effects in NM rats.[23] However, there are no reports of the antihyperglycemic activity-guided fractionation of ethanol extract of leaves from *A. diversifolia* (EEL*Ad*) using alloxan-induced Type 2 diabetic (AITD) and NM mice. Thus, as part of our search to obtain antidiabetic agents from the plants used in Mexican traditional medicine, this study reports the antihyperglycemic properties of the EEL*Ad*.

MATERIALS AND METHODS

Chemicals

All the solvents used were of analytical grade and were obtained from JT Baker México. Acarbose (Glucobay, tablets of 50 mg, Bayer Mexico S.A. DE C.V.) and canagliflozin (Invokana, tablets of 300 mg, Janssen-Ortho LLC, Puerto Rico) were used as oral antidiabetic drugs and purchased from the local pharmacy. Glibenclamide (Glibenclamide, tablets of 5 mg, Silanes, Mexico) was used to confirm that pancreatic β-cells were viable in AITD mice and purchased from the local pharmacy. Alloxan (2,4,5,6-(1H,3H)-pyrimidinetetrone, Sigma-Aldrich) was used to induce AITD in mice. Glucose, sucrose, and lactose (Sigma-Aldrich) were used to induce hyperglycemia in oral glucose tolerance test (OGTT), oral sucrose tolerance test (OSTT), and oral lactose tolerance test (OLTT).

Plant material

A. diversifolia leaves were collected in February 2016 by a chemical engineer Jorge Ebrard Maure in Metapa de Domínguez, Chiapas, México. The leaves were authenticated by MS Abigail Aguilar Contreras of the Herbarium IMSSM of Mexican Institute of Social Security (IMSS) where the voucher specimen (No: 16248) was deposited.

Isolation and identification of farnesol from *Annona diversifolia*

The air-dried and finely powdered leaves (3 kg) were extracted by maceration at room temperature with EtOH (17 L × 2 times). After filtration, the extracts were combined and evaporated *in vacuo* to 175 g (EEL*Ad*, 5.86% yield) of a green residue. The antihyperglycemic extract (47.5 g) was suspended in 10% EtOH-water (100 mL) and successively partitioned with $CHCl_3$ (100 mL × 3 times, 43.31 g, $CHCl_3$ fraction) and EtOAc (100 mL × 3 times, 1.69 g, EtOAc fraction). The aqueous residual layer was concentrated under reduced pressure to give 2.5 g of AcR fraction. The most antihyperglycemic activity was associated with $CHCl_3$ fraction, and then, a portion (25 g) was subjected to a silica gel column (172 g, 70–230 mesh, E. Merck, Germany) and eluted with dichloromethane to give five fractions: Fr1 (450 mL), Fr2 (210 mL), Fr3 (1380 mL), Fr4 (150 mL), and Fr5 (3000 mL). The most active fraction (Fr5, 800 mg) was purified by preparative TLC (Merck, TLC silica gel $60F_{254}$, 2 mm, $CHCL_3$) using $CHCl_3$ (100%) as mobile phase to give farnesol [306 mg, Figure 1]. EtOAc and AcR fractions were discarded. Farnesol was readily identified by comparing its spectroscopic data (NMR, MS, IR, and UV) with those described in the literature[24] as well as by direct comparison with authentic sample (Sigma-Aldrich).

Animals

Balb-c female mice, aged 8–10 weeks (25 ± 5 g) and glucose level values of 161.5 ± 5.3 mg/dL, were used. Animals were raised in the Animal House of the National Medical Center "Siglo XXI" from IMSS. Investigations using experimental animals were conducted in accordance with the Official Mexican Rules for Animal Experimentation and Care. The mice were maintained at room temperature (22°C ± 2°C) on a 12 h light-dark natural cycle. Rodents were fed with standard diet and water *ad libitum*. All assays were conducted with the approval of the Specialty Hospital Ethical Committee of National Medical Center "Siglo XXI" from IMSS (Register: R-2014-3601-213, CNIC R-2017-785-071, and R-2016-3601-193).[25]

Acute oral toxicity study

The study was performed according to the guidelines on acute oral toxicity assay 423 of the OECD.[26] Twenty-four Balb-c mice (aged 8–10 weeks, 25.0 ± 5.0 g) fasted overnight but allowed free access to water *ad libitum* were randomly assigned into the following groups of six mice of either sex (three males and three females). Three groups received the extract at doses of 30, 300, and 3000 mg/kg and control group received 2% tween 20 in water. For the test, the animals were not fed for 4 h following administration. The signs of toxic effects and mortality were observed 4 h after administration and then for the next 48 h. The behavior of mice was observed daily in a period of 14 days for toxic effects, mortality, and changes in behavioral pattern. At the end of the experiments, the animals were sacrificed in a CO_2 chamber, and the internal organs (stomach, lungs, gut, kidney, heart, spleen, liver, and pancreas) were extracted, and the pathological observations were performed.

