European Journal of Medicinal Plants
17(3): 1-10, 2016; Article no.EJMP.30697
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Aqueous Extract of *Momordica charantia*, Reduces Hyperglycemia in Alloxan-induced Diabetic Wistar Rats

Alejandra Castellanos-Campos[1], Marco Antonio González Coronel[1], Samuel Treviño[2], Ulises Peña-Rosas[3], Berenice Venegas[4], José Albino Moreno Rodríguez[5], Teresa Marquez-Cabrera[1], José Ausencio Moran-Dominguez[1], Alfonso Díaz[1*] and Guadalupe Muñoz-Arenas[1*]

[1]Departamento de Farmacia, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Mexico.
[2]Departamento de Análisis Cínicos, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Mexico.
[3]Departamento de Química Analítica, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Mexico.
[4]Facultad de FES-Zaragoza, Universidad Nacional Autónoma de México, Mexico.
[5]Departamento de Química General, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Mexico.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AD, MAGC and JAMR designed the study. Authors ST, UPR and GMA performed the statistical analysis. Authors GMA and AD wrote the protocol and wrote the first draft of the manuscript. Authors ACC and BV managed the analyses of the study. Authors GMA, JAMD and TMC managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/30697
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Viduranga Waisundara, National Institute of Fundamental Studies, Sri Lanka.
(2) F. B. Miikue-Yobe Togenu, Rivers State Polytechnic, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/17363

Original Research Article

Received 25th November 2016
Accepted 21st December 2016
Published 27th December 2016

**Corresponding author: E-mail: alfonso.diaz@correo.buap.mx, gmunozarenas@gmail.com;*

ABSTRACT

The *Momordica charantia* (bitter melon) is a widely used plant in the traditional medicine for the treatment of diabetes mellitus (DM). It has been shown that *Momordica charantia* (Mc) has hypoglycemic effects on animals and humans, however, we don´t know if this effect is present in a chronic time and if the plant extract (stem and leaves) participates in the antihyperglycemic effect.

Aims: The aim of this study was to investigate the composition of *Momordica charantia* (Mc) and to study the hypoglycemic effect of Mc in alloxan-induced diabetic rats.

Study Design: Methods of characterization of Mc like HPLC, FITR and UV-VIS. *In vivo* antidiabetical assays in Wistar rats.

Place and Duration of Study: Department of general chemistry and department of pharmacy in the FCQ-BUAP, between January-August 2015.

Methodology: Collection of Mc was in Santiago Tuxtla, Veracruz, Mexico and ethanolic extract was made for characterizations studies. 5 mg of ethanolic extract of Mc were analyzed with HPLC. Posteriorly, in FITR studies, The Mc extract was mixed with KBr and pressed and recorded with Fourier transform infrared spectroscopy. UV visible methods was recorded in a Varian Cary 100. For pharmacological studies, diabetic Wistar male rats were administered with aqueous extract of Mc during 30 days in different doses (10, 20, 40, 80 and 160 mg/kg orally). Subsequently, glucose, insulin and glycated hemoglobin concentration were measured.

Results: The composition analysis showed that the Mc ethanolic extract has a great number of secondary metabolites which may be responsible of many plant properties. The dose-response study of Mc aqueous extract in alloxan-induced diabetic rats, showed that the hypoglycemic effect depends of the Mc dose (173.8 ± 11 mg/dL to 63.8 ± 2.8 mg/dL in rats administered with 160 mg/kg of Mc). Finally, we found that the Mc aqueous extract decreases the hyperglycemia (148.5 ± 5.7 mg/dLof alloxan group to 112.5 ± 5 mg/dL with Mc extract) and caused an increment in the insulin concentration (14.4 ± 0.05 mU/mL to 23.7 ± 1.2 mU/mL), 30 days after administration of Mc.

Conclusion: Mc aqueous extract, has antihyperglycemic effects in alloxan-induced diabetic rats. This study shows the importance of the knowledge about traditional medicine and different alternatives for diabetes treatment.

Keywords: Diabetes; secondary metabolites; hypoglycemia; glycosylated hemoglobin; insulin.

1. INTRODUCTION

The *Momordica charantia* (Mc) belongs to the *Cucurbitaceae* family and commonly is known as bitter melon. The Mc is a climbing grass with thin and resistant stalks. Leaves are divided into five or seven parts; its flowers are tubular divided into five lobes which are yellow and small [1]. The fruits are fleshy and orange when ripe, and the seeds are wrapped in a red pulp. This grass is originally from Africa and tropical Asia; however, it can grow in American countries such as Colombia, Cuba, Panama, Peru and Mexico [1].

