



Antidiabetic, hypolipidemic, antioxidant and anti-inflammatory effects of *Momordica charantia* L. foliage extract

[Efectos antidiabéticos, hipolipemiantes, antioxidantes y anti-inflamatorios del extracto foliar de *Momordica charantia* L.]

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Abstract

Context: *Momordica charantia* (Cucurbitaceae) is extensively distributed in tropical areas, and it is traditionally used as antidiabetic. Nevertheless, few studies supporting the metabolic effects of its foliage extracts have been reported elsewhere.

Aims: To evaluate the effects of *M. charantia* foliage extract (MCh) on blood glucose and lipids levels in animal models of hyperglycemia. The anti-inflammatory and antioxidant properties together with the acute toxicity and genotoxic effects were also assessed.

Methods: An MCh powder was obtained by spray-drying techniques. The anti-hyperglycemic and hypolipidemic effects of MCh were evaluated in the glucose tolerance test and the alloxan-induced diabetes, respectively. The *in vitro* and *ex vivo* MCh antioxidant effects were assessed against the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), peroxy, and superoxide anion radicals. The MCh anti-inflammatory effects were also assessed together with its toxicological profile by means of the Acute Toxic Class Method, and the chromosome aberration assay.

Results: MCh administered orally diminished the glucose and lipids levels in normoglycemic healthy rats and alloxan-induced hyperglycemic mice. Strong *in vitro* free radical scavenging effects was demonstrated by MCh against ABTS, peroxy, and superoxide anion radicals. The extract also improved the free radical scavenging ability of diabetic mice serum. Moreover, MCh reduced the weight of cotton-induced granulomas in mice, the exudate volume, and its leukocyte counts in carrageenan-induced pleurisy in rats. Very low MCh toxicity was also verified.

Conclusions: The hypoglycemic, hypolipidemic, anti-inflammatory, and antioxidant activities here observed make the spray-dried powder from *M. charantia* foliage extract an interesting alternative for the correction of diabetes and its complications.

Keywords: antidiabetic; anti-inflammatory; antioxidant; foliage extract; toxicity; *Momordica charantia*.

Resumen

Contexto: La *Momordica charantia* (Cucurbitaceae) se encuentra ampliamente distribuida en las áreas tropicales y se usa tradicionalmente como antidiabético. Sin embargo, pocos estudios sustentan los efectos metabólicos de sus extractos foliares.

Objetivos: Evaluar los efectos de un extracto foliar de *M. charantia* (MCh) sobre los niveles de glucosa y lípidos en modelos animales de hiperglicemia. También se evaluaron sus propiedades anti-inflamatorias y antioxidantes, así como su toxicidad aguda oral y efectos genotóxicos.

Métodos: Se obtuvo un polvo de MCh por técnicas de secado por aspersión. Sus efectos anti-hiperglicémicos e hipolipemiantes se evaluaron en modelos de tolerancia a la glucosa y de diabetes inducida por aloxano, respectivamente. Los efectos antioxidantes *in vitro* y *ex vivo* se evaluaron frente a los radicales 2,2'-azinobis(3-ethylbenzotiazolina-6-ácido sulfónico, peróxido, y el radical anión superóxido. Se evaluaron además los efectos anti-inflamatorios de MCh junto a su perfil toxicológico a través del Método de las Clases para la toxicidad aguda oral y el ensayo de aberración cromosómica.

Resultados: La administración oral de MCh disminuyó los niveles de glucosa y lípidos en ratas saludables normo-glicémicas y en ratones hiperglicémicos por acción del aloxano. Se demostró una potente actividad secuestradora de los radicales ABTS, peróxido y superóxido, *in vitro*. El extracto también mejoró la actividad secuestradora de radicales libres del suero de ratones diabéticos. Además, el MCh redujo el peso de los granulomas inducidos por algodón en ratones, y en el modelo de pleuresía inducida por carragenina en ratas, redujo el volumen de exudado y su conteo de leucocitos. Se demostró una baja toxicidad para el MCh.

Conclusiones: Las actividades hipoglicémicas, hipolipemiantes, anti-inflamatorias y antioxidantes aquí observadas, hacen del extracto de *M. charantia* obtenido a partir de un secado por aspersión, una alternativa interesante para corregir la diabetes y sus complicaciones.

Palabras Clave: antidiabético; anti-inflamatorio; antioxidante; extracto foliar; toxicidad; *Momordica charantia*.

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INTRODUCTION

Momordica charantia L. (*Cucurbitaceae*) is a climbing shrub highly allocated in tropical zones of America, Asia, and Africa. In Cuba, it can be found widely distributed throughout the island, and it is popularly known as "cundeamor". Its traditional use as medicine against various diseases dates back to ancient times (Roig y Mesa, 1974; Garau et al., 2003; Singh et al., 2011), been the fruits the most used portion of the plant, especially for diabetes treatments (Alam et al., 2015; Jia et al., 2017; Fan et al., 2019).