Induction of experimental Type 2 diabetes in mice

Experimental Type 2 DM was induced by an intraperitoneal injection of single dose of 150 mg/kg of alloxan. The development of AITD was determined by measuring the postprandial blood glucose level 2 days after the administration of alloxan, using glucose oxidase method (Evolution Blood Glucometer Glucose Monitoring System, Infopia Co., Ltd. USA). Animals with glucose level values of 312.6 ± 19.8 mg/dL of glucose were considered with AITD and used in this study.[27-30] In addition, AITD mice responded to glibenclamide confirming that pancreatic β-cells were viable.[30]

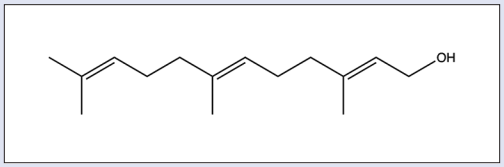

Figure 1: Structure of farnesol isolated from *Annona diversifolia*

Single oral administration of *Annona diversifolia* extract, subsequent fractions, and farnesol

AITD mice were randomly divided into 18 groups (n = 6 per group) as follows: Normoglycemic (NM) mice treated with vehicle (2% tween 20 in water), AITD mice treated with vehicle, AITD treated with EEL*Ad*, primary fractions, secondary fractions, farnesol, acarbose, canagliflozin and glibenclamide. Blood samples were collected from the tail vein at intervals 0, 2, and 4 h. Glucose level in each sample was assessed using the glucose oxidase method. The effects on blood glucose level of EEL*Ad*, subsequent fractions, farnesol, and oral antidiabetic drugs were also evaluated on NM mice. Blood sample analysis was done as previously mentioned.[16,30]

Subchronic effects of *Annona diversifolia* extract, $CHCl_3$ fraction, and farnesol

This study was carried out to determine the effect of the subchronic administration of EEL*Ad*, $CHCl_3$ fraction, and farnesol on AITD mice. The AITD mice were put in a stability period by 7 days to confirm and maintain the chronic hyperglycemic state.[16,30] The animals were randomly grouped into six groups (n = 6 per group). Group 1 consisted of NM treated with vehicle (2% tween 20 in water), Group 2 consisted of AITD mice treated with vehicle (2% tween 20 in water), Group 3 consisted of AITD mice treated with EEL*Ad* (200 mg/kg), Group 4 consisted of AITD mice treated with $CHCl_3$ fraction (200 mg/kg), and Groups 5 and 6 consisted of AITD mice treated with farnesol or acarbose (50 mg/kg), respectively. Groups 3–6 received treatment orally for 28 consecutive days. The hyperglycemia of the animals was determined weekly by glucose oxidase method.

The oral glucose tolerance test of *Annona diversifolia* extract, $CHCl_3$ fraction, farnesol, and canagliflozin in fast normoglycemic mice

The OGTT was performed in NM mice with a previous fasting of 18 h. The animals divided into six groups (n = 6 per group) were administered orally with 2% tween 20 in water, EEL*Ad* (200 mg/kg), $CHCl_3$ fraction (200 mg/kg), and farnesol (50 mg/kg); canagliflozin (50 mg/kg) was used as a SGLT2 inhibitor. Time 0 was adjusted at the beginning of treatment and 30 min later; a glucose load (1.5 g/kg) was administered to the mice. Subsequently, glycemia values were determined at 2 and 4 h using the glucose oxidase method.[16]

The oral sucrose tolerance and oral lactose tolerance tests of *Annona diversifolia* extract, $CHCl_3$ fraction, farnesol, and acarbose in fast normoglycemic mice

The OSTT was carried out under the same conditions as OGTT, and in this case, a sucrose load (3 g/kg) was given to the fast NM (FNM) mice; acarbose (50 mg/kg) was used as an α-glucosidase inhibitor. The glycemia values were determined at 2 and 4 h using the glucose oxidase method.[16] In the case of OLTT, a lactose load (3 g/kg) was used and blood glucose levels were determined using glucose oxidase method.

