

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

Fernando Calzada, Jesús Iván Solares-Pascasio, R. M. Ordoñez-Razo¹, Claudia Velazquez², Elizabeth Barbosa³, Normand García-Hernández¹, David Mendez-Luna⁴, José Correa-Basurto⁴

Medical Research Unit in Pharmacology, UMAE Speciality Hospital-2° Floor CORCE National Medical Center Siglo XXI, IMSS, Av. Cuauhtémoc 330, Col. Doctores, CP 06725, México City, ¹Medical Research Unit in Human Genetics UMAE Pediatric Hospital. Medical Center Siglo XXI, IMSS, ²Institute of Health Sciences, Autonomous University of the State of Hidalgo, Km. 4.5 Carretera Pachuca-Tulancingo, Unidad Universitaria, C. P. 42076 Pachuca, Hidalgo, México, ³Postgraduate Studies and Research, Superior School of Medicine of IPN, Plan de San Luis y Díaz Mirón, CP 11340, México City, ⁴Laboratory of Molecular Modeling and Bioinformatics/ Drug Design, Superior School of Medicine of IPN, Plan de San Luis y Díaz Mirón s/n, 11340 México City, México

ABSTRACT

Background: *Annona cherimola*, known as “chirimoya” has been reported in Mexican traditional medicine for the treatment of diabetes.

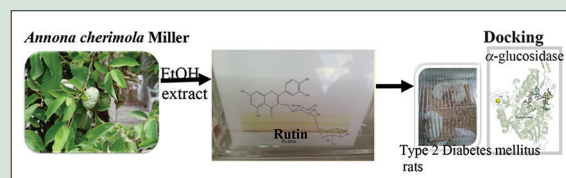
Objective: The aims of the present study were to validate and assess the traditional use of *A. cherimola* as an antidiabetic agent.

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

Correspondence:

Dr. Fernando Calzada Bermejo,
Unidad de Investigación Médica en
Farmacología-2° piso CORCE Centro Médico
Nacional Siglo XXI, IMSS, Av. Cuauhtémoc 330,
Col. Doctores, CP 06725, D. F., México.
E-mail: fercalber10@gmail.com
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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 indicate 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 México; 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 EECa. 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 $\times$ 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 $\times$ 2 times) and EtOAc (EtOAc fraction, 0.69 g, 100 mL $\times$ 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 $\pm$ 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 $\pm$ 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 CO₂ 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)-pyrimidinetrone, Sigma-Aldrich) at intervals of 48 h (2 $\times$ 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 EECa, 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 EECa 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 EECa 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 EECa 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 $\pm$ 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 $126 \times 126 \times 126 \text{ \AA}^3$ in each space coordinate, with a grid points spacing of $0.375 \text{ \AA}$, 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 $\pm$ 1.1*	111.6 $\pm$ 1.2*	112.2 $\pm$ 1.1*
EEAc (300 mg/kg)	109.3 $\pm$ 1.9*	118.8 $\pm$ 1.1*	118.7 $\pm$ 1.2*
Rutin (30 mg/kg)	114.2 $\pm$ 1.2	100.0 $\pm$ 11.1	104.0 $\pm$ 7.0
Acarbose (30 mg/kg)	111.0 $\pm$ 1.3*	101.0 $\pm$ 2.6*	116.0 $\pm$ 2.9*
AITD control	324.4 $\pm$ 3.4*	314.6 $\pm$ 10*	310.7 $\pm$ 17**
EEAc (300 mg/kg)	331.5 $\pm$ 3.2*	272.5 $\pm$ 13	149.2 $\pm$ 2.8***
DCM fraction (100 mg/kg)	333.0 $\pm$ 2.2*	314.0 $\pm$ 10*	317.0 $\pm$ 17*
EtOAc fraction (100 mg/kg)	344.0 $\pm$ 2.5*	319.0 $\pm$ 18*	316.0 $\pm$ 19*
AR fraction (100 mg/kg)	334.0 $\pm$ 2.4*	314.0 $\pm$ 13.2	155.0 $\pm$ 11
Rutin (30 mg/kg)	317.0 $\pm$ 2.1	161.0 $\pm$ 13.3	150.0 $\pm$ 12.8
Acarbose (30 mg/kg)	320.0 $\pm$ 1.2*	234.6 $\pm$ 13	151.3 $\pm$ 16***

Data are expressed as mean $\pm$ 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

