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Antioxidant potential, anti-inflammatory activity, antidiabetic and cardioprotective effect of *Microsechium helleri* (Peyr.) Cong.

[Potencial antioxidante, actividad anti-inflamatoria, efecto antidiabético y cardioprotector de *Microsechium helleri* (Peyr.) Cong.]

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Abstract: *Microsechium helleri* (Cucurbitaceae) has been used in ethnopharmacological as a lotion to prevent hair loss, diuretic and cathartic, in the region of central Veracruz, Mexico is used as antidiabetic. The antioxidant properties of the hexanic (EHex), chloroformic ($ECHCl_3$) and ethanolic (EEtOH) extracts, were evaluated by 2,2diphenyl-1-pychrylhydrazyl (DPPH) test, the Ferric Reducing/Antioxidant Power (FRAP) and the total phenolic content test. The anti-inflammatory effect was evaluated in the acute ear edema induced with phorbol 12-myristate 13-acetate (TPA) in mouse and the hypoglycemic and cardioprotective effects of the EEtOH were determined in rats. The EEtOH was the most active in the antioxidant potential DPPH test and the $ECHCl_3$ was the best in the FRAP assay and the total polyphenols content. In the anti-inflammatory assay, the $ECHCl_3$ showed the most activity. The EEtOH had the decreased the glucose levels and reduced myocardial damage. The results support the use of this plant in folk medicine in Mexico as antioxidant, anti-inflammatory, hypoglycemic and cardioprotective.

Keywords: Mexican Traditional Medicine; *Microsechium helleri* (Peyr.) Cong.; Anti-inflammatory; Hypoglycemic; Cardioprotective.

Resumen: *Microsechium helleri* (Cucurbitaceae) se utiliza en etnofarmacología como una loción para prevenir la caída del cabello, como diurético y catártico, en la región del centro de Veracruz, México es usado como antidiabético. Las propiedades antioxidantes de los extractos hexánico (EHex), clorofórmico ($ECHCl_3$) y etanólico (EEtOH), se evaluaron mediante la prueba de 2,2difenil-1-psililhidrazilo (DPPH), el poder reductor férrico/poder antioxidante (FRAP) y el contenido fenólico total. El efecto anti-inflamatorio se evaluó en el edema agudo de la oreja inducido con forbol 12-miristato 13-acetato (TPA) en ratones y se determinaron los efectos hipoglucémicos y cardioprotectores del EEtOH en ratas. El EEtOH fue el más activo en la prueba DPPH de potencial antioxidante y el $ECHCl_3$ fue el mejor en el ensayo FRAP y el contenido total de polifenoles. En el ensayo antiinflamatorio, el $ECHCl_3$ mostró la mayor actividad. El EEtOH disminuyó los niveles de glucosa y redujo el daño miocárdico. Los efectos hipoglucémicos y cardioprotector del extracto de EEtOH se determinaron en ratas, donde el extracto disminuyó los niveles de glucosa y redujo el daño miocárdico. Los resultados apoyan el uso de esta planta en la medicina popular en México como antioxidante, anti-inflamatorio, hipoglucemiante y cardioprotector.

Palabras clave: Medicina traditional mexicana; *Microsechium helleri* (Peyr.) Cong.; Anti-inflamatorio; Hipoglucemiante; Cardioprotector.

INTRODUCTION

Microsechium helleri (Peyr.) Cong. (*M. helleri*) is a perennial plant, it belongs to the botanic family (Cucurbitaceae) and specifically it is known as "liana amole" or "chicamole" (Dieterle, 1976; Lira *et al*., 1998; Villaseñor, 2016). This climbing plant is endemic to the highlands of Mexico and Guatemala); *M. helleri* has been used as a soap substitute and ornamental plant (Lira & Caballero, 2002). In ethnopharmacological tradition, this plant has been used as a lotion to prevent hair loss, diuretic and cathartic (Dieterle, 1976). Besides, the inhabitants of the region of central Veracruz refer to it as antidiabetic (Cano-Asseleih, 1997).

León *et al*. (1998), isolated two saponins from *M. helleri* roots named amoles F and G, as oleanane-type triterpene glycosides. Also, another two new saponins were evaluated as antifeedant, nematicidal and phytotoxic (Hernández-Carlos *et al*., 2011). However, there is no information regarding the ethnopharmacological properties, toxicity data in the animals or phytochemical studies.

These plants show high allelopathic activities due o presence of secondary metabolites like saponins that are essential for survival and contribute selective suppressing of the growth of neighboring plants and protecting it against pests, pathogens and stress (Haralampidis *et al*., 2002; Nielsen *et al*., 2010). Many cucurbits are traditionally used as an anti-inflammatory, hepatoprotective, cardiotonic, immunoregulatory, antidiabetic, fungicidal, bactericidal or antitumor agents (Rajasree, 2016).

Therefore, the secondary metabolites present in the root extracts of *M. helleri* could possess different pharmacological properties. Considering the information above, the study aimed to evaluate the effectiveness of Hexane (Hex), chloroform ($CHCl_3$) and ethanol (EtOH) extracts from *M. helleri* roots on animal models of inflammation, hyperglycemia and myocardial ischemia-reperfusion and the relation with antioxidant potential.

MATERIAL AND METHODS

Chemicals

The chemicals employed in this study were analytical grade. Ethanol (EtOH), chloroform ($CHCl_3$), Hexane (Hx), Methanol (MeOH), 2,2diphenyl-1-pychrylhydrazyl (DPPH), sodium acetate, acetic acid, sodium carbonate, ferric chloride, chloride acid, acetone, tween 80, sodium citrate (Cit Na) buffer pH 4.5, glucose, were purchased from Merck Co (Germany). Folin-Ciocalteu reagent, gallic acid, 2,4,6-tripyridyl-5-triazine (TPTZ), phorbol 12-myristate 13-acetate (TPA), indomethacin, 2,3,5-triphenyltetrazolium chloride (TTC), p-formaldehyde, streptozotosin (STZ) (St. Louis, MO, USA).

Plant Collection

Microsechium helleri (Peyr.) were collected from La Perla, Veracruz State, Mexico in March 2012. The plants were identified by M.S. Jorge A. Alejandre Rosas and a voucher specimen (IBUNAM-MEXU-PV714342) was deposited at the herbarium of the Instituto de Biología, Universidad Nacional Autónoma de Mexico.

Preparation of Plant Extracts

103 g of the dried root of *M. helleri* were macerated at room temperature 25°C with solvents of ascending polarity, obtained the hexane (Hex, 0.20 g), chloroform ($CHCl_3$