Diabetes mellitus (DM) has a great incidence in global population and it is characterized by a metabolic disorder that produces hyperglycemia and dyslipidemia due to an impairment in the insulin secretion and/or a deficiency in the signal cascade mediating its action (Singh et al., 2011). DM is classified into type 1 or 2 according to the insulin dependence profile (Singh et al., 2011). DM affects around 6% of Cuban population, and it is responsible for some chronic complications like neuropathies, hypertension, retinopathies, heart diseases, predisposition to infections, nephropathies and eventually death, reaching the 8th place among all death causes (MINSAP, 2020). Oxidative stress and inflammation have crucial role in DM progression, since they can contribute to beta cells destruction (Baynes and Thorpe, 1999; Matough et al., 2012).

M. charantia has been used for the cure of various diseases and infections due to its antiviral and antibacterial properties (Jia et al., 2017). The pharmacological properties of the fruits have been extensively studied but there are very few reports concerning the effects and safety of the *M. charantia* foliage extracts. Supported by the traditional use of the plant and the reported properties for diabetes treatment (Alam et al., 2015; Krawinkel and Keding, 2006), we have proposed the design of a phytopharmaceutical using *M. charantia* foliage extract desiccated by a spray-drying technique as an active pharmaceutical ingredient (Lagarto et al., 2008; 2014). This technological intervention improved the physico-chemical and stability properties of the extract (Lagarto et al., 2008; 2014), but

could interfere with its pharmacological properties. Here we aimed to evaluate the metabolic effects of this powdered *M. charantia* foliage extract (MCh) on murine models of diabetes by determining blood glucose and lipids status. The anti-inflammatory and antioxidant properties together with its oral acute toxicity and genotoxic effects were also assessed.

MATERIAL AND METHODS

Plant extract and preliminary phytochemical characterization

Foliage of *M. charantia* was collected in May at the Medicinal Plant Experimental Station "Dr. Juan Tomás Roig" (Güira de Melena, Artemisa, Cuba). Voucher specimen (N^o 4778) was deposited at the "Dr. Juan Tomás Roig" herbarium in the cited Experimental Station. Solar dryer was used to dry the plant material. The aqueous extract was obtained as described previously (Lagarto et al., 2014). A Büchi B191 spray dryer (Flawil, Switzerland) was used for drying the extract. Entry temperature between 100 and 180°C, outlet temperature between 60 and 80°C and atomization between 400 and 700 L/h were fixed as parameters of drying process. Microcrystalline cellulose 20% (w/v) MC-101 was used as additive with the objective of increasing the vitrea transition temperature. The *M. charantia* powder (MCh) obtained was packed and kept protected from light and moisture.

The total alkaloids in the extract were analyzed by gravimetric method (Maxwell et al., 1995). Two hundred mL of 10% acetic acid in ethanol were mixed with 5 g of MCh and incubated for four hours at room temperature. The resulting solution was filtered through Whatman filter paper and evaporated to 50 mL, alkalized with ammonium hydroxide 28-32% to total precipitation and evaporated to dryness at 60°C. The mass of the residual solid was accurately determined. The total alkaloid content of MCh was 275.30 ± 0.85 mg in 100 g of the dry sample (RSD = 0.31%).

Charantin content in the extract was determined by High Performance Thin Layer Chroma-

tography. Briefly, one mg of accurately weighed amount of standard charantin (purity >98%) and MCh (100 mg) were dissolved with methanol, sonicated, and filtered. Charantin standard stock solution (0.1 mg/mL) and MCh (20 mg/mL) were applied as band ranging from 2-10 μ L for standard and 20 μ L for extract, on TLC plate with Camag TLC Scanner 3. Mobile phase of dichloromethane: methanol (9:1) was used and developed was performed in a normal vertical tank with a distance of 85 mm. Evaluation was carried out using a reflectance mode densitometer at 530 nm. Calibration curve was obtained, and regression equation was calculated. A peak corresponding to charantin was seen at R_f value 0.32. A linear calibration equation $Y = 6.186x + 381.657$ was obtained for charantin in the concentration range of 200-1000 ng/spot with a correlation coefficient and standard deviation of 0.9989, and 2.09, respectively. The charantin concentration in MCh was set at 0.156%.

Animals

The experiments were carried out with healthy (male and female) Wistar rats and (male) NMRI mice coming from Centro Nacional para la Producción de Animales de Laboratorio (CENPALAB, Havana, Cuba). Animals were quarantined for seven days before starting the experiments. They were fed a conventional rodent diet and water *ad libitum*. The environmental conditions during all the experiments were: temperature: $22 \pm 1^\circ\text{C}$, relative humidity: 40-70%, light/dark cycle of 12h. Before the start of the study, the protocols were rigorously reviewed for approval by the CIDEM ethics committee (CIDEM-ECD 037-2017). Compliance with the UE Directive 2010/63/EU for animal experiments was guaranteed throughout the study.