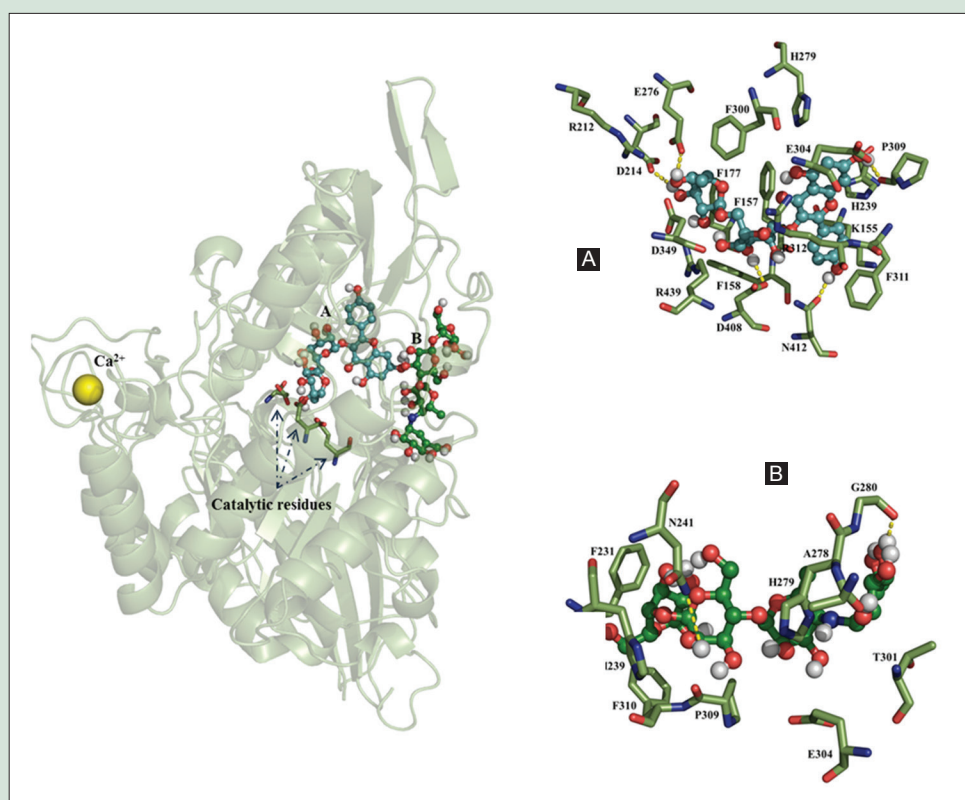


Figure 1: Binding mode of rutin (A) and acarbose (B) inside of the active site of α -glucosidase

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 $\pm$ 1.1*	116.9 $\pm$ 1.9*	111.1 $\pm$ 1.6*	106.2 $\pm$ 1.8*	109.3 $\pm$ 1.9*
AITD control	255.0 $\pm$ 4.1*	343.0 $\pm$ 1.6**	482.0 $\pm$ 1.3**	552.0 $\pm$ 1.1**	550.0 $\pm$ 1.7**
EEAc (300 mg/kg)	250.0 $\pm$ 3.1*	142.0 $\pm$ 1.2**	113.0 $\pm$ 1.5**	119.0 $\pm$ 1.2**	116.0 $\pm$ 1.9**
Rutin (30 mg/kg)	251.0 $\pm$ 2.0	155.0 $\pm$ 2.2	112.0 $\pm$ 2.0	117.0 $\pm$ 5.0	113.0 $\pm$ 2.5

Data are expressed as mean $\pm$ 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; EECa: 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 $\pm$ 1.1*	111.6 $\pm$ 1.2**	112.2 $\pm$ 1.1*
NR + G (1.5 g/kg)	113.1 $\pm$ 1.1*	148.9 $\pm$ 2.7**	111.1 $\pm$ 2.1*
NR + G + EECa (300 mg/kg)	114.7 $\pm$ 1.3*	117.6 $\pm$ 2.3*	110.3 $\pm$ 1.8*
NR + G + rutin (30 mg/kg)	116.0 $\pm$ 2.0	116.0 $\pm$ 2.0	110.0 $\pm$ 2.8
NR + G + acarbose (30 mg/kg)	114.5 $\pm$ 1.3*	113.8 $\pm$ 1.7*	112.1 $\pm$ 1.3*
NR + S (3.0 g/kg)	114.5 $\pm$ 1.2*	166.8 $\pm$ 1.2**	114.3 $\pm$ 1.3*
NR + S + EECa (300 mg/kg)	114.6 $\pm$ 1.1*	115.6 $\pm$ 2.5*	115.5 $\pm$ 2.5*
NR + S + rutin (30 mg/kg)	112.8 $\pm$ 2.0	112.8 $\pm$ 2.3	112.7 $\pm$ 1.3
NR + S + acarbose (30 mg/kg)	114.0 $\pm$ 1.2*	115.6 $\pm$ 1.4*	115.7 $\pm$ 1.9*

Data are expressed as mean $\pm$ 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; EECa: 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 LD₅₀ 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.