The Mc is a very used plant in the traditional medicine, it is used for different diseases such as dysmenorrhea, pneumonia, psoriasis, rheumatism [2] and in addition, the plant has antibacterial [3] and antiviral properties [4]. However, actually, the principal use of Mc is for the treatment of diabetes mellitus (DM) which is characterized by a chronic oxidative stress disorder which occurs due to the insulin deficiency or resistance. This resistance to insulin caused hyperglycemia that produces glycosylation of different proteins such as hemoglobin [5]. The DM is one of the most prevalent and serious diseases worldwide, for these reason, the finding of different treatment that help or decreases the effects of DM, is crucial to improve the patients' life quality. Many studies have demonstrated that pulp juice of Mc, lowered fasting blood glucose (FBG) in noninsulin dependent diabetic (NIDDM) rats, however, it doesn´t have effect in insulin-dependent diabetes mellitus (IDDM) model rats [6]. In the same way, it has been demonstrated, that the administration of 375 mg/kg of methanolic fruit extract of Mc, decreased the FBG at 12 hours after Mc administration in alloxan-induced diabetic rats [7]. One of the principal complications in DM is the degree of hyperglycemia and the apparition of microvascular complication [8]. In 2014, it has been demonstrated that the Mc fruit extract (1.5 g/kg vo), can decrease FBG level and produces a significant increase in cardiac superoxide dismutase (SOD), glutathione (GSH) and

catalase in streptozotocin-induced diabetic rats [9], and exerts vasculoprotective effects and increase in nitric oxide (NO) levels in aortic tissue in the same model of DM [10]. Although, Mc aqueous extract, increased the SOD and decreased reactive oxygen species (ROS) concentration in hyperglycemia- induced human umbilical vein endothelial cells (HUVECs) [11].

It has recently shown that the administration of 6 mL/kg of Mc juice, apparently decreases blood glucose levels, total cholesterol and triglycerides through a decrement in PKC-β activity in streptozotocin-induced diabetic rats [12].

In this work, we first characterized the Mc ethanolic extract and secondly, we probed the antihyperglycemic effect of the Mc aqueous extract in alloxan-induced diabetic rats. We show the Mc composition and propose that secondary metabolites could participate in the hypoglycaemic effect of Mc.

2. MATERIALS AND METHODS

2.1 Preparation of the *Momordica charantia* Extract

The *Momordica charantia* (Mc) was grown in Santiago Tuxtla, municipality of Veracruz, México. Dr. Albino Moreno confirmed the identification. Is important to mention that Mc was harvested in the month of April (spring); season in which it has been reported that the plant have a greater antidiabetic activity [13]. Mc Leaves (3.5 g) are washed and dried in an Elisa-550 oven for 24 h. Then are pulverized and macerated with 100 mL of ethanol and heated to 78°C to obtain soluble compounds. The extraction was made in a soxhlet equipment. For pharmacological studies, 100 g of fresh Mc was heated with 1000 mL of water to boiling.

Finally, the decantation was made to obtain the liquid. The Mc extract were conserved to 4°C in a 24 h period.

2.2 Characterization of Mc Extract

2.2.1 HPLC analysis

5 mg of ethanolic extract of Mc were analyzed with HPLC equipment (Agilent Technologies 1200 Series Binary SL), RP-C18 column (Zorbax Bonus 100 x 2.1 mm id, 3.5 µm). The mobile phase was of 10:90 MeOH:H2O with a flow rate of 0.2 mL/min. We report only the chromatographic profile of the used extract, but without determining of the active fraction or secondary metabolites responsible of therapeutic effects.

2.2.2 Infrared spectroscopy (FITR)

The Mc extract, was mixed with KBr (5 wt%) and pressed in transparent wafers. Fourier transform infrared spectroscopy was recorded using a Digilab Scimitar Series FTS 3000 MX spectrophotometer in the 4000–400 cm−1 range, and 32 scans were run for each measurement.

2.2.3 UV visible

For the characterization, Mc extract samples were deposited in 5 mL of quartz cells. UV-VIS was recorded in a Varian Cary 100.

2.3 Pharmacological Studies

2.3.1 Animals

Wistar male rats (150-250 g) were obtained from Bioterio “Claude Bernard” of BUAP. Animals were individually caged in an environment with temperature, humidity and light (12:12) controlled with food, water ad libitum. All procedures described in this study were performed in accordance to the Mexican Law of Animal Treatment and Protection Guidelines (NOM-062-ZOO-1999) and the Research Committee uses of laboratory animals of the BUAP (VIEP-3450-2013). All efforts were made for to minimize the number of animals.

2.3.2 Induction of diabetes

For the diabetes induction, animals were injected with alloxan, which was dissolved in citrate buffer (0.1 M, pH 4.5) and it was administered in a single dose of 150 mg/kg ip. Three days after alloxan administration, we took blood samples to measure glucose levels. Animals with more than 150 mg/dL of glucose were considered for the next experiment [14,15].

