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ORIGINAL ARTICLE

Antihyperglycemic Activity of the Leaves from *Annona cherimola* Miller and Rutin on Alloxan-induced Diabetic Rats

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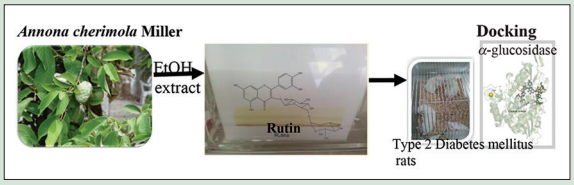
ABSTRACT

Background: *Annona cherimola*, known as "chirimoya" has been reported in Mexican traditional medicine for the treatment of diabetes. **Objective:** The aims of the present study were to validate and assess the traditional use of *A. cherimola* as an antidiabetic agent. **Materials and Methods:** The ethanol extract from *A. cherimola* (300 mg/kg, EEAc), subsequent fractions (100 mg/kg), and rutin (30 mg/kg) were studied on alloxan-induced type 2 diabetic (AITD) and normoglycemic rats. In addition, oral glucose tolerance test (OGTT) and oral sucrose tolerance test (OSTT) were performed in normoglycemic rats. Molecular docking technique was used to conduct the computational study. **Results:** Bioassay-guided fractionation of EEAc afforded as major antihyperglycemic compound, rutin. EEAc attenuated postprandial hyperglycemia in acute test using AITD rats (331.5 mg/dL) carrying the glycemic levels to 149.2 mg/dL. Rutin after 2 h, attenuated postprandial hyperglycemia in an acute assay using AITD rats such as EEAc, with maximum effect (150.0 mg/dL) being seen at 4 h. The antihyperglycemic activities of EEAc and rutin were comparable with acarbose (151.3 mg/dL). In the subchronic assay on AITD rats, the EEAc and rutin showed a reduction of the blood glucose levels since the 1st week of treatment, reaching levels similar to normoglycemic state (116.9 mg/kg) that stayed constant for the rest of the assay. OGTT and OSTT showed that EEAc and rutin significantly lowered blood glucose levels in normoglycemic rats at 2 h after a glucose or sucrose load such as acarbose. Computational molecular docking showed that rutin interacted with four amino acids residues in the enzyme α-glucosidase. **Conclusion:** The results suggest that rutin an α-glucosidase inhibitor was responsible in part of the antihyperglycemic activity of *A. cherimola*. Its *in vivo* antihyperglycemic activity is in good agreement with the traditional use of *A. cherimola* for the treatment of diabetes.

Key words: α-glucosidase, *Annona cherimola* Miller, *Annonaceae*, rutin, type 2 diabetes mellitus

SUMMARY

The ethanol extract from *Annona cherimola* (300 mg/kg, EEAc), subsequent fractions (100 mg/kg) and rutin (30 mg/kg) were studied on alloxan-induced type 2 diabetic (AITD) and normoglycemic rats. The results suggest that rutin; an α-glucosidase inhibitor was responsible in part of the antihyperglycemic activity of *A. cherimola*. Its *in vivo* antihyperglycemic activity is in good agreement with the traditional use of *A. cherimola* for the treatment of diabetes.



Abbreviations Used: EEAc: The ethanol extract from Annona cherimola, AITD: Alloxan-induced type 2 diabetic rats, OGTT: Oral glucose tolerance test, OSTT: Oral sucrose tolerance test, DM: Diabetes mellitus

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INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous metabolic disorder that is characterized by high levels of blood glucose with disturbances of carbohydrate, lipid, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1-6] DM affects more than 371 million people worldwide and accounts for >4.8 million deaths each year.[7,8] In the case of México, the estimates indicates that the number of diabetic patients will increase from >2 million in 2002 to >132 million in 2030.[9] According to the Mexican health services, among 2001–2014, DM was the first cause of mortality among women and the second in men.[9-11] Treatment with oral blood glucose-lowering drugs such as metformin, glibenclamide, rosiglitazone, voglibose, miglitol, and acarbose are used for the control of DM. However, DM and its secondary complications continue to be a major problem in the world population. On the other the pharmaceutical drugs are either too expensive or have undesirable side effects. In the case of acarbose is a well-known α-glucosidase inhibitor that is currently used in Mexico; it is effective; however, hepatotoxicity and abdominal discomfort such as gas, abdominal distention, meteorism, bloating, and loose stool has been reported for this drug.[12] In addition, tolerance usually occurs after continued administration for 3 months suggesting an adaptive response within the intestinal tract.[13,14] Clearly,