Glucose tolerance in normal healthy rats

Male rats (6-8 weeks of age, 150-200 g of body weight, n = 8 per group) were divided into five groups: (1) Control group received vehicle (Microcrystalline cellulose 1%), treated groups received single daily doses of (2) 25, (3) 50, and (4) 100 mg/kg of MCh in purified water, and (5) positive control group received single dose of

glibenclamide 10 mg/kg. All treatments were administered by oral route for four weeks. The animals were then orally administered with 3 g/kg of glucose. Blood was obtained from ocular plexus under anesthesia (thiopental 35 mg/kg i.p.) before treatment and at 15, 30, 60, 120 and 240 minutes after treatment (Silva et al., 2002). Glucose was determined spectrophotometrically (Spectronic Genesys 2, Spectronic Instruments Inc, Rochester, NY) as previously reported (Silva et al., 2002).

Assessment of hypoglycemic activity in diabetic mice

Male mice (5-6 weeks of age, 20-25 g of body weight, n = 8 per group) were fasted overnight and divided into six groups: (1) Normal control and (2) diabetic control groups received vehicle, treated groups received the oral doses of (3) 25, (4) 50, (5) 100 mg/kg of MCh suspended in distilled water and (6) positive control group received glibenclamide 10 mg/kg. Single intravenous injection of alloxan 50 mg/kg body weight was used for inducing diabetes (Ighodaro et al., 2017); it was prepared in water for injection just before dosing. After 48 h, blood glucose concentration was measured (Silva et al., 2002). Animals with blood glucose concentration higher than 8.0 mmol/L were selected for the test and distributed in experimental groups. Animals received the oral treatments for 8 days and after that, blood samples were obtained for the determination of blood glucose concentration. Additionally, ABTS radical scavenging effect were measured in animals sera (Silva et al., 2002).

All blood samples collected were centrifuged and the supernatant was used immediately for blood glucose determination. A spectrophotometer Spectronic Genesys 2 was used for absorbance measurement.

Lipids profile

Additionally, serum lipids profile was measured in diabetic animals administered with 10 mg/kg glibenclamide or 50 mg/kg MCh. Total cholesterol (CHOL) and triglycerides (TG) were determined by HEL-FA diagnostic kits. Cholesterol in high-density lipoprotein (HDL-C) was esti-

mated by precipitation with PG 6000 (Gomez and Rodriguez Llach, 1984). The concentration of cholesterol in β -lipoprotein fraction (β -LP CHOL) was determined by subtraction of serum total cholesterol from HDL-C.

Assessment of the anti-inflammatory effects

Effect on cotton pellet granuloma in mice

Male mice (5-6 weeks of age, 20-25 g of body weight, n = 10 per group) were divided into five groups. (1) Control group (vehicle), (2) the positive control group (dexamethasone 3 mg/kg), and treated groups receiving the oral doses of (3) 25, (4) 50, and (5) 100 mg/kg of MCh in purified water. Sterilized cotton pellet of 10 mg of weight, were implanted in anesthetized (thiopental 40 mg/kg) animals. Oral treatments were administered for 7 days, starting the same day of cotton implantation. Then, the pellets with the adhered fibrovascular tissue were removed and weighed. The difference between the pellets initial weight and the wet and dry weight at the end of the study were determined (Bertollo et al., 2006).

Effect on carrageenan-induced pleurisy in rat

Male rats (7-8 weeks of age, 150-200 g of body weight, n = 10 per group) were divided into five groups. (1) Control group (vehicle), (2) positive control group (indomethacin 10 mg/kg in a single dose at last day), and treated groups receiving the oral doses of (3) 50, (4) 100, and (5) 200 mg/kg of MCh in distilled water. Oral treatments were administered for two weeks except indomethacin. One hour after the last dosing, rats were anaesthetized (thiopental 40 mg/kg) and pleurisy induced with 1% carrageenan injection. Four hours later, animals were killed under overdose of anesthesia, the pleural exudate was collected, and its volume was measured. A washing of the pleural cavity (2 mL of buffer saline phosphate pH 7.2 and 5 UI/mL heparin) was obtained to determine total leukocyte count in Neubauer chamber (Mikami and Miyasaka, 1983).

Free radical scavenging assays

ABTS radical scavenging activity

The test was carried out as explained previously (Miller and Rice-Evans, 1997). The ABTS radical was prepared by mixing same volumes of 8 mM of ABTS with 3 mM of potassium persulfate in distilled water. The assay medium was made up with 990 μ L of ABTS \bullet^+ and 10 μ L of MCh (0.78-200 μ g/mL) or positive control (quercetin 0.015-2.5 μ g/mL). Absorbance was read at 734 nm.

Peroxyl radical scavenging capacity

The assay was performed according to previous report with some modifications (Cai et al., 2003). Brain homogenate, previously inactivated, was used as fatty acid source. The peroxidation was initiated with 50 μ L of 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPH) 20 μ M added to a mixture of 500 μ g of brain homogenate, 500 μ L of PBS pH 7.4 and 20 μ L of MCh (6.25-100 μ g/mL) or quercetin (0.05- 50 μ g/mL). Thiobarbituric acid reactive substances (TBARS) in the supernatant was measured at 535 nm with an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Superoxide radical scavenging capacity

The assay was done as reported previously (Marklund and Marklund, 1974). Autoxidation of pyrogallol was determined spectrophotometrically (420 nm) for 1 min after addition of 40 μ L of pyrogallol 0.124 mM, 1.5 mL of Tris-HC buffer solution, 25 μ L of EDTA 1 mM, and 20 μ L of MCh (1.5-32.9 μ g/mL) or quercetin (0.15-12.5 μ g/mL). The linear slope (with $r^2 > 0.99$) was calculated, and the autoxidation initial rate was taken as the increment of absorbance using purified water. Linear slope inhibition was indicative of scavenging activity. For all free radical scavenging assays, test compound was compared with quercetin and the IC_{50} for sample and reference were calculated by triplicate from the graph plotting inhibition percentage versus log concentration using a non-linear regression algorithm.