2.3.3 Determination of hypoglycemic effect of *Momordica charantia*

The dose orally administrated in rats in the subsequent experiments, were established by a dose response curve made in diabetic rats (previously treated with alloxan 150 mg/kg ip). We measured the hyperglycemia in a blood sample from the tail vein of the rats separated in the control group, alloxan group only and Mc groups (Mc was administrated vo in 10, 20, 40,

80 and 160 mg/kg) (n= 6 per group). In the experiment the blood glucose level of animals was estimated by Glucose Oxidase–Peroxidase Enzymatic Method using a digital glucometer (ACCU-CHEK brand active).

Subsequently, we formed four groups (n=6): group 1: normal rats (vehicle group); group 2: vehicle + Mc (40 mg/kg); group 3: Alloxan (150 mg/kg) + vehicle; group 4: Alloxan + Mc. The administration of vehicle or Mc, was v.o. during 30 days. During this time, we measured blood glucose concentration every day in the same way as mentioned above.

2.3.4 Effect of the Mc administration on hyperglycemia induced by alloxan

At 30 days after the Mc administration, animals were deprived of food for 9 h, and were sacrificed by dislocation and blood samples were taken by cardiac puncture to measure glucose, insulin and glycated hemoglobin. Collected samples were centrifuged at 2 x g for 5 min. The Serum determination of glucose were developed by enzymatic colorimetric assay, following the protocol according the BioSystem kit and analyzed in a semi-automated spectrophotometer (Bayer RA-50). The plasma insulin concentration was determined by an ELISA immunoassay (Diagnóstica Internacional), and antibody-antigen complex was determined at 415 nm in a Stat fax 2600 plate reader (WinerLab group). The glycated hemoglobin measuring was carried out by immuno-fluorescence method, following the protocol according the i-Chroma kit and analyzed in a detector of the same brand.

2.3.5 Statistical analysis

For all experiments, the data were expressed as mean ± standar error (SE). Statistical differences between different treatments data were analyzed using a one-way ANOVA and a confidence level of 95% and, when it is necessary, the means were compared using the Bonferroni post-test.

3. RESULTS

3.1 *Momordica charantia* Characterization

3.1.1 HPLC analysis

Our results of chromatographic profile of ethanolic extract of *Momordica charantia*, indicate that this extract contains a wide variety of polar secondary metabolites, which are located at retention times between 18 and 34 minutes; the chromatographic profile of the extract shows two abundant peaks (8.66 and 8.2% area under curve), which they may be associated with secondary metabolites responsible for the hypoglycemic effect of the ethanolic extract. In another aspect, if the therapeutic effect is related to the concentration of polar metabolites, for this reason, ethanolic extract was very effective (Fig. 1).

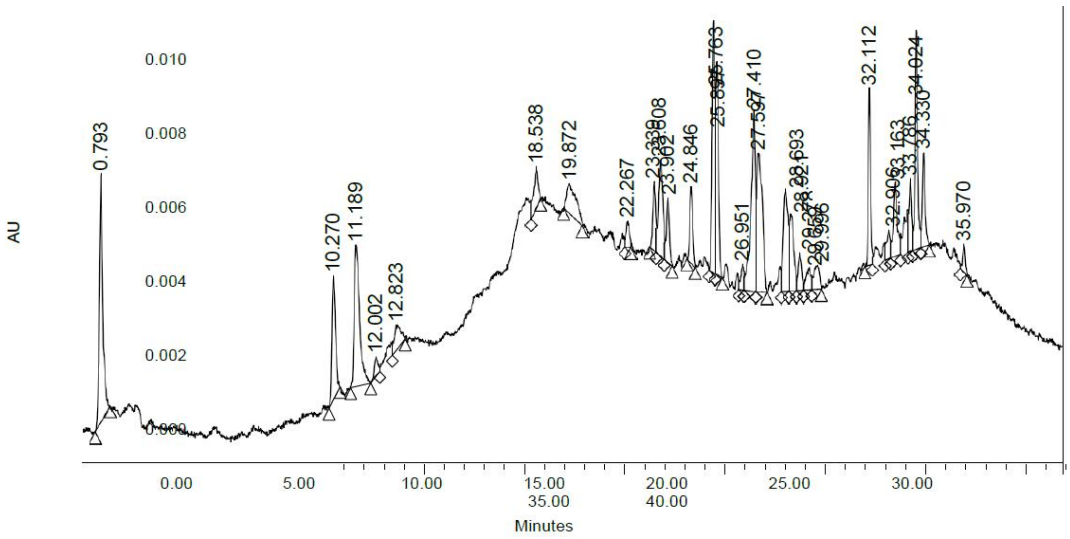

Fig. 1. HPLC analysis of the ethanolic extract of *Momordica charantia*. At 34 minutes approximately, the extract contains polar secondary metabolites which could be responsible of the ethanolic extract hypoglycemic effect