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there is need for novel drugs such as α-glucosidase inhibitors devoid of side effects, especially hepatotoxicity, they are required to improve the patients' quality of life. In this sense, medicinal plants are one of the useful areas of this research since they constitute an important source of new compounds with potential therapeutic effects.[15] In the case of México, a total of 306 species are used for the treatment of diabetes.[16]

Annona cherimola Miller is one of the many edible fruits species in the *Annona* genus that belongs to the *Annonaceae* family in the Magnoliales order. It is a semi-deciduous, erect, but low-branched tree, frequently branched off at ground level. The plant is native of Ecuador and Peru distributed widely in the tropical or subtropic regions from America, Africa, and Asia and even in the South of Europe.[17,18] In México is popularly known as "chirimoya, atish (Michoacan), tzon te chkia (Oaxaca), lamatzapotl (Puebla), and yati (Veracruz)". This species, alone or in combinations with others have been used in Mexican traditional medicine for the treatment of several diseases such as fever, cough, worms, and headache as well as anti-inflammatory. In addition, to treat gastrointestinal disorders such stomach pain, diarrhea, and dysentery; at present, it is used to treat diabetes.[16,19,20] Phytochemical investigations revealed the presence of alkaloids, flavonoids, sterols,[20-24] terpenoids,[25-27] cyclic peptides,[28,29] and acetogenins.[30,31] With regard to pharmacological investigations have been reported that *A. cherimola* extracts possess genotoxic, cytotoxic,[31-33] antihypercholesterolemic,[23] antihyperlipidemic[34] antidepressant,[22] cryoprotective,[35] anxiolytic,[25] antiprotozoal,[36] antisecretory,[37] antiarthritic,[38] antibacterial,[17,20] antifungal,[17] anti-inflammatory, antioxidant,[39,40] and inhibitor of mitochondrial complex I properties.[30] In addition, antihyperglycemic activities of the ethanol extract of the leaves from *A. cherimola* (EEAc) have been reported.[41,42] However, there are no reports of the antihyperglycemic activity-guided fractionation of EEAc. Thus, as part of continuing search to discover novel therapies for the treatment of DM derived from plants commonly used in Mexican traditional medicine,[43] the goals of the present study were to validate and assess the traditional use of *A. cherimola* collected in Mexico as antidiabetic agent and to identify α-glucosidase inhibitors that could efficiently control postprandial glucose levels.

MATERIALS AND METHODS

Plant material

A. cherimola leaves were collected by Dr. Fernando Calzada in December 2011 in San Jose, Tláhuac, Mexico. The plant material was authenticated by MS Abigail Aguilar-Contreras of the Herbarium IMSSM of Mexican Institute of Social Security (IMSS) where the voucher specimen is conserved under reference number: 15,795.

Isolation and identification of rutin from *Annona cherimola*

The air-dried and finely powdered leaves (2.9 kg) were extracted by maceration at room temperature with EtOH (2 times × 10 L). After filtration, the extract were combined and evaporated *in vacuo* to yield 131 g (yield 4.5%) of green residue. The active extract (40 g) was suspended in 10% EtOH-water (120 mL) and successively partitioned with dichloromethane (DCM fraction, 35 g, 100 mL × 2 times) and EtOAc (EtOAc fraction, 0.69 g, 100 mL × 2 times). The aqueous residual (AR) layer was concentrated under reduced pressure to give (AR fraction, 15.1 g). The activity was associated with AR fraction and then a portion (30 mg) was purified by preparative TLC (Silica Gel 60F-254 Merck) using EtOAc-MeOH-water (100: 16.5: 13.5) mixture as the mobile phase to give rutin (20 mg). The process was repeated as needed. The structure of rutin was ascertained by comparison of the spectroscopic data (NMR and UV) reported in the literature.[44]

Animals

A 3-month-old male albinos Sprague-Dawley rats, (250 and 300 g) and Balb-C mice of either sex (20 ± 4 g) 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 by the Official Mexican Rule.[45] They were maintained in a temperature room (22°C ± 2°C) on a 12 h light-dark natural cycle. Rodents were fed with standard diet and water *ad libitum*. These studies were conducted with the approval of the Specialty Hospital Ethical Committee of the National Medical Center "Siglo XXI" from IMSS (Register: R-2012-3601-18).