Toxicological evaluation

Acute toxicity test

The limit test described in the OECD guideline was carried out at dose of 2000 mg/kg. Female fasting rats (7-8 weeks of age, 150–200 g of body weight, n = 6 per group) received the oral dose of MCh by gavage in aqueous solution 10% (w/v) of the plant extract. Clinical exam was performed daily for toxic signs. On day fourteen, animals were killed by lethal dose of thiopental and macroscopic examination of organs and tissues was performed to detect possible toxic effects (OECD, 2002).

Genotoxicity evaluation

Genotoxic effects of MCh was investigated by means of chromosome aberration (CA) test systems, and was conducted according to methods described elsewhere (Scott et al., 1990). Whole blood (1 mL) from healthy donors was added to 1 mL fetal bovine serum F5023 supplemented with 2% phyto-hemagglutinin and 1% antibiotic (penicillin/streptomycin) in culture medium RPMI 1640. Cultures were incubated at 37°C for 48 h. The cells were treated with 0.31, 0.62, 1.2 and 2.5 mg/mL concentrations of MCh for 17 h. Negative and positive control received purified water and Mitomycin-C 0.6 µM, respectively. Then, colchicine 0.4 µg/mL was added for later treat the cells with KCl (75 mM) as the hypotonic solution, fixed with methanol: glacial acetic acid (3:1) and staining with Giemsa 5%. Structural and/or numerical alterations of metaphases were used to obtain the quantity of CA. The structural aberrations were sorted as chromatid and chromosome type. Genotoxicity was evaluated according to structural CA.

Statistical analysis

Data were expressed as the mean ± SEM. All statistical analysis was assessed using the GraphPad Prism Version 7 (GraphPad Software, San Diego, California, USA). Each test group was compared with control. Bartlett's test was applied to determine the homogeneity of variance. When

the variance was homogenous, one-way ANOVA was applied. When the variance was not homogenous, the Kruskal–Wallis test (nonparametric ANOVA) was performed. Statistical differences among groups were analyzed by means of Dunnett's multiple comparison test. Statistical significance was considered at $p < 0.05$.

RESULTS

Evaluation of MCh extract on oral glucose tolerance test in normal rats

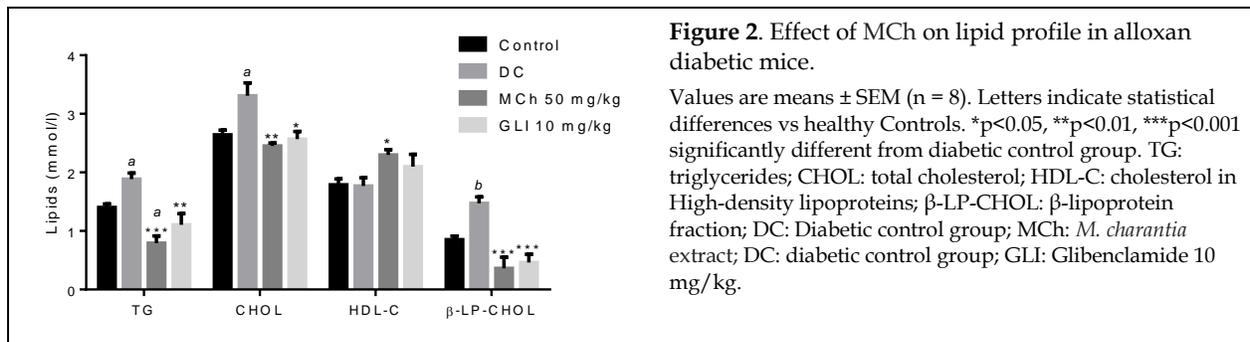
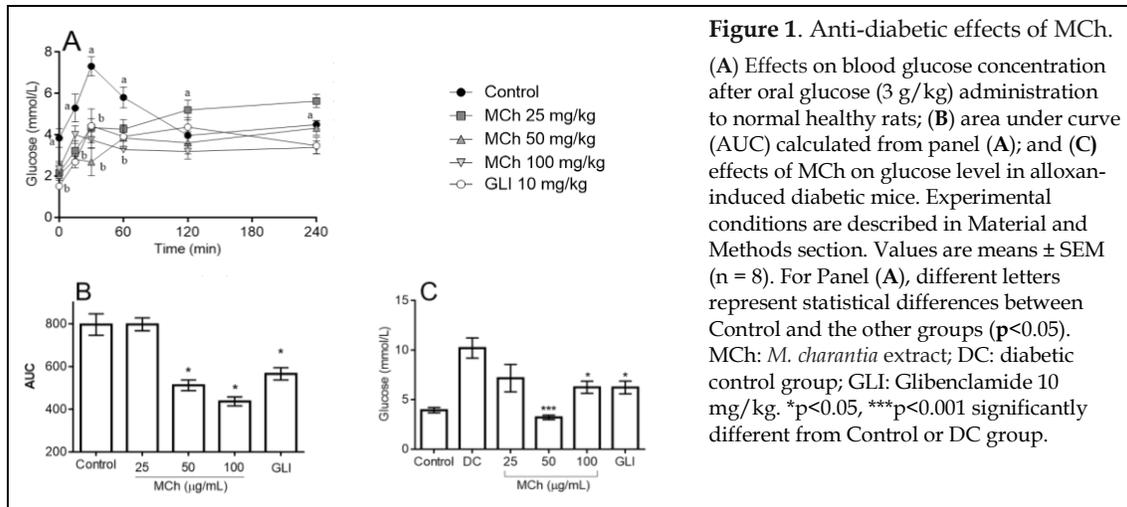
After 30 min of oral glucose administration, a hyperglycemic peak was observed in healthy Control rats (Fig. 1A). The glucose tolerances curves were flattened by 4 weeks of daily MCh (25, 50, and 100 mg/kg), and GLI (10 mg/kg) treatments. MCh at 50 mg/kg was more effective than GLI for glucose peak reduction (63.3% versus 39.4% reduction after 30 min glucose administration). The total body glucose levels, here estimated by the calculation of the Area Under Curve (AUC), also showed a significant reduction of 35.8, 45.2, and 29% by 50, 100 mg/kg MCh, and 10 mg/kg GLI treatments, respectively (Fig. 1B).