Statistical analysis

The results were expressed as the mean values ± standard error mean. The statistical evaluation was carried out by analysis of variance followed by Bonferroni test for multiple comparisons. $P < 0.05$ was considered statistically significant difference between the group means. We used GraphPad Prism version 6.0 for Macintosh (GraphPad software. San Diego. CA, USA).

RESULTS

Bioassay-guided fractionation of the EEL*Ad* was carried out using acute and subchronic tests on AITD and NM mice [Tables 1-3]. *A. diversifolia* is used to control postprandial hyperglycemia in people with DM in Mexican traditional medicine.[23] In this context, medicinal plants used in traditional medicine are an important source of biologically active compounds and have potential for the development of novel antidiabetic agents such as α-glucosidase inhibitors or SGLT2 inhibitor or enhance peripheral glucose uptake. We have initiated a screening program to evaluate the antidiabetic potential of some species of *Annonaceae* family and isolate the active constituents. These plants are used in Mexican traditional medicine as an antihyperglycemic agent.[16]

The results showed that, in acute tests [Table 1] using AITD mice (312.6 ± 19.8 mg/dL of glucose) and administration at four different doses, the best effect was observed after the administration of the EEL*Ad* at a dose of 200 mg/kg; it induced a significant reduction ($P < 0.05$) in hyperglycemia values at 4 h after the treatment (237.0 ± 14.0 mg/dL of glucose). In contrast, when the EEL*Ad* was evaluated in NM mice, at the same doses, it did not affect the blood glucose level. Then, the active EEL*Ad* was fractionated into fractions by organic solvent extraction with $CHCl_3$ followed by EtOAc. All fractions ($CHCl_3$, EtOAc, and AcR) were tested for antihyperglycemic activity on AITD and NM mice. After the administration, only of $CHCl_3$ fraction at a dose of 200 mg/kg on AITD mice induced a significant reduction ($P < 0.05$) in hyperglycemia values at 2 (149.6 ± 33.6 mg/dL of glucose) and 4 h (153.8 ± 12.0 mg/dL of glucose) after treatment comparable to NM mice (161.5 ± 5.3 mg/

Table 1: Effect of a single oral administration of *Annona diversifolia* products on blood glucose levels of normoglycemic and alloxan-induced Type 2 diabetic mice

Treatment	Blood glucose levels (mg/dL)		
	0 h	2 h	4 h
NM control	154.3±1.2	151.3±3.1	146.6±5.2
NM + EEL*Ad* (200 mg/kg)	143.6±5.8	136.6±10.2	124.6±16.3
NM + $CHCl_3$ fraction (200 mg/kg)	161.7±6.8	129.2±5.8*	168.5±7.2
NM + EtOAc fraction (200 mg/kg)	163.5±5.2	160.0±5.7	164.0±8.5
NM + AcR fraction (200 mg/kg)	153.6±4.1	180.8±3.4*	167.2±1.6
NM + F5 (50 mg/kg)	145.6±3.8	154.6±3.3	125.3±4.9*
NM + Farnesol (50 mg/kg)	148.0±6.3	143±8.0	139.6±7.1
NM + Acarbose (50 mg/kg)	177.5±8.8	156.7±3.7*	160.7±2.7*
NM + Canagliflozin (50 mg/kg)	144.0±4.3	123.9±5.5*	126.3±2.8*
NM + Glibenclamide (50 mg/kg)	152.3±11.0	120.5±2.5*	128.3±2.2*
AITD control	352.0±29.0	317.6±6.2	328.6±27.0
AITD + EEL*Ad* (150 mg/kg)	310.3±23.4	308.3±23.7	299.0±21.0
AITD + EEL*Ad* (200 mg/kg)	325.0±11.8	293.0±21.0	237.0±14.0*,Δ
AITD + EEL*Ad* (450 mg/kg)	348.8±7.6	321.5±21.0	307.0±26.9
AITD + EEL*Ad* (600 mg/kg)	327.3±38.2	292.5±38.0	287.7±37.0
AITD + $CHCl_3$ fraction (200 mg/kg)	317.6±22.0	149.6±33.6*,+	153.8±12.0*,Δ
AITD + EtOAc fraction (200 mg/kg)	321.0±25.0	393.0±32.0	420.0±25.0
AITD + AcR fraction (200 mg/kg)	322.6±31.0	371.6±27.0	498.0±28
AITD + F5 (50 mg/kg)	396.0±33.0	304.8±15.1*	311.1±10.5*
AITD + Farnesol (50 mg/kg)	361.0±5.7	271.6±20.6*,+	182.0±3.0*,Δ
AITD + Acarbose (50 mg/kg)	325.5±23.0	266.5±13.8*,+	285.0±16.5*,Δ
AITD + Canagliflozin (50 mg/kg)	321.0±28.3	181.0±34.5*,+	120.6±9.9*,Δ
AITD + Glibenclamide (50 mg/kg)	308.0±4.8	228.5±24.4*,+	179.5±16.5*,Δ