3.1.2 Infrared spectroscopy (FTIR)

The FTIR spectrum of Mc extract is presented in the Fig. 2. The absorption band located at 3 386 cm^{-1}, it corresponds to the stretching vibration mode ν_{O-H}, which correspond to hydroxyl groups (OH^-) of water (H-OH) and stretching vibration mode ν_{N-H} of Mc. To 2975 cm^{-1}, it can be observed the stretching vibration mode ν_{Csp2-H} of C-H groups of methyl and methylene. The absorption band located at 1 647 cm^{-1} correspond to stretching vibration mode $\nu_{C=N}$, the flexion type ν_{OH} and deformation δ_{HOH} of coordinated water. It can be seen too, the stretching vibration mode ν_{C-C} and $\nu_{C=O}$ of quinones. At 1 456 cm^{-1}, corresponding to stretching vibration mode ν_{COO^-} and bending deformation vibration mode out of degenerate phase $\delta^{as}{}_{CH3}$. The absorption band in 1 379 cm^{-1}, correspond to the formation vibration mode in δ_{CH3} tortion type. Band located in 1 322 cm^{-1} shows bending deformation vibration mode δ_{CH3} and vibration mode ν_{C-CO-C}. The absorption band located at 1 157 cm^{-1}, correspond to the stretching vibration mode of C-C (ν_{C-C}) and (ν_{C-O}) C-O groups which are asigned to the monomer. At 1 068 cm^{-1}, it can be observed the band which correspond to the swinging vibration mode (ν_{CH2} and ν_{O-C-C}). Finally, the absorption band at 1 049 cm^{-1}, it is located vibration mode ν_{O-C-C}. To low vibration regions of IR, at 631.6 cm^{-1}, it is found the out plane bending vibration mode $\gamma_{C.H}$, which correspond to olephyns. The infrared spectrum of fractions obtained from Mc, expressed in Fig. 2A, shows the same vibration mode of Mc.

3.1.3 UV-visible

The Fig. 2B), shows de UV-VIS spectrum obtained from Mc and fractions obtained in cromathography.

In the UV-VIS spectrum of Mc, it can be observed three peaks of maximun absorption. One in the region of visible spectrum with a wavelenght in 664.6 nm which correspond to oxipolienates O=CH-(CH=CH) n-O- of terpens. In 406.8 nm (limits of UV spectrum and visible) is present the second peak of maximum absorption which correspond to cianines: (CH3)2N-(CH=CH)n-CH=N+(CH3)2 of steroids and alcaloids. Finally, in 265.1 nm, is present the third peak of maximun absorption (in UV spectrum) which correspond to auxocromes or cromophores of five member's aromatic heterocycles, to homoanular homodiene, monosustituides bencene and bencenic derivatives type YArCOX.

The UV-VIS spectrum of obtained fractions in the column chromatography of Mc, only presented the peak in 265.1 nm.

3.2 Hypoglycemic Effect of the *Momordica charantia*

3.2.1 Dose response study of the *Momordica charantia*

The hypoglycemic effect of the Mc was examined in alloxan treated rats in the glucose concentration measure in seven groups of six animals. The Fig. 3 shows the glucose concentration of different group of alloxan treated rats and the hypoglycemic effect when dose of Mc increase. Rats of the control group presented a glucose concentration of 84.4 ± 2 mg/dL, however, rats administrated with alloxan only (150 mg/kg), presented a glucose concentration of 173.8 ± 11 mg/dL; in the group with 10 mg/kg of Mc, glucose concentration was of 155.6 ± 7 mg/dL; in the group with 20 mg/kg of Mc, the glucose concentration was of 142.2 ± 7.6 mg/dL; rats administrated with 40 mg/kg of Mc, presented a glucose concentration of 101.2 ± 4.1 mg/dL; in the group administrated with 80 and 160 mg/kg of Mc, the glucose concentration was of 90.6 ± 4.7 mg/dL and 63.8 ± 2.8 mg/dL respectively (one-way ANOVA, Bonferroni post-test *p< 0.05 and **p< 0.01).

The daily glucose concentration in rats (Fig. 4) shows a hyperglycemic effect of alloxan administration, however, the administration of Mc during 30 days causes a decrement in the glucose concentration (one-way ANOVA, Bonferroni post-test).

3.2.2 Effect of the Mc administration on hyperglycemia induced by alloxan

After 30 days of treatments administration, blood glucose concentration in the control group was of 82.7 ± 1.6 mg/dL, however, the group alloxan+SSI showed a significant increase of 79% (148.5 ± 5.7 mg/dL). At the same form, glycosylated hemoglobin was of 7.5 ± 0.04 g/dL (increment of 74% with respect to the control) (Fig. 5A and C; one-way ANOVA, post-Bonferroni test, *p<0.05). Moreover, single administration of Mc doesn´t cause effect in glucose and glycosylated hemoglobin concentration. On the another hand, the Mc

administration in hyperglycemic rats, caused a decrease of 24% in the blood glucose concentration (112.5 ± 5 mg/dL) and glycosylated hemoglobin (34%; 5 ± 0.17 g/dL) (one-way ANOVA, post-Bonferroni test **p<0.01).