REFERENCES

- Grupo de trabajo ALAD. Guías ALAD de diagnóstico, control y tratamiento de la diabetes mellitus tipo 2 (GALAD). Rev Asoc Latinoam Diabetes 2006;14:96-140.
- Umamahesh B, Veeresham C. Antihyperglycemic and insulin secretagogue activities of *Abrus precatorius* leaf extract. Pharmacognosy Res 2016;8:303-8.
- Atal S, Atal S, Vyas S, Phadnis P. Bio-enhancing effect of piperine with metformin on lowering blood glucose level in alloxan induced diabetic mice. Pharmacognosy Res 2016;8:56-60.
- Dzib-Guerra WD, Escalante-Erosa F, García-Sosa K, Derbré S, Blanchard P, Richomme P, *et al.* Anti-advanced glycation end-product and free radical scavenging activity of plants from the Yucatecan flora. Pharmacognosy Res 2016;8:276-80.
- Menon N, Sparks J, Omoruyi FO. Oxidative stress parameters and erythrocyte membrane adenosine triphosphatase activities in streptozotocin-induced diabetic rats administered aqueous preparation of *Kalanchoe pinnata* leaves. Pharmacognosy Res 2016;8:85-8.
- Parasuraman S, Balamurugan S, Christapher PV, Petchi RR, Yeng WY, Sujithra J, *et al.* Evaluation of antidiabetic and antihyperlipidemic effects of hydroalcoholic extract of leaves of *Ocimum tenuiflorum* (Lamiaceae) and prediction of biological activity of its phytoconstituent. Pharmacognosy Res 2015;7:156-65.
- International Federation of Diabetes (IFD). Diabetes Atlas. 5th ed. 2012. Available from: <http://www.idf.org/diabetesatlas/5e/Update2012>.
- World Health Organization (WHO). 2004. Available from: <http://www.who.org>.
- Secretaría de Salud (SSA). Página de la Secretaría de Salud, Gobierno de México; 2004. Available from: <http://www.ssa.gob.mx>.
- Guzmán JR, Lyra R, Aguilar-Salinas CA, Cavalcanti S, Escano F, Tambasia M, *et al.* Treatment of type 2 diabetes in Latin America: A consensus statement by the medical associations of 17 Latin American countries. Latin American Diabetes Association. Rev Panam Salud Publica 2010;28:463-71.
- Cervantes-Castañeda RA, Menchaca-Díaz R, Alfaro-Trujillo B, Guerrero-Gutiérrez M, Chayet-Berdowsky AS. Deficient prevention and late treatment of diabetic retinopathy in Mexico. Gac Med Mex 2014;150:518-26.
- Andrade RJ, Lucena M, Vega JL, Torres R, Salmerón FJ, Bellot V, *et al.* Acarbose-associated hepatotoxicity. Diabetes Care 1998;21:2029-30.
- Krentz AJ, Bailey CJ. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. Drugs 2005;65:385-411.
- Rabasa-Lhoret R, Chiasson JL. Potential of alpha-glucosidase inhibitors in elderly patients with diabetes mellitus and impaired glucose tolerance. Drugs Aging 1998;13:131-43.