Data are expressed as mean±SEM, n=6. *P<0.05 versus the initial value; +P<0.05 versus AITD control 2 h. NM; ΔP<0.05 versus AITD control 4 h. NM: Normoglycemic mice; SEM: Standard error of mean; AITD: Alloxan-induced Type 2 diabetic mice; EEL*Ad*: Ethanol extract from *Annona diversifolia;* $CHCl_3$ fraction: Chloroformic fraction; EtOAc fraction: Ethyl acetate fraction; AcR fraction: Aqueous residual fraction

Table 2: Effect of repeated administration of ethanol extract from *Annona diversifolia*, chloroformic fraction, farnesol, and acarbose on alloxan-induced Type 2 diabetic mice and normoglycemic mice

Treatment	Blood glucose levels (mg/dL)				
	0	1st week	2nd week	3nd week	4th week
AITD control	301.0±2	282.3±10.7	274.7±6.9	305±1.8	305±17.1
AITD + EEL*Ad* (200 mg/kg)	291.5±22.8	212.0±8.6$^{*,\Delta}$	183.5±1.4$^{*,\Delta}$	190.0±8.6$^{*,\Delta}$	181.5±2.02$^{*,\Delta}$
AITD + $CHCl_3$ fraction (200 mg/kg)	310.6±22.6	194.0±11.9$^{*,\Delta}$	153.8±8.6$^{*,\Delta}$	165.2±7.7$^{*,\Delta}$	167.0±7.4$^{*,\Delta}$
AITD + Farnesol (50 mg/kg)	300.3±10.6	208.3±35$^{*,\Delta}$	201.3±28.3$^{*,\Delta}$	180.0±19.5$^{*,\Delta}$	153.7±16.2$^{*,\Delta}$
AITD + Acarbose (50 mg/kg)	320.0±39	336.0±4.0	225.3±35.5$^{*,\Delta}$	251.0±43.2$^{*,\Delta}$	313.0±69.0
NM control	168.5±0.5	162.0±5.6	170±6.4	177.0±4.0	174.5±3.5
NM + EEL*Ad* (200 mg/kg)	176.3±5.5	166.0±10.0	172.6±5.7	176.0±2.1	173.6±10.0
NM + $CHCl_3$ fraction (200 mg/kg)	179.6±8.1	174.2±9.2	162.0±10.0	175.2±9.3	171.4±10.0
NM + farnesol (50 mg/kg)	176.3±5.5	166.0±10.0	172.6±5.7	176.0±2.1	173.6±10.0

Data are expressed as mean±SEM, n=6. *P<0.05 versus the initial value; $^{\Delta}P$<0.05 versus AITD. NM: Normoglycemic mice; SEM: Standard error of mean; AITD: Alloxan-induced type 2 diabetic mice; EEL*Ad*: Ethanol extract from *Annona diversifolia*; $CHCl_3$ fraction: Chloroformic fraction; EtOAc fraction: Ethyl acetate fraction; AcR fraction: Aqueous residual fraction

Table 3: Effect of ethanol extract from *Annona diversifolia*, chloroformic fraction, farnesol, and acarbose in glucose, lactose, and sucrose oral tolerance assays