Evaluation of MCh extract on alloxan-induced diabetic mice

After 8 days of alloxan exposure, the blood glucose levels in diabetic Controls (DC) almost duplicate those of Controls (Fig. 1C). Daily administration of 25, 50 and 100 mg/kg MCh and 10 mg/kg GLI reduced blood glucose concentration by 29.8, 68.7, 38.8, and 39.0%, respectively.

Evaluation of MCh effects on lipids profile in diabetic mice

Besides rising blood glucose, alloxan exposition increased triglycerides (TG) levels, total cholesterol (CHOL), and cholesterol in β-lipoprotein fraction (β-LP-CHOL) when compared to healthy Control (Fig. 2). MCh supplementation decreased cholesterol while HDL-C was increased significantly, even beyond Control levels.



Evaluation of MCh free radical scavenging and antioxidant effects

MCh scavenges the ABTS⁺, peroxy and superoxide anions radicals in a concentration-dependent manner with IC₅₀ values of 20.20, 69.99 and 20.63 μ g/mL, respectively (Fig. 3A-C). Quercetin, the classical antioxidant flavonoid here used as control, shows lower IC₅₀ values for each radical assayed, as expected.

The ABTS radical scavenging capacity decreased in control diabetic mice serum when compared with the normal control group ($p < 0.05$) as indicative of a depletion of endogenous antioxidant defenses (Fig. 3D). MCh treatment at 50 mg/kg restores the serum free radical scavenging capacity to values similar to the normal control group, suggesting an *in vivo* antioxidant effect (Fig. 3D).

Evaluation of MCh anti-inflammatory effects

MCh treatments inhibited the granuloma formation in mice at all tested doses similarly as dexamethasone treated group (Fig. 4A). Likewise, significant reduction in the exudate volumes (Fig. 4B), and the total leukocyte count (Fig. 4C) were observed after the natural extract treatments to rats exposed to carrageenan. Indomethacin (10 mg/kg), the classical anti-inflammatory drug, was used here as positive control.

Toxicological evaluation

Neither death nor toxic signs were observed after the acute oral toxicity test performed to MCh. Normal tendency in body weight gain was observed according to the experimental animals used (29.0 \pm 2.5 g and 28.3 \pm 3.2 g for MCh-treated and control rats, respectively). None of the tissues examined showed evidence of toxic effects.

Data of structural chromosomal aberrations (CA) induced by MCh on human peripheral blood lymphocytes are presented in Table 1. No statisti-

cally significant increases were obtained in CA frequency after 17 h treatment with MCh.