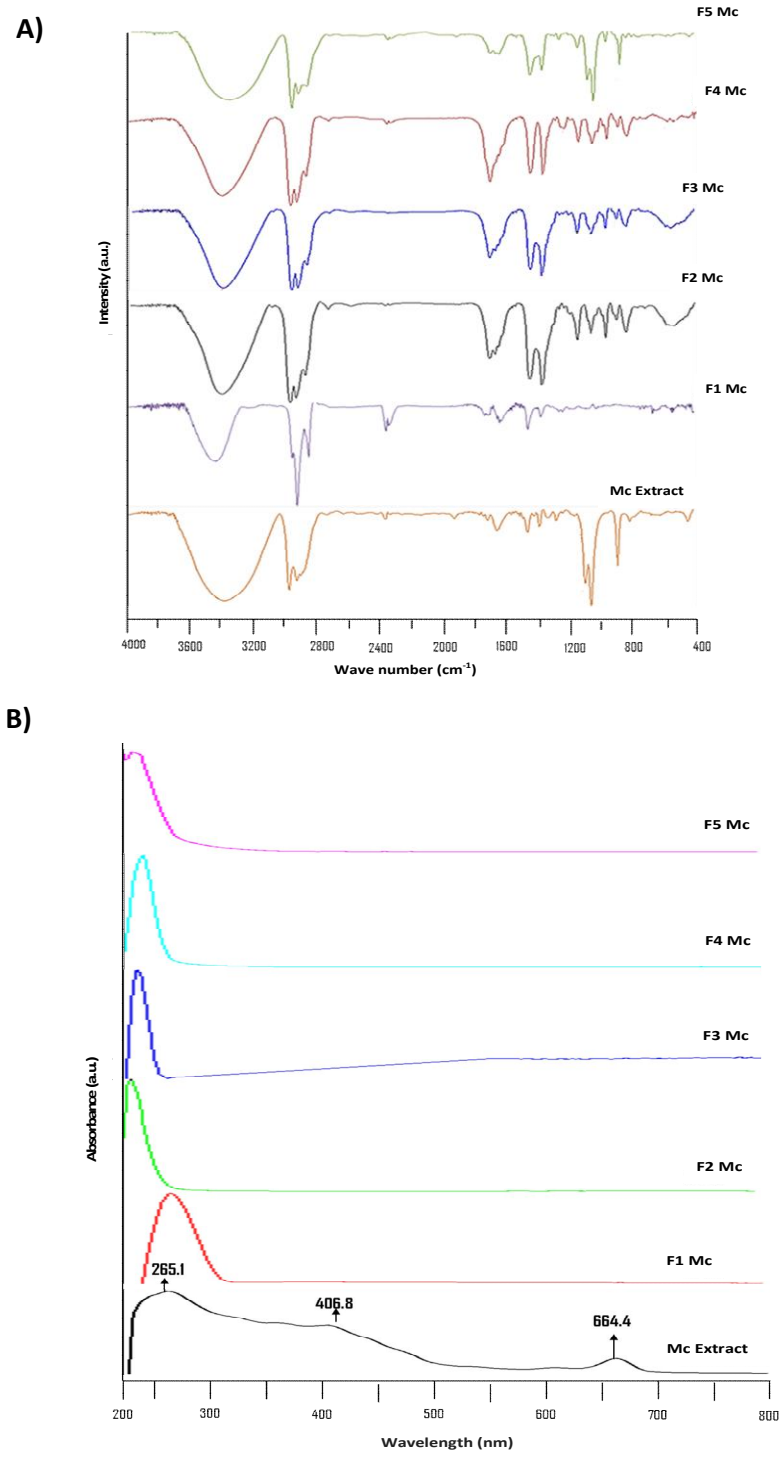

Fig. 2. (A) FITR spectrum of the ethanolic extract of *Momordica charantia*; (B) UV-vis spectrum of the ethanolic extract of *Momordica charantia* and fractions obtained in chromatography

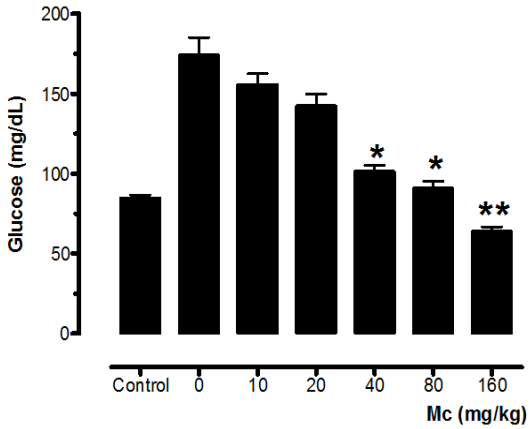

Fig. 3. Dose-response curve of Mc over blood glucose levels in diabetic rats induced by alloxan. The administration of Mc was made orally in a daily form and glucose levels were measured 5 hours after administration of Mc