Acute oral toxicity study

The acute oral toxicity study was conducted using test guidelines on acute oral toxicity test 423 according to OCDE (2001).[46] Twenty-four Balb-C mice fasted overnight but allowed free access to water *ad libitum* were randomly assigned into the following four groups of six mice of either sex (three males and three females). Control received distilled water and three groups received the extract at the doses of 30 mg/kg, 300 mg/kg and 3000 mg/kg. The mice were not fed for 4 h following administration. The signs of toxic effects and/or mortality were observed 4 h after administration then, for next 48 h. The general behavior of mice was observed daily in a period of 14 days for mortality, toxic effects, and/or changes in behavioral pattern. At the end of the experiments, the animals were sacrificed in a CO_2 chamber. Then, the internal organs (stomach, gut, lungs, kidney, heart, spleen, and liver) were extracted, and the pathological observations were performed.

Induction of experimental diabetes in rats

Experimental type 2 DM (TDM) was induced by two intravenous injections of alloxan (Alloxan monohydrate (2, 4, 5, 6-(1H, 3H)-pyrimidinetetrone, Sigma–Aldrich) at intervals of 48 h (2 × 125 mg/kg). Blood glucose levels were determined 7 days after the last administration using glucose oxidase method (Evolution Blood Glucometer Glucose Monitoring System, Infopia Co., Ltd. USA). Animals with hyperglycemia values >200 mg/dL were considered diabetic and used in this study. In addition, animals responded to glibenclamide treatment in agree with TDM rats. It was reported that glibenclamide is ineffective when β-cells are destroyed.[43,47-49]

Single oral administration of *Annona cherimola* extract and rutin

Normoglycemic rats were assigned to four different groups of six rats each. The EEAc, subsequent fractions, and pure compounds were administrated orally. Group 1 made up of control rats treated with 1 mL/kg of distilled water. Group 2 and Group 3 received 30 mg/kg of the acarbose (Glucobay, tablets of 50 mg, Bayer) and rutin, respectively. Group 4 received EEAc at the dose of 300 mg/kg. Blood samples were collected from the tail vein at intervals 0, 2, and 4 h. Glucose content in each sample was assessed using the glucose oxidase method. The antidiabetic effects of EEAc and rutin were also evaluated on alloxan-induced type 2 diabetic (AITD) rats. Group I of AITD rats were treated with distilled water (1 ml/kg). Group II and Group III of diabetic rats received either acarbose (30 mg/kg) or rutin. Groups IV to VI were treated with DCM, EtOAc, and AR fractions (100 mg/kg) while Group VII was administered a single dose of EEAc at 300 mg/kg. Blood sample collection and analysis were done as previously mentioned.

Subchronic effects of *Annona cherimola* extract and rutin

In this study, we examine the effects of long-term administration of the plant extract and rutin on AITD rats. Rats were kept for 7 days before

the beginning of the treatment to stabilize the diabetic conditions and to allow a permanent and chronic hyperglycemia.[49] The animals were randomly divided into three groups containing six rats each: Diabetic control of AITD rats were treated with distilled water, one group of diabetic rats treated with EEAc at the dose of 300 mg/kg, and other with rutin at the dose of 30 mg/kg. The animal received treatment orally for 28 consecutive days. Glycemia was determined weekly.

The oral glucose tolerance test of ethanol extract from *Annona cherimola* in normoglycemic rats

The oral glucose tolerance test[45] was performed in overnight fasted (18 h) normoglycemic rats. Rats divided into four groups (n = 6) were administered drinking water, EEAc (300 mg/kg), rutin (30 mg/kg), or acarbose (30 mg/kg), respectively. Time 0 h was set before treatment with the extract; 30 min later, a glucose load (1.5 g/kg) was administered to the rats. Blood samples were obtained 2 and 4 h after the carbohydrate load then glucose levels were determined using glucose oxidase method.