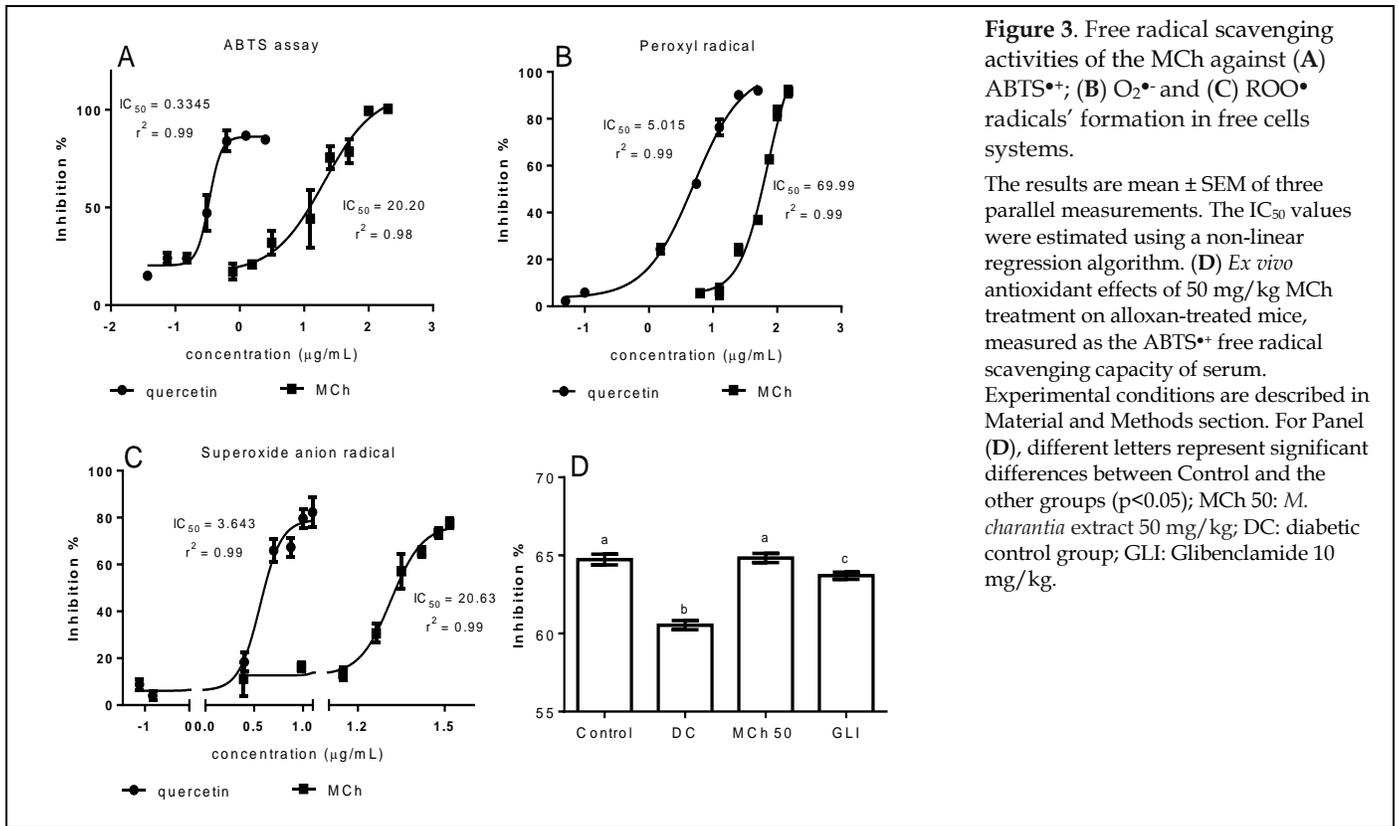


Figure 3. Free radical scavenging activities of the MCh against (A) ABTS^{•+}; (B) O₂^{•-} and (C) ROO[•] radicals' formation in free cells systems.

The results are mean ± SEM of three parallel measurements. The IC₅₀ values were estimated using a non-linear regression algorithm. (D) *Ex vivo* antioxidant effects of 50 mg/kg MCh treatment on alloxan-treated mice, measured as the ABTS^{•+} free radical scavenging capacity of serum. Experimental conditions are described in Material and Methods section. For Panel (D), different letters represent significant differences between Control and the other groups (p<0.05); MCh 50: *M. charantia* extract 50 mg/kg; DC: diabetic control group; GLI: Glibenclamide 10 mg/kg.