*Values are mean ± SE of each dose respectively. Data were analyzed with a one-way ANOVA and Bonferroni post-test, *P<0.05 and **P<0.01 compared to control*

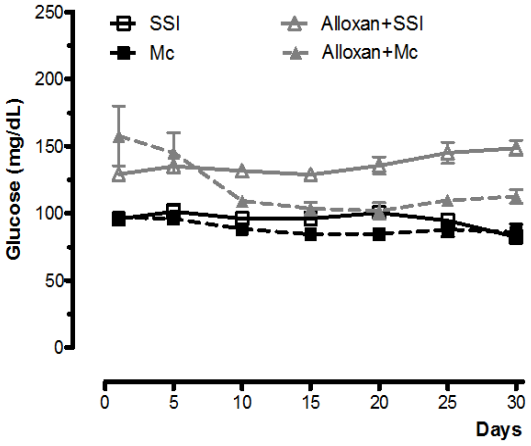

Fig. 4. Effect of the Mc administration on daily blood glucose levels of rats. The Mc administration was made between 7:00 to 9:00 during 30 days

Values are mean ± SE of each dose respectively in three experiments. Data were analyzed with a one-way ANOVA and Bonferroni post-test

In concentration of insulin determination, SSI group showed a value of 37 ± 0.8 mU/mL), and alloxan group showed a decrease of 62 % (14.4 ± 0.05 mU/mL) with respect to the control. However, in the group alloxan+Mc, insulin concentration was of 23.7 ± 1.2 mU/mL (Fig. 5B; one-way ANOVA, post-Bonferroni test, ***p < 0.001).

4. DISCUSSION

In the present study, we characterized by HPLC, FITR and UV-Vis, the ethanolic extract of *Momordica charantia*, subsequently, we evaluated the antihyperglycemic effect of the aqueous extract of the same plant in alloxan induced diabetic rats. We found that Mc is composed by several secondary metabolites that could be responsible of hypoglycemic effects observed in rats. We propose that the use of Mc, is a good alternative of traditional medicine in the treatment of diabetes.

Several studies had indicated that Mc fruit, is composed by different structures such as of polysaccharides [16], saponines [17,18], antioxidants [19], among others. Different fractions were characterized by FITR and UV-Vis and it share important characteristics with Mc ethanolic extracts.

On the other hand, the plant aqueous extract was administrated in alloxan induced diabetic rats, to evaluate the pharmacological activity. Alloxan is a good tool to induce diabetes [20]; this drug induces beta cells dead through oxidative stress and excitotoxicity [21] and in few hours, alloxan produces a total suppression of the islet response to the glucose [22]. The oral administration of Mc (40, 80 and 160 mg/kg), decreased glucose concentration in the blood of diabetic rats. The dose dependent effect of Mc in this study is according with Fernandes [23], however, doses employed were higher compared with our doses. This event is important because we demonstrated that the hypoglycemic effect of Mc is present in lower doses and possible toxic events could decrease.

In these sense, the blood glucose concentration in alloxan induced diabetic rats administrated with Mc during 30 days, decreased in important form. Currently, the mechanism by which glucose decreased in diabetic rats is not known, however, it is proposed that Mc inhibits the glucose uptake in rat everted gut sacs *in vitro* [24]; in other study it has been demonstrated that the aqueous extract of Mc increase the glucose uptake and secretion of adiponectin from fat cells [25]. Glycosylated hemoglobin decreased too, this effect is related with the blood glucose decrease and increment of peripheral glucose utilization [23]. It has been proposed that Mc stimulate the insulin receptor (IR) in muscle and adipocyte tissue; this activation regulates IR-downstream pathway and the translocation of GLUT-4 which perhaps internalized glucose [26].