- Yin Z, Zhang W, Feng F, Zhang Y, Kang W. α -glucosidase inhibitors isolated from medicinal plants. Food Sci Hum Wellness 2014;3:136-74.
- Andrade-Cetto A, Heinrich M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. J Ethnopharmacol 2005;99:325-48.
- Rios MY, Castrejón F, Robledo N, León I, Rojas G, Navarro V. Chemical composition and antimicrobial activity of the essential oils from *Annona cherimola* (Annonaceae). Rev Soc Quím Méx 2003;47:139-42.
- Jyothi AB, Venkatesh K, Chakrapani P, Roja AR. Phytochemical and pharmacological potential of *Annona cherimola* – A review. Int J Phytomed 2011;3:439-47.
- Aguilar A, Camacho JR, Chino S, Jáquez P, López ME. Herbario Medicinal del Instituto Mexicano de Seguro Social. México City: IMSS; 1994. p. 17.
- Argueta A, Cano L, Rodarte M. Atlas de las Plantas de la Medicina Tradicional Mexicana. Vol. I-III. Mexico City: Instituto Nacional Indigenista; 1994.
- Martínez-Vázquez M, De la Cueva Lozano DG, Estrada-Reyes R, González-Lugo NM, Ramírez Apan T, Heinze G. Bio-guided isolation of the cytotoxic corytenchone and isocoreximine from roots of *Annona cherimola*. Fitoterapia 2005;76:733-6.
- Martínez-Vázquez M, Estrada-Reyes R, Araujo Escalona AG, Ledesma Velázquez I, Martínez-Mota L, Moreno J, *et al.* Antidepressant-like effects of an alkaloid extract of the aerial parts of *Annona cherimola* in mice. J Ethnopharmacol 2012;139:164-70.
- Falé PL, Ferreira C, Maruzzella F, Helena Florêncio M, Frazão FN, Serralheiro ML. Evaluation of cholesterol absorption and biosynthesis by decoctions of *Annona cherimola* leaves. J Ethnopharmacol 2013;150:718-23.
- Ugandhar DR, Sudhakar KB, Rao SR, Golakoti T. Isoquinoline alkaloid, flavonoids, from leaves of *Annona cherimola*. J Appl Chem 2015;4:120-6.
- López-Rubalcava C, Piña-Medina B, Estrada-Reyes R, Heinze G, Martínez-Vázquez M. Anxiolytic-like actions of the hexane extract from leaves of *Annona cherimola* in two anxiety paradigms: Possible involvement of the GABA/benzodiazepine receptor complex. Life Sci 2006;78:730-7.
- Guillapé R, Escobar-Khondiker M, Guérineau V, Laprévotte O, Höglinger GU, Champy P. Kaurenoic acid from pulp of *Annona cherimola* in regard to Annonaceae-induced parkinsonism. Phytother Res 2011;25:1861-4.
- Miyashita H, Nishida M, Okawa M, Nohara T, Yoshimitsu H. Four new ent-kaurane diterpenoids from the fruits of *Annona cherimola*. Chem Pharm Bull (Tokyo) 2010;58:765-8.
- Wélé A, Zhang Y, Brouard JP, Pousset JL, Bodo B. Two cyclopeptides from the seeds of *Annona cherimola*. Phytochemistry 2005;66:2376-80.
- Wéle A, Zhang Y, Dubost L, Pousset JL, Bodo B. Cyclic peptides from the seeds