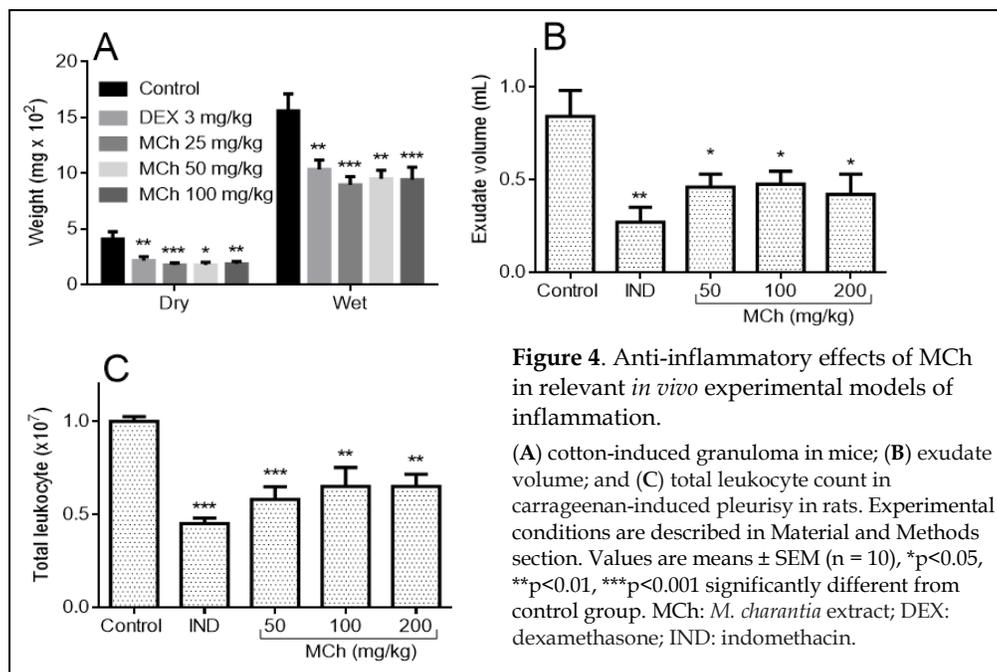


Figure 4. Anti-inflammatory effects of MCh in relevant *in vivo* experimental models of inflammation.

(A) cotton-induced granuloma in mice; (B) exudate volume; and (C) total leukocyte count in carrageenan-induced pleurisy in rats. Experimental conditions are described in Material and Methods section. Values are means ± SEM (n = 10), *p<0.05, **p<0.01, ***p<0.001 significantly different from control group. MCh: *M. charantia* extract; DEX: dexamethasone; IND: indomethacin.

Table 1. Effect of MCh extract on chromosome aberration (CA) in human peripheral blood lymphocytes.

Concentration (mg/mL)	MI (%)	Aberrant cells (%)		Aberrations/200 cells					
		-Gaps	+Gaps	Gaps	Chromosome type		Chromatid type		
					Breaks	Exchange	Breaks	Exchange	
0	13.8	0.0	0.0	0	0	0	0	0	0
0.31	13.2	0.5	0.5	0	0	0	1	0	0
0.62	11.3	0.5	0.5	1	1	0	0	0	0
1.2	10.9	0.0	1.5	3	2	0	0	0	0
2.5	10.6	1.0	1.5	2	0	1	1	1	0
Mitomycin-C	1.3	39.5 **	43.0	7	8	11	9	14	14

**p<0.01. MI: Mitotic index.

DISCUSSION

The evaluation of MCh effects in our experimental conditions evidenced anti-hyperglycemic as well as hypolipidemic actions in normal rats and alloxan-induced diabetic mice, respectively. The most effective dose in both normoglycemic healthy rats and diabetic mice was 50 mg/kg bw. Further doses increase did not improve the glycemic effects. Some of the reported mechanisms involved in the hypoglycemic effect of the plant have been previously reported and include an insulin-like effects, an increase in glucose utilization by muscles and peripheral cells, a decrease of intestinal uptake of glucose, an inhibition of gluconeogenic enzymes, and protection of pancreatic cells (Singh et al., 2011). Nevertheless, the effects on both glucose and lipids levels suggest a more centrally controlled mechanisms of metabolic regulation by MCh. AMP-activated protein kinase (AMPK) is a conserved eukaryotic protein serine/threonine kinase that senses the energy status of the cell and coordinates a global metabolic response to restore energy homeostasis (Hardie, 2007). Its activation has been shown to restore normal glycemia (Viana et al., 2006), and lower hepatic glucose production and plasma triglycerides levels in animal models of obesity (Cool et al., 2006) and type 2 diabetic patients (Boon et al., 2008). In this sense it has been reported that the plant extracts may modulate fat metabolizing kinases such as AMPKs, and the expression of its downstream target genes involved in mitochon-

drial fuel oxidation like carnitine palmitoyltransferase-1 (CPT-1), peroxisome proliferator-activated receptors, (PPARs), liver X receptors (LXR), and the peroxisome proliferator activated receptor- γ coactivator 1 α (PGC-1 α), in liver and skeletal muscle and affected adipocyte differentiation (Krawinkel and Keding, 2006; Alam et al., 2015). We have also observed an increase in resting respiration, and a dissipation of mitochondrial membrane potential after MCh incubation with isolated hepatic mitochondria from rats (unpublished results). This strongly suggest a mitochondrial uncoupling mechanism, which could increase the AMP/ATP ratio, the master ignitor of AMPK signaling (Hardie, 2007).

Oxidative stress has been implicated in the pathology of many diseases and antioxidants may offer resistance against oxidative impairment by scavenging free radicals, decreasing the lipid peroxidation and by preserving some other biomolecules from oxidative damage (Youdim and Joseph, 2001). Alloxan is known to induce ROS formation, resulting in the selective necrosis of β -cells (Lensen, 2008). Here we demonstrated a potent free radical scavenging capacity of MCh *in vitro*, and antioxidant effects *ex vivo*. Thus, the antioxidant protection of β -cells could partly explain the anti-diabetic mechanism of MCh. Several studies have pointed out that oxidative stress is strongly linked to diabetic complications. Oxidative stress derived from chronic hyperglycemia negatively influence the structure and function of many or-

gan systems, particularly the cardiovascular, nervous, and renal system (Ceriello, 2003). Therefore, our results suggest the beneficial effect of the foliage extract against oxidative process, including those associated to diabetes (Tripathi and Chandra, 2009; 2010). Several *in vitro* studies have shown that the extracts with the maximum antioxidant effect are those obtained from the bitter ground pulp and seed extracted with ethanol and water (Jia et al., 2017; Sandikapura et al., 2018). Our results suggest that *M. charantia* foliage is another source of antioxidants, although further studies should be done to compare its antioxidant potency versus fruits, and seeds. Other research with *M. charantia* in diabetic rats has also shown antioxidant actions, with none effects on normal glucose and lipids levels (Chaturvedi and George, 2010). The differences in relation to our results could be ascribed to different methodological approaches, particularly the spray-drying of the extract, which significantly reduced its particle size, and probably improved its pharmacokinetic/pharmacodynamics properties.