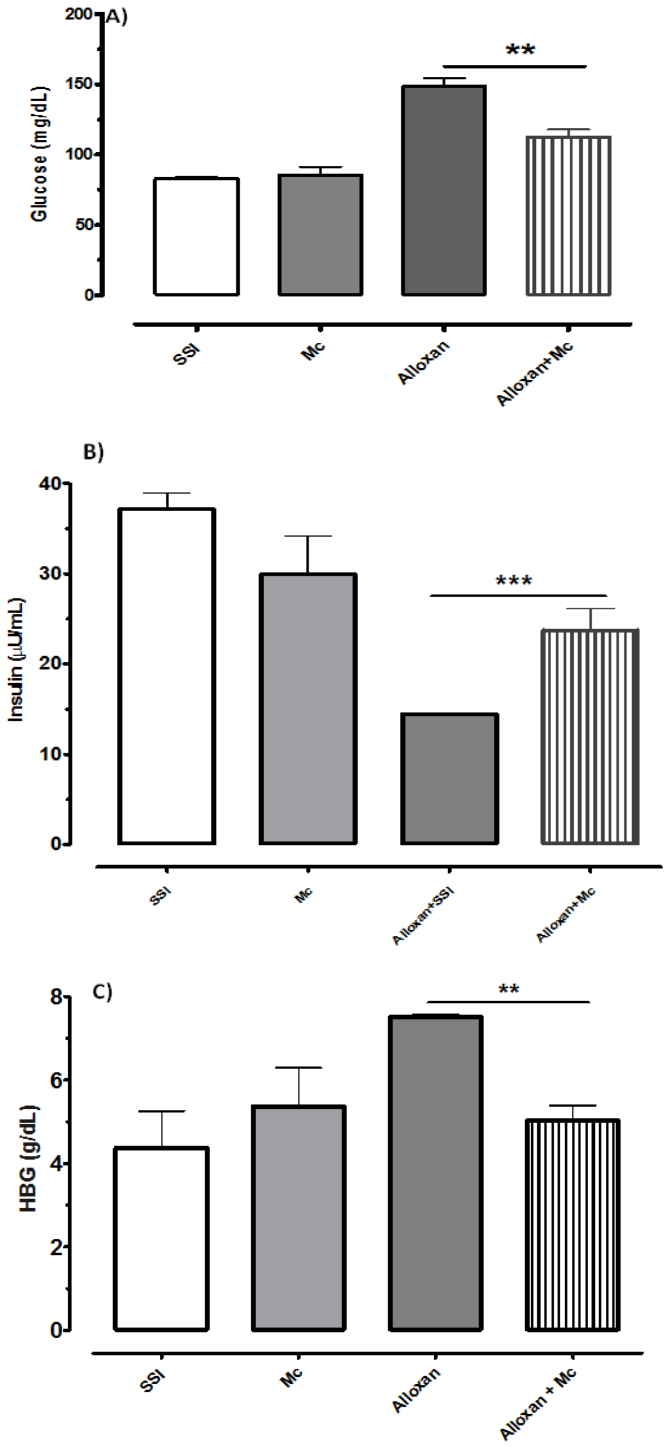

Fig. 5. Effect of the Mc administration on glucose, insulin and glycosylated hemoglobin in rats treated with alloxan. Quantification was performed in plasma at 30 days after administration of Mc. (A) plasma glucose concentration. (B) Insulin concentration and (C) Glycated hemoglobin in different groups of rats

*Values are mean ± SE of each group respectively in three experiments. Data were analyzed with a one-way ANOVA and Bonferroni post-test, **p<0.01 and ***p<0.001 compared to control*

The insulin is an essential substance for the homeostasis of glucose; according to Keller [18], the antidiabetic effect of Mc is due to high content of saponins which induce the insulin secretion *in vitro*. We measured the insulin concentration in alloxan induced diabetic rats after 30 days of the Mc administration and we found like Keller, a significant increment in the insulin. In the other hand, it has been shown that acute administration of the Mc aqueous extract increases the release of glucagon-like peptide-1 (GLP-1), which is an important hormone that stimulate the secretion of insulin, and the antagonism of the GLP-1 receptor with exendin-9, blocked the hypoglycemic effect of the Mc extract [27]. We propose that saponins in the Mc aqueous extract are agonist of GLP-1 receptor and produces hypoglycemic effects observed in this work; however, this inference must be demonstrated.

5. CONCLUSION

Mc aqueous extract, has antihyperglycemic effects in alloxan-induced diabetic rats. This study shows the importance of the knowledge about traditional medicine and different alternatives for diabetes treatment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Research Committee uses of laboratory animals of the BUAP (VIEP-3450-2013).

FUNDING

Funding for this study was provided by grants from VIEP-BUAP grant (No. DIFA-NAT16-G, 17I and TRMS-NAT16I).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Grover JK, Yadav SP. Pharmacological actions and potential uses of *Momordica charantia*: A review. J Ethnopharmacol. 2004;93(1):123-132.
2. Kwatra D, Dandawate P, Padhye S, Anant S. Bitter melon as a therapy for diabetes, inflammation, and cancer: A Panacea? Curr Pharmacol Reports. 2016;2(1):34-44.
3. Khan MR, Omoloso AD. *Momordica charantia* and *Allium sativum*: Broad spectrum antibacterial activity. K J Pharmacog. 1998;29(3):155-158.
4. Lee-Huang S, Huang PL, Chen HC. Anti-HIV and anti-tumor activities of recombinant MAP30 from bitter melon. Gene. 1995;161(2):151-156.
5. Virdi J, Sivakami S, Shahani S, Suthar AC, Banavalikar MM, Biyani MK. Antihyperglycemic effect of three extracts from *Momordica charantia*. J Ethnopharmacol. 2003;88(1):107-111.
6. Ali L, Khan AK, Mamun MI, Mosihuzzaman M, Nahar N, Nur-e-Alam M, Rokeya B. Studies on hypoglycemic effects of fruit pulp, seed, and whole plant of *Momordica charantia* on normal and diabetic model rats. Planta Medica. 1993;59(5):408-412.
7. Nkambo W, Anyama NG, Onegi B. *In vivo* hypoglycemic effect of methanolic fruit extract of *Momordica charantia*. L. Afr Health Sci. 2013;13(4):933-939.
8. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type2 diabetes (UKPDS 35): Prospective observational study. BMJ. 2000;321(7258): 405- 412.
9. Abas R, Othman F, Thent ZC. Protective effect of *Momordica charantia* fruit extract on hyperglycaemia-induced cardiac fibrosis. Oxid Med Cell Longev. 2014; 2014:429060.
10. Abas R, Othman F, Thent ZC. Effect of *Momordica charantia* fruit extract on vascular complication in type 1 diabetic rats. Excli J. 2015;14:179-189.
11. Choo C, Waisundara VY, Hoon LY Bittergourd (*Momordica charantia*) scavenges free radicals by enhancing the expression of superoxide dismutase in in vitro models of diabetes and cancer. CyTA - J of Food. 2014;12(4):378-382.
12. Kolawole OT, Ayankunle AA. Seasonal variation in the anti-diabetic and hypolipidemic effects of *Momordica charantia* fruit extract in rats. EJMP. 2012; 2(2):177-185.