The oral sucrose tolerance test of ethanol extract from *Annona cherimola* in normoglycemic rat

Oral sucrose tolerance test was executed in the same way as for the oral glucose tolerance test (OGTT); however in this case, sucrose (3 g/kg) was used as carbohydrate load and acarbose (30 mg/kg) as positive control. Blood glucose levels were determined using glucose oxidase method.

Statistical analysis

Data are expressed as the mean ± standard error mean. Statistical significance was determined by the one-way analysis of variance followed by the Bonferroni test (GraphPad Prism Version 5.03; GraphPad Software Inc., La Jolla, CA, USA) as the post-test. $P < 0.05$ indicates significant difference between group means.

Docking of α-glucosidase inhibitors

To known the potential binding mode at molecular level of rutin as α-glucosidase inhibitor, it was compared versus acarbose in molecular docking experiments. We carried out docking studies employing yeast α-glucosidase as target (crystal structure, PDB ID: 3A47), total molecules of water and ions no needed to catalytic activity were stripped to preserve the entire protein. First, all the chemical structures of the ligands tested (rutin and acarbose) were drawn with ChemBioDraw Ultra 11.0 program, later, the Z matrix of the ligands previously drawn were created using the GaussView 5.0 graphical viewer and submitted to energetic and geometrical minimization with Gaussian 09 package, the computed output topologies from the previous step were used as input files to docking simulations. AutoDock 4.2 software was employing to carry out the docking simulations with the follow search parameters: Kollman charges were computed considering the entire protein, and polar hydrogen atoms were added in those atoms capable to establish H bond interactions (O and N atoms), a grid-based procedure was employed to generate the affinity maps delimiting a grid box of 126 × 126 × 126 Å3 in each space coordinate, with a grid points spacing of 0.375 Å, the Lamarckian genetic algorithm was employed as scoring function with a randomized initial population of 100 individuals and maximum number of energy evaluations of 1×10^7 cycles, the analysis of the interactions established in the enzyme/inhibitor complex was done with Pymol graphical viewer.

RESULTS AND DISCUSSION

The use of *A. cherimola* in Mexican traditional medicine prompted us to validate and assess its efficacy as an antidiabetic agent using bioactivity-guided fractionation of the EEAc on well-known animal models and computational experiments. First, an EEAc (300 mg/kg) was tested on AITD and normoglycemic rats. Experimental type 2 diabetes was achieved by treatment rats with alloxan.[38,44] In the acute preliminary experiments, a single oral administration of EEAc at the dose of 300 mg/kg did not affect significantly blood glucose levels in normoglycemic rat [Table 1] such as acarbose (30 mg/kg) antidiabetic drug used as control. In contrast, single administrations of the plant extract at dose of 300 mg/kg on AITD rats induced a reduction in the levels of hyperglycemia (331.5 mg/dL), carrying the glycemic levels to 149.2 mg/dL at the 4th h after the administration ($P < 0.05$); it was comparable to the acarbose (151.3 mg/dL) an α-glucosidase inhibitor currently is used to treat TDM. In the subchronic assay, AITD rats showed [Table 2] a reduction in the blood glucose levels since the 1st week of treatment (142 mg/dL), reaching levels similar to normoglycemic levels (113 mg/dL) that stayed constant for the rest of the assay ($P < 0.05$). In the oral glucose (1.5 g/kg) and sucrose (3.0 g/kg) tolerance tests, the treatment of EEAc provoked significant ($P < 0.05$) decrease of the postprandial peak [Table 3] in normoglycemic rats at 2 h after a glucose or sucrose load ($P < 0.05$) such as acarbose. In relation to the subchronic assay on AITD rats with the treatment of EEAc (300 mg/kg) during 28 days have shown a significant reduction of blood glucose levels implying that the plant extract may act in long-term. These tests suggest that antihyperglycemic action of EEAc involved inhibition of intestinal α-glucosidase. The enzyme reduces the rate of digestion of polysaccharides by preventing of their immediate break down into monosaccharides, which are absorbed into the bloodstream. The inhibition of enzyme cause slow absorption of monosaccharides and gives to the β-cell in the pancreas more time to secrete adequate insulin to cover the meal.[46,47] Ours results are in agreement with the previous reports on antihyperglycemic properties of *Annonaceae* family[36,37,47-60] and confirm the antidiabetic properties of EEAc.[36,37] In these sense, antihyperglycemic properties of these species have been associated with the presence of polyphenols and flavonoids. However, it is important to point that antihyperglycemic activity-guided fractionation to isolate the compounds responsible of antidiabetic properties of several of these species have not been reported including *A. cherimola*.