Treatment	Blood glucose levels (mg/dL)		
	0 h	2 h	4 h
FNM control	110.3±2.9	114.7±2.7	112.7±0.6
FNM + G (1.5 g/kg)	116.0±3.2	149.7±1.5^{+}	145.7±0.8^{++}
FNM + G + EEL*Ad* (200 mg/kg)	117.3±5.0	116.0±5.5*	111.3±1.2**
FNM + G + $CHCl_3$ fraction (200 mg/kg)	114.7±1.2	110.3±8.9*	98.6±10.1**
FNM + G + farnesol (50 mg/kg)	118.7±1.8	112.0±5.5*	102.7±8.5**
FNM + G + canagliflozin (50 mg/kg)	112.6±8.6	105.3±1.5*	83.3±1.6**
FNM + G + acarbose (50 mg/kg)	109.5±0.8	97.0±4.0*	119.0±2.8**
FNM + L (3.0 g/kg)	115.0±5.6	140.3±4.2^{+}	121.3±3.0^{++}
FNM + L + EEL*Ad* (200 mg/kg)	116.0±4.0	97.3±2.8$^{¥}$	89.0±2.7$^{¥¥}$
FNM + L + $CHCl_3$ fraction (200 mg/kg)	115.6±6.0	94.3±4.1$^{¥}$	78.3±1.9$^{¥¥}$
FNM + L + farnesol (50 mg/kg)	116.6±4.5	109.3±2.0$^{¥}$	105.0±1.4$^{¥¥}$
FNM + L + acarbose (50 mg/kg)	114.0±1.2	127.3±1.4$^{¥}$	112.3±4.0
FNM + S (3.0 g/kg)	119.0±3.1	135.3±4.6^{+}	140.3±1.3^{++}
FNM + S + EEL*Ad* (200 mg/kg)	111.0±4.3	113.3±3.7$^{\Delta}$	115.3±2.1$^{\Delta\Delta}$
FNM + S + $CHCl_3$ fraction (200 mg/kg)	121.0±3.7	111.0±3.0$^{\Delta}$	106±1.7$^{\Delta\Delta}$
FNM + S + farnesol (50 mg/kg)	117.0±5.1	112.0±2.0$^{\Delta}$	125.7±2.0$^{\Delta\Delta}$
FNM + S + acarbose (50 mg/kg)	112.0±3.6	105.0±2.0$^{\Delta}$	122±3.6$^{\Delta\Delta}$

Data are expressed as mean±SEM, n=6. ^{+}P<0.05 versus FNM control 2 h; ^{++}P<0.05 versus FNM Control 4 h; *P<0.05 versus FNM + G 2 h; **P<0.05 versus FNM + G 4 h; $^{¥}P$<0.05 versus FNM + L 2 h; $^{¥¥}P$<0.05 versus FNM + L 4 h,$^{\Delta}P$<0.05 versus FNM + S 2 h; ,$^{\Delta}$,$^{\Delta}P$<0.05 versus FNM + S 4 h. FNM: Fasted normoglycemic mice; SEM: Standard error of mean; G: Glucose; S: Sucrose; L: Lactose; AITD: Alloxan-induced Type 2 diabetic mice; EEL*Ad*: Ethanol extract from *Annona diversifolia*; $CHCl_3$ fraction: Chloroform fraction

dL of glucose). The administration of AcR and EtOAc fractions on AITD mice increased the hyperglycemia values at 2 and 4 h after the treatment. When the three fractions were evaluated in NM mice, only the $CHCl_3$ fraction induced a significant reduction (P < 0.05) in the glycemia at 2 h after the treatment. In the case of the EtOAc and AcR fractions did not affect the blood glucose levels [Table 1], therefore, these fractions were discarded. Considering that $CHCl_3$ fraction showed the best antihyperglycemic effect on both mice, it was purified by column chromatography and preparative TLC to yield an acrylic sesquiterpene alcohol and farnesol [Figure 1].

The administration of farnesol at a dose of 50 mg/kg on AITD mice induced a significant reduction (P < 0.05) in blood glucose values at 4 h of the test, as similar to canagliflozin and glibenclamide (two oral antidiabetic drugs used to treat Type 2 DM). In contrast, farnesol at a dose of 50 mg/kg on NM mice did not affect the blood glucose levels [Table 1].

In the subchronic assays on AITD mice [Table 2], the EEL*Ad*, $CHCl_3$ fraction, and farnesol induced a significant reduction of postprandial hyperglycemia levels since the 1st week and with maximum effect at the 4th week, with blood glucose levels comparable to NM mice. The group treated with acarbose showed a decrease in glycemia levels with significant values (P < 0.05) until the 2nd week of treatment; however, the values began to increase in the 3rd week of treatment, returning to values of an AITD mice in the 4th week of treatment.