- of *Annona glauca* and *A. cherimola*. Chem Pharm Bull (Tokyo) 2006;54:690-2.
30. Barrachina I, Neske A, Granell S, Bermejo A, Chahboune N, El Aouad N, *et al.* Tucumanin, a beta-hydroxy-gamma-lactone bistetrahydrofuranic acetogenin from *Annona cherimola*, is a potent inhibitor of mitochondrial complex I. Planta Med 2004;70:866-8.
 31. García-Aguirre KK, Zepeda-Vallejo LG, Ramón-Gallegos E, Álvarez-González I, Madrigal-Bujaidar E. Genotoxic and cytotoxic effects produced by acetogenins obtained from *Annona cherimola* Mill. Biol Pharm Bull 2008;31:2346-9.
 32. Quispe-Mauricio A, Callaconda Riva D, Rojas-Camayo J, Zavala Curso D, Posso Rivera MC, Vaisberg Wolach AJ. Cytotoxic effect of *Annona cherimola* seeds on cell cultures of cervical and breast cancer and chronic myeloid leukemia. Acta Med Peru 2009;26:156-61.
 33. Khalifa NS, Barakat HS, Elhallouty S, Salem D. Do cancer cells in human and meristematic cells in plant exhibit similar responses toward plant extracts with cytotoxic activities? Cytochemistry 2015;67:123-33.
 34. Verma AM, Kumar AP, Secar RK, Kumar KA, Rani RA. Pharmacological screening of *Annona cherimola* for antihyperlipidemic potential. J Basic Clin Pharm 2011;2:63-9.
 35. Goñi O, Sanchez-Ballesta MT, Merodio C, Escribano MI. A cryoprotective and cold-adapted 1,3-β-endoglucanase from cherimoya (*Annona cherimola*) fruit. Phytochemistry 2011;72:844-54.
 36. Calzada F, Yépez-Mulia L, Aguilar A. *In vitro* susceptibility of *Entamoeba histolytica* and *Giardia lamblia* to plants used in Mexican traditional medicine for the treatment of gastrointestinal disorders. J Ethnopharmacol 2006;108:367-70.
 37. Velázquez C, Calzada F, Torres J, González F, Ceballos G. Antisecretory activity of plants used to treat gastrointestinal disorders in Mexico. J Ethnopharmacol 2006;103:66-70.
 38. Ugandhar DR, Raghavendra HG, Sridhar C, Kumar SS, Reddy SY, Udayasree K, *et al.* Anti-arthritis activity of methanolic extract of leaves of *Annona cherimola*. Int J Pharma Bio Sci 2012;6:35-44.
 39. Verma AM, Kumar A, Kavitha D, Anurag AD. Antidenaturation and antioxidant activities of *Annona cherimola in-vitro*. Int J Pharma Bio Sci 2011;2:1-6.
 40. Goncalves TG, Santos F, Sanches-Silva A, Oliveira MB, Bento AC, Costa HS. Nutritional and phytochemical composition of *Annona cherimola* Mill. fruits and by-products: Potential health benefits. Food Chem 2016;193:187-95.
 41. Rani RA, Venkatesh K, Chakrapani P, Arunjiyothi B, Amareshwari P, Saraswathi S. Indian medicinal plants *Annona cherimola*, *Erythrina indica*, *Gymnema* and *Andrographis* species. The best cure for diabetes. 2nd World Congress on Bioavailability and Bioequivalence: Pharmaceutical R and D; 2011. p. 23-6.
 42. Chakrapani P, Rani RA. Antidiabetic activity of *Annona cherimola*. The 5th International Conference on Advanced Technologies and Treatments of Diabetes. Barcelona, Spain; 2012. p. 8-11.
 43. Hernández-Galicia E, Calzada F, Roman-Ramos R, Alarcón-Aguilar FJ. Monoglycerides and fatty acids from *Ibervillea sonora* root: Isolation and hypoglycemic activity. Planta Med 2007;73:236-40.
 44. Markham KR. Techniques of Flavonoid Identification. London: Academic Press; 1982. p. 46, 80.
 45. NOM-062-ZOO-1999 (Revised in 2001), 2001. Norma Oficial Mexicana, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Diario Oficial de la Federación. 6 de diciembre de 1999.
 46. OECD/OCDE. Guideline for Testing of Chemicals. Acute Oral Toxicity-acute Toxic Class Method. OECD/OCDE; 2001. p. 1-14. Available from: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf. [Last accessed on 2016 Nov 06].
 47. Hosseinzadeh H, Ramezani M, Danaei AR. Antihyperglycaemic effect and acute toxicity of *Securigera Securidaca* L. seed extracts in mice. Phytother Res 2002;16:745-7.
 48. Li Y, Nishimura T, Teruya K, Maki T, Komatsu T, Hamasaki T, *et al.* Protective mechanism of reduced water against alloxan-induced pancreatic beta-cell damage: Scavenging effect against reactive oxygen species. Cytochemistry 2002;40:139-49.
 49. Alarcón-Aguilar FJ, Calzada-Bermejo F, Hernandez-Galicia E, Ruiz-Angeles C, Roman-Ramos R. Acute and chronic hypoglycemic effect of *Ibervillea sonora* root extracts-II. J Ethnopharmacol 2005;97:447-52.
 50. Bonner-Weir S. Morphological evidence for pancreatic polarity of beta-cell within islets of Langerhans. Diabetes 1988;37:616-21.