To identify the α-glucosidase inhibitors of EEAc, it was subject to antihyperglycemic activity-guided fractionation using AITD rats. The EEAc was divided into organic and soluble fractions by solvent partition with DCM and EtOAc. All fractions (DCM, EtOAc, and AR fractions)

Table 1: Effect of a single oral administration of *Annona cherimola on* blood glucose levels of normoglycemic and alloxan-induced type 2 diabetic rats

Treatment	Blood glucose levels (mg/dL)		
	0 h	2 h	4 h
NR	114.1±1.1*	111.6±1.2*	112.2±1.1*
EEAc (300 mg/kg)	109.3±1.9*	118.8±1.1*	118.7±1.2*
Rutin (30 mg/kg)	114.2±1.2	100.0±11.1	104.0±7.0
Acarbose (30 mg/kg)	111.0±1.3*	101.0±2.6*	116.0±2.9*
AITD control	324.4±3.4*	314.6±10*	310.7±17*,**
EEAc (300 mg/kg)	331.5±3.2*	272.5±13	149.2±2.8*,**
DCM fraction (100 mg/kg)	333.0±2.2*	314.0±10*	317.0±17*
EtOAc fraction (100 mg/kg)	344.0±2.5*	319.0±18*	316.0±19*
AR fraction (100 mg/kg)	334.0±2.4*	314.0±13.2	155.0±11
Rutin (30 mg/kg)	317.0±2.1	161.0±13.3	150.0±12.8
Acarbose (30 mg/kg)	320.0±1.2*	234.6±13	151.3±16*,**

Data are expressed as mean±SEM, n=6; *P<0.05 compared to the initial value; **P<0.05 compared to AITD control. NR: Normoglycemic rats; SEM: Standard error of mean; AITD: Alloxan-induced type 2 diabetic; EEAc: Ethanol extract from *Annona cherimola*; DCM: Dichloromethane; EtOAc: Ethyl acetate; AR: Aqueous residual