The results of OLTT and OSTT [Table 3] showed that the EEL*Ad*, $CHCl_3$ fraction, and farnesol significantly lowered blood glucose levels in FNM at 2 h after a lactose or sucrose load. In the case of OGTT was observed similar effect in FNM mice at 2 h after a glucose load.

Acute toxicity studies revealed the moderate toxicity of the EEL*Am*. The oral LD_{50} value was of 1587.1 mg/kg.

DISCUSSION

This study was to evaluate the acute and subchronic antihyperglycemic properties of the leaves from *A. diversifolia*, on AITD and NM mice. The evaluation of the EEL*Ad* on AITD mice showed that it possesses acute activity at doses of 200 mg/kg and when it was evaluated in NM mice at the same dose did not induce decrease in the blood glucose values, behaving as an antihyperglycemic agent, eliminating the hypoglycemia main adverse effect derived from the use of oral antidiabetic drugs such as sulfonylureas and meglitinides.[8-10,31] Some species of the *Annonaceae* family have been evaluated to demonstrate their activity as antidiabetics;

in most cases, they have been demonstrated that the activities of extracts are at doses higher than 300 mg/kg behaving as antihyperglycemic agents.[32-35] In acute and subchronic tests, the administration of the *A. diversifolia* extract on NM mice did not affect their blood glucose levels at 2–4 h posttreatment. In contrast to similar conditions, the EEL*Ad* induced a significant reduction on blood glucose values in AIDT mice at a dose of 200 mg/kg. Its effect may be associated with enhancing peripheral glucose uptake. Similar findings were observed with *A. muricata*, *Pterocarpus santalinus,* and *Trema orientalis* that had not effect on blood glucose levels in normal rats.[36] In agreement with these results, glibenclamide caused hypoglycemia on NM mice, and on AITD mice, the effect was moderated and significant [Table 1], supporting that pancreatic β-cells were viable in AITD mice used to our tests. It is known that sulfonylurea agents as glibenclamide induced hypoglycemia in NM rats by their ability to stimulate the pancreas β-cells to liberate insulin. Furthermore, it was reported that glibenclamide is ineffective when the pancreas β-cells are destroyed. Our results suggest that part of the action mechanism of *A. diversifolia* ethanol extract may be different from that of sulfonylurea. In contrast, part of the effect may be like *A. muricata*, *Pterocarpus santalinus,* and *Trema orientalis*.[36]

OLTT and OSTT showed that the EEL*Ad* induced a significant reduction of blood glucose levels in FNM at 2 h after a lactose or sucrose load comparable to acarbose, an α-glucosidase inhibitor. In the case of OGTT was observed similar effect in FNM mice at 2 h after a glucose load like to canagliflozin an SGLT2 inhibitor. In addition to a possible effect as enhance peripheral glucose uptake, our results suggest that the control of postprandial hyperglycemia may also be mediated by the regulation of absorption of glucose and inhibition of disaccharide digestion such as sucrose and lactose in agreement with the results obtained in OGTT, OLTT, and OSTT. In this sense, part of the mechanism of *A. diversifolia* extract attenuated postprandial hyperglycemia in acute and subchronic assays may be associated with a potential effect on α-glucosidase enzymes and/or SGLT2.[16,36,37]

The fraction with the best activity on acute and subchronic glycemia levels on AITD mice was $CHCl_3$ fraction at a dose of 200 mg/kg. Our results differ with previous work where the authors reported that an aqueous extract of the leaves from *A. diversifolia* had a significant activity over the glycemia in NM rats; it contains flavonoids, such as rutin and isoquercitrin, which are attributed to antihyperglycemic activity. The results obtained differ with the previous reported because the plant materials were collected from two different areas of Mexico and different months. In this sense, maybe that the concentration of flavonoids in our sample was lower than the previous report.[23]