The different types of diabetes have in common auto-inflammatory processes that cause inflammation of the pancreatic Langerhans β -cells islets and therefore a reduction in the amount and function of this cells (Tsalamandris et al., 2019). Reductions in inflammatory markers is an effect of anti-diabetic agents related to their prime mechanism of action (Tsalamandris et al., 2019). Since metabolic dysregulation itself causes inflammation, efficient antidiabetic treatments may improve inflammation by virtue of improving the metabolic condition (Pollack et al., 2016). In addition, it is currently known that diabetes and hyperglycemia develop a proinflammatory microenvironment that may lead to several microvascular complications, nephropathy, retinopathy, and neuropathy (Nguyen et al., 2012). In our study, the foliage extract of *M. charantia* produced *per se* anti-inflammatory effects in carrageenan-induced pleurisy in rats; a well-recognized model of acute inflammation (Saleh et al., 1999), and cotton pellet granuloma in mice; an excellent model of chronic inflammation (Goldstein et al., 1976). Thus, this broad spectrum of anti-inflammatory activities

may also contribute to the beneficial actions elicited by the *M. charantia* foliage extract, not only acting against diabetes development but also on its associated late inflammatory complications.

Hyperlipidemia is a common finding in patients with diabetes mellitus and the main risk factor of cardiovascular diseases and vascular complications. High triglycerides (TG), low HDL-C, and increased low-density lipoprotein (LDL) cholesterol are the characteristic feature of diabetic dyslipidemia (Schofield et al., 2016). Furthermore, oxidative stress and inflammation interact in the development of diabetic atherosclerosis. In this regard, it is known that inflammation and oxidative stress within the vasculature establish a vicious cycle capable of amplify each other, giving rise to a process that initiates and consequently lead to the progression and the rupture of the atherosclerotic lesion (Hajjar and Gotto, 2013). We have shown here that *M. charantia* extract decreased total cholesterol and those associated to β -lipoproteins meanwhile increased HDL-C in alloxan-diabetic mice. Besides, the extract showed antioxidant and anti-inflammatory properties. Thus, it is reasonable to expect that *M. charantia* extract, by normalizing the lipid profile and inhibiting the inflammatory and oxidant response within the vasculature, may in turn reduce the risk of heart diseases such as atherosclerosis, which could be a complication of uncontrolled diabetes mellitus.

Some of the main components of *M. charantia* extracts are the steroidal glycoside charantin, and the alkaloid momordicin, both with hypoglycemic properties (Pitipanponga et al., 2007). The combination of both metabolites could produce a greater effect similar to that of the crude water extract (Taylor, 2002). We found alkaloids and charantin in the MCh that could be the responsible phytochemicals for the hypoglycemic and antilipidemic effects herein observed.

No toxic effects of *M. charantia* extract was observed in the performed acute toxicological test as observed elsewhere (Lagarto et al., 2008; Thiagarajan et al., 2019). The acute toxicity study with the fruit extract also shown no deleterious effects at doses up to 5 g/kg (Abdel-Rahman et al., 2019).

Moreover, the *M. charantia* foliage extract was not genotoxic as evidenced by results of CA assay.

Overall, these results expand current knowledge on the beneficial metabolic effects of *M. charantia* extract, and proposed new formulation obtained from the plant foliage extract that preserved the described anti-diabetic effects for the seeds and fruits, combined with potent antioxidant and anti-inflammatory effects.

CONCLUSIONS

M. charantia foliage extract previously desiccated by spray-drying intervention exhibited a significant anti-hyperglycemic, hypoglycemic and antilipidemic effects in healthy and diabetic animals, together with anti-inflammatory and antioxidant actions, without toxic effects. These properties point MCh as an effective active principle for pharmaceutical formulations designed to correct the metabolic impairments in diabetes and its inflammatory complications. Further studies should be conducted in order to demonstrate the advantage of spray-dried MCh extract *versus* non-desiccated extract, from a pharmacodynamics, pharmacokinetic and physic and chemical stability considerations.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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Concepts or ideas	x	x	x	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x	x	x	x	x	x	x
Literature search	x	x								x	x	x
Experimental studies	x	x	x	x	x	x	x	x	x		x	x
Data acquisition	x	x	x	x	x	x					x	x
Data analysis	x	x	x	x	x	x					x	x
Statistical analysis	x										x	x
Manuscript preparation	x	x									x	x
Manuscript editing	x	x									x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x

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