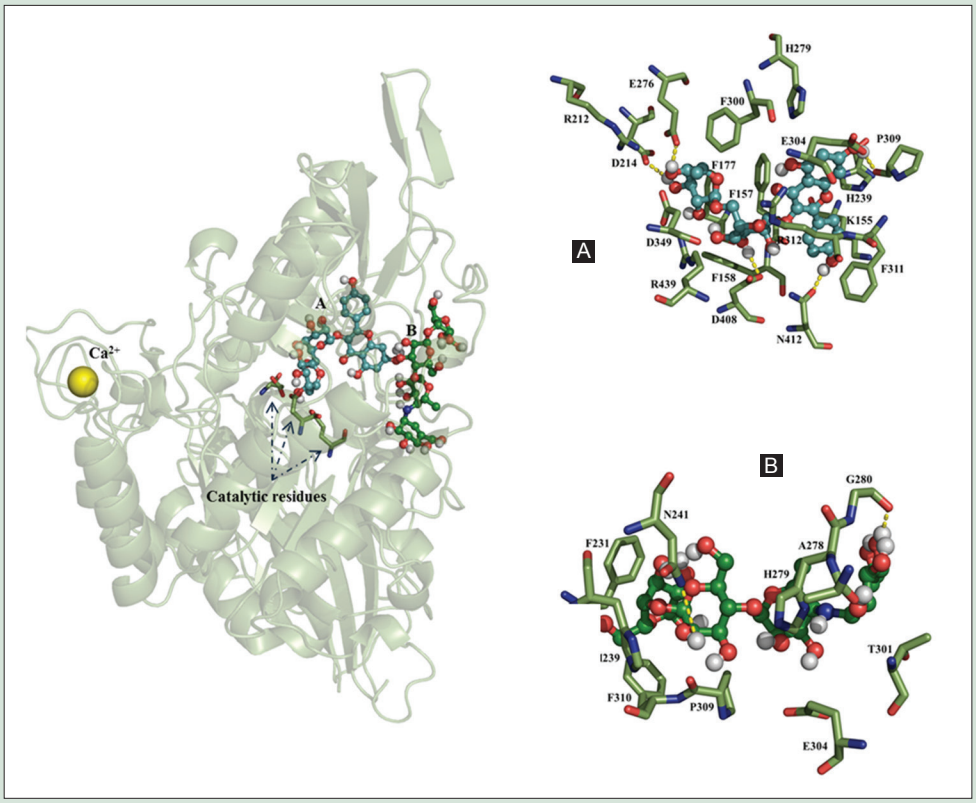

Figure 1: Binding mode of rutin (A) and acarbose (B) inside of the active site of α-glucosidase

Table 2: Effect of repeated administration of ethanol extract of *Annona cherimola* and rutin in diabetic rats with a chronic hyperglycemia

Treatment	Blood glucose levels (mg/dL)				
	Day 0	Day 7	Day 14	Day 21	Day 28
NR control	114.1±1.1*	116.9±1.9*	111.1±1.6*	106.2±1.8*	109.3±1.9*
AITD control	255.0±4.1*	343.0±1.6*,**	482.0±1.3*,**	552.0±1.1*,**	550.0±1.7*,**
EEAc (300 mg/kg)	250.0±3.1*	142.0±1.2*,**	113.0±1.5*,**	119.0±1.2*,**	116.0±1.9*,**
Rutin (30 mg/kg)	251.0±2.0	155.0±2.2	112.0±2.0	117.0±5.0	113.0±2.5

Data are expressed as mean±SEM, n=6. *P<0.05 compared to the initial value; **P<0.05 compared to AITD control. NR: Normoglycemic rats; SEM: Standard error of mean; AITD: Alloxan-induced Type 2 diabetic; EEAc: Ethanol extract from *Annona cherimola*

Table 3: Effect of ethanol extract of *Annona cherimola* on oral glucose and sucrose tolerance tests

Treatment	Blood glucose levels (mg/dL)		
	0 h	2 h	4 h
NR	114.1±1.1*	111.6±1.2*,**	112.2±1.1*
NR + G (1.5 g/kg)	113.1±1.1*	148.9±2.7**	111.1±2.1*
NR + G + EEAc (300 mg/kg)	114.7±1.3*	117.6±2.3*	110.3±1.8*
NR + G + rutin (30 mg/kg)	116.0±2.0	116.0±2.0	110.0±2.8
NR + G + acarbose (30 mg/kg)	114.5±1.3*	113.8±1.7*	112.1±1.3*
NR + S (3.0 g/kg)	114.5±1.2*	166.8±1.2**	114.3±1.3*
NR + S + EEAc (300 mg/kg)	114.6±1.1*	115.6±2.5*	115.5±2.5*
NR + S + rutin (30 mg/kg)	112.8±2.0	112.8±2.3	112.7±1.3
NR + S + acarbose (s0 mg/kg)	114.0±1.2*	115.6±1.4*	115.7±1.9*

Data are expressed as mean±SEM, n=6, *P<0.05 compared to the initial value; **P<0.05 compared to normal control. G: glucose; S: sucrose; NR: Normoglycemic rats; SEM: Standard error of mean; EEAc: Ethanol extract from *Annona cherimola*