Purification of $CHCl_3$ fraction led to the isolation of an acrylic sesquiterpene alcohol, farnesol [Figure 1], it sesquiterpene, acarbose, canagliflozin, and glibenclamide were evaluated in acute test [Table 1] on AITD mice at a dose of 50 mg/kg, results showed that at 4 h after treatment farnesol had a better acute activity on glycemia levels than acarbose, similar to glibenclamide and less than canagliflozin. When they were evaluated on NM mice, farnesol had not altered the blood glucose levels. These results suggest that part of antihyperglycemic properties of farnesol may be enhancing peripheral glucose uptake.[36] It is important to mention that the administration of farnesol at dose of 50 mg/kg did not produce decrease of blood glucose level in acute test, thus eliminating one of the main adverse effects of the drugs used to the treatment of DM which is the hypoglycemia.[8,31] There are no reports in the literature of the isolation of farnesol in species belonging to the *Annonaceae* family; most studies have reported the presence of antihyperglycemic flavonoids such as rutin, isoquercitrin, and astragalin; alkaloids such as liriodenine; acetogenins such as rolliniastatin-2, laherradurin, and cherimolin-2, among other metabolites.[23,35-41]

Subchronic evaluation [Table 2] of farnesol (50 mg/kg) for 28 days on AITD mice showed a significant reduction of postprandial hyperglycemia levels since the 1st week and with maximum effect at the 4th week, with blood glucose levels similar to NM mice.

When farnesol was evaluated in OSTT and OLTT significantly decreased of blood glucose levels in fast NM mice at 2 h after of a load of sucrose or lactose, its effect was like acarbose, these results suggest that the antihyperglycemic activity observed in farnesol may be associated with α-glucosidases inhibition.[23,42] Similar effect was observed in the case of OGTT where blood glucose level significantly decreased at 2 h after load of glucose; its effect was comparable to canagliflozin, suggesting that part of antihyperglycemic activity showed by farnesol may be associated with SGLT2 inhibition.[37] However, additional tests must be realized to confirm a possible effect of the EEL*Ad* and farnesol as SGLT2 inhibitors.

These results provide novel information about acrylic sesquiterpenes as farnesol with antihyperglycemic activity present in a species of *Annonaceae* family; most studies of antihyperglycemic activity in species of this family describe that the flavonoids present in the leaves possess the antihyperglycemic activity,[36,41] being rutin the flavonoid to which the activity is attributed; it is known that the way in which rutin exerts the antihyperglycemic activity is by the inhibition of the enzyme α-glucosidase, by union to four residues of amino acids (His 239, His 279, Glu 304, and y Pro 309) presenting better affinity for the enzyme than the acarbose control drug itself.[16] Several studies indicate the antihyperglycemic activity of sesquiterpene lactones such as calein A, calein C, and cariophilene isolated from plant species, which have been indicated to have activity on the enzyme α-glucosidase generating delay in the digestion of hydrates of carbon with a reduction in the postprandial peaks of glucose, these suggest that the farnesol maybe acts partly in this way maintaining the levels of glycemia; nevertheless, it is necessary to realize studies of enzymatic inhibition to confirm the observed.[41-44] Additional tests must be realized to confirm a possible effect of the farnesol as an α-glucosidase inhibitor.

Acute toxicity analysis of the extract showed a LD_{50} of 1587 mg/kg for either sex; these values classify the EEL*Am* in category 4 of toxicity indicating the potential for significant acute effects; it may be harmful if swallowed.[30] Although the EEL*Ad* showed significant effects as antihyperglycemic agent, its use in Mexican traditional medicine must be with precaution. Furthermore, additional experimental works of acute and chronic toxicity of the EEL*Ad* must be realized. In contrast, one study carried out on *A. cherimola* showed that this species possesses an LD_{50} >3000 mg/kg, classifying it in category 5 of toxicity;[16] it is widely known that the toxicity of the species of this family is due to its acetogenins content.[38,40]

CONCLUSION

The EEL*Ad* and farnesol significantly decrease postprandial hyperglycemia on AITD mice in acute and subchronic assays. Our results suggest that the control of postprandial hyperglycemia may be mediated by the regulation of absorption of glucose and inhibition of disaccharide digestion such as sucrose and lactose in agreement with the results obtained in OGTT, OLTT, and OSTT. In this sense, part of the mechanism of *A. diversifolia* extract and farnesol attenuated postprandial hyperglycemia in acute and subchronic assays may be associated with a potential effect on α-glucosidase enzymes and SGLT2. Furthermore, their effect may be associated with enhancing peripheral glucose uptake. Finally, the results support the use of *A. diversifolia* in Mexican traditional medicine as an antihyperglycemic agent.[23]

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Conflicts of interest

There are no conflicts of interest.

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