 51. Bilous R, Donnelly R. Handbook of Diabetes. Oxford: Wiley-Blackwell; 2010. p. 238.
 52. Brindis F, González-Trujano ME, González-Andrade M, Aguirre-Hernández E, Villalobos-Molina R. Aqueous extract of *Annona macrophyllata*: A potential α-glucosidase inhibitor. Biomed Res Int 2013;2013:591313.
 53. Shirwaikar A, Rajendran K, Kumar DC, Bodla R. *In vivo* evaluation of anti-oxidant and anti-lipidemic potential of *Annona squamosa* aqueous extract in type 2 diabetic models. J Ethnopharmacol 2008;118:21-5.
 54. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G. Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa* L. in experimental animals. J Ethnopharmacol 2005;99:75-81.
 55. Gupta RK, Kesari AN, Watal G, Murthy PS, Chandra R, Tandon V. Nutritional and hypoglycemic effect of fruit pulp of *Annona squamosa* in normal healthy and alloxan-induced diabetic rabbits. Ann Nutr Metab 2005b;49:407-13.
 56. Gupta RK, Kesari AN, Diwakar S, Tyagi A, Tandon V, Chandra R, *et al.* *In vivo* evaluation of anti-oxidant and anti-lipidemic potential of *Annona squamosa* aqueous extract in Type 2 diabetic models. J Ethnopharmacol 2008;118:21-5.
 57. Panda S, Kar A. Antidiabetic and antioxidative effects of *Annona squamosa* leaves are possibly mediated through quercetin-3-O-glucoside. Biofactors 2007;31:201-10.
 58. Kaleem M, Asif M, Ahmed QU, Bano B. Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Singapore Med J 2006;47:670-5.
 59. Kaleem M, Medha P, Ahmed QU, Asif M, Bano B. Beneficial effects of *Annona squamosa* extract in streptozotocin-induced diabetic rats. Singapore Med J 2008;49:800-4.
 60. Adeyemi DO, Komolafe OA, Adewole OS, Obuotor EM, Adenowo TK. Anti hyperglycemic activities of *Annona muricata* (Linn). Afr J Tradit Complement Altern Med 2008;6:62-9.
 61. Adewole OS, Caxton-Martins EA. Morphological changes and hypoglycemic effects of *Annona muricata* Linn. (*Annonaceae*) leaf aqueous extract on pancreatic-cells of streptozotocin-treated diabetic rats. Afr J Biomed Res 2006;9:173-87.
 62. Adewole SO, Ojewole JA. Protective effects of *Annona muricata* Linn. (*Annonaceae*) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. Afr J Tradit Complement Altern Med 2008;6:30-41.
 63. Davis JA, Sharma S, Mittra S, Sujatha S, Kanaujia A, Shukla G, *et al.* Antihyperglycemic effect of *Annona squamosa* hexane extract in type 2 diabetes animal model: PTP1B inhibition, a possible mechanism of action? Indian J Pharmacol 2012;44:326-32.
 64. Formaggio AS, Kassuya CA, Neto FF, Volobuff CR, Iriguchi EK, Vieira Mdo C, *et al.* The flavonoid content and antiproliferative, hypoglycaemic, anti-inflammatory and free radical scavenging activities of *Annona dioica* St. Hill. BMC Complement Altern Med 2013;13:14.
 65. Florence NT, Benoit MZ, Jonas K, Alexandra T, Désiré DD, Pierre K, *et al.* Antidiabetic and antioxidant effects of *Annona muricata* (*Annonaceae*), aqueous extract on streptozotocin-induced diabetic rats. J Ethnopharmacol 2014;151:784-90.
 66. Jo SH, Ka EH, Lee HS, Apostolidis E, Jang HD, Kwon YI. Comparison of antioxidant potential and rat intestinal α-glucosidases inhibitory activities of quercetin, rutin, and isoquercetin. Int J Appl Res Nat Prod 2009;2:52-60.
 67. Li YQ, Zhou FC, Gao F, Bian JS, Shan F. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of alpha-glucosidase. J Agric Food Chem 2009;57:11463-8.
 68. Kapetanovic IM. Computer-aided drug discovery and development (CADD): In silico-chemico-biological approach. Chem Biol Interact 2008;71:165-76.
 69. Ha CH, Fatima A, Gaurav A. In silico investigation of flavonoid as potential trypanosomal nucleoside hydrolase inhibitors. Adv Bioinform 2015;2015:10. [Doi: 10.1155/2015/826047].
 70. Khan KH, Rahim F, Wadood A, Kosar N, Taha M, Lalani S, *et al.* Synthesis and molecular docking studies of potent α-glucosidase inhibitor based on biscoumarin skeleton. Eur J Med Chem 2014;81:245-52.



Fernando Calzada Bermejo

ABOUT AUTHOR

PhD Fernando Calzada Bermejo is positioned as a Principal Senior Researcher of Medical Research Unit in Pharmacology at Speciality Hospital, National Medical Center Siglo XXI, of Mexican Institute of Social Security. PhD Calzada is working on metabolic disorders as diabetes mellitus, kidney damage and obesity, as well as diarrhoea; with experience in Pharmacognosy and Chemistry of Natural Products. He is SNI level II.