were tested for acute antihyperglycemic activity at doses of 100 mg/kg. As result of this process, the AR fraction showed the best inhibitory activity [Table 1], it was purified by preparative TLC to give rutin.[44] The DCM and EtOAc were discarded because they were inactive. The effects of the flavonol glycoside on blood glucose levels were studied on AITD and normoglycemic rats. In addition, OGTT (1.5 g/kg) and oral sucrose (3.0 g/kg) tolerance tests (OSTT) were performed in normoglycemic rats [Tables 1 and 3]. Rutin after 2 h attenuated postprandial hyperglycemia in acute assay using AITD rats, with maximum effect (150.0 mg/dL, $P < 0.05$) being seen at 4 h. The antihyperglycemic activity of rutin was close that of to acarbose (151.3 mg/dL, $P < 0.05$). In the subchronic assay with AITD rats, rutin showed a reduction of the blood glucose levels, since the 1st week of treatment, reaching levels similar to normoglycemic state (113 mg/kg) that stayed constant for the rest of the assay ($P < 0.05$). OGTT and OSTT showed that rutin significantly lowered blood glucose levels in normoglycemic rats at 2 h after a glucose or sucrose load ($P < 0.05$) like acarbose. Ours results are in agreement with the previous reports as an α-glucosidase inhibitor and antihyperglycemic properties of rutin.[66,67]

In the case of the docking experiments, they are based on the fact that this class of studies has recently contributed to the discovery of novel drug candidates. *In silico* approaches have been applied to search the possible interaction between the potential targets and molecules obtained from

Table 4: ΔG (kcal/mol), Ki (μM) and receptor-ligand interactions from molecular dynamics simulations

Ligand	ΔG	Ki	Nonbonded interactions and distances in Å
Rutin	−7.85	1.75	Lys 155$^{3.20}$, Phe 157$^{3.46}$, Phe 158$^{2.69}$, Phe 177$^{3.92}$, Arg 212$^{3.05}$, Asp 214$^{2.21}$, His 239$^{4.00}$, Glu 276$^{1.88}$, His 279$^{4.19}$, Phe 300$^{3.25}$, Glu 304$^{3.50}$, Pro 309$^{2.10}$, Phe 311$^{3.10}$, Arg 312$^{2.59}$,Asp 349$^{2.63}$, Asp 408$^{2.85}$, Asn 412$^{1.89}$, and Arg 439$^{2.98}$
Acarbose	−4.07	1.04	Phe 231$^{3.75}$, His 239$^{3.68}$, Asn 241$^{3.41}$, Ala 278$^{2.25}$, His 279$^{2.37}$, Gly 280$^{1.78}$, Thr 301$^{3.80}$, Glu 304$^{2.73}$, Pro 309$^{2.27}$, and Phe 310$^{3.70}$

Asp: Aspartate; Trp: Tryptophan; Lys: Lysine; Phe: Phenylalanine; Arg: Arginine; His: Histidine; Glu: Glutamate; Pro: Proline; Asn: Asparagine; Ala: Alanine; Gly: Glycine; Thr: Threonine; Val: Valine

natural products as flavonoids yielding new pharmacological horizons.[68-70] Computational molecular docking showed that rutin interacted with four amino acids residues (His 239, His 279, Glu 304, and Pro 309) in the enzyme α-glucosidase, revealing its potential binding mode at molecular level in the catalytic site to acarbose [Figure 1 and Table 4]. Furthermore, showed that it flavonol glycoside have high affinity for the enzyme α-glucosidase with lowest free binding energy from −7.85 kcal/mol like to acarbose (−4.07 kcal/mol). In addition, theoretical inhibition constants (Ki) were similar [Table 4].

Acute toxicity studies revealed the nontoxic nature of the EEAc. A single dose of 30 mg/kg or 300 mg/kg or 3000 mg/kg did not indicate modification of behavior. No mortality was recorded during the study. After sacrifice on the 14th day, macroscopic pathology observations revealed no visible lesions in any animals. The oral LD_{50} value of EEAc must be >3000 mg/kg.

It can be concluded from the data that EEAc is beneficial in controlling the blood glucose level in experimental diabetic rats and according to *in vivo* studies it acts as α-glucosidase inhibitor. This effect can be attributed in part to the presence of flavonol glycoside, rutin. In addition, results validate the use of *A. cherimola* in Mexican traditional medicine for the treatment of diabetes. Finally, it is the first bioassay-guided about the chemistry and antidiabetic properties of *A. cherimola*.

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Conflicts of interest

There are no conflicts of interest.

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