13. Chandru S, Vishwanath P, Devegowda D, Ramasamudra SN, Prashant A, Hathur B. Evaluation of protein Kinase Cβ and PPARγ activity in diabetic rats supplemented with *Momordica charantia*. J Clin Diagn Res. 2016;10(4):1-4.
14. Langle A, González-Coronel MA, Carmona-Gutiérrez G, Moreno-Rodríguez JA, Venegas B, Guadalupe Muñoz, Samuel Treviño, Alfonso Díaz. Stevia rebaudiana loaded titanium oxide nanomaterials as an antidiabetic agent in rats. Rev Bras Farmacogn. 2015;25(2): 145-151.
15. Treviño S, Velázquez-Vázquez D, Sánchez-Lara E, Diaz-Fonseca A, Flores-Hernandez JÁ, Pérez-Benítez A, Brambila-Colombres E, González-Vergara E. Metforminium decavanadate as a potential Metallopharmaceutical drug for the treatment of diabetes mellitus. Oxid Med Cell Longev. 2016;2016:1-14.
16. Deng YY, Yi Y, Zhang LF, Zhang RF, Zhang Y, Wei ZC, Tang XJ, Zhang MW. Immunomodulatory activity and partial characterization of polysaccharides from *Momordica charantia*. Molecules. 2014; 19(9):13432-13447.
17. Kang J, Zeng B, Tang S, Wang M, Han X, Zhou C, Yan Q, He Z, Liu J, Tan Z. Effects of *Momordica charantia* Saponins on *in vitro* ruminal fermentation and microbial population. Asian-Australas J Anim Sci. 2016;29(4):500-508.
18. Keller AC, Ma J, Kavalier A, He K, Brillantes AM, Kennelly EJ. Saponins from the traditional medicinal plant *Momordica charantia* stimulate insulin secretion *in vitro*. Phytomed. 2011;19(1):32-37.
19. Lin KW, Yang SC, Lin CN. Antioxidant constituents from the stems and fruits of *Momordica charantia*. Food Chem. 2011; 127(2):609-614.
20. Szkudelski T. The mechanism of alloxan and streptozotocin action in B-cells of the rat pancreas. Physiol Res. 2001;50:537-546.
21. Heikkila RE, Winston B, Cohen G, Barden H. Alloxan induced diabetes, evidence for hydroxyl radicals as a cytotoxic intermediate. Biochem Pharmacol. 1976; 25:1085-1092.
22. Kliber A, Szkudelski T, Chichlowska J. Alloxan stimulation and subsequent inhibition of insulin release from in situ perfused rat pancreas. J Physiol Pharmacol. 1996;47(2):321-328.
23. Fernandes NP, Lagishetty CV, Panda VS, Naik SR. An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized *Momordica charantia* fruit extract. BMC Complement Altern Med. 2007;24:7-29.
24. Mahomoodally MF, Fakim AG, Subratty AH. *Momordica charantia* extracts inhibit uptake of monosaccharide and amino acid across rat everted gut sacs *in-vitro*. Biol Pharm Bull. 2004;27(2):216-218.
25. Dhiman K, Gupta A, Sharma DK, Gill NS, Goyal A. A review on the medicinally important plants of the family Cucurbitaceae. As J of Clin Nut. 2012;4: 16-26.
26. Lo HY, Ho TY, Lin C, Li CC, Hsiang CY. *Momordica charantia* and its novel polypeptide regulate glucose homeostasis in mice via binding to insulin receptor. J Agric Food Chem. 2013;61(10):2461-2468.
27. Huang TN, Lu KN, Pai YP, Chin H, Huang CJ. Role of GLP-1 in the hypoglycemic effects of wild bitter gourd. Evid Based Complement Alternat Med. 2013;625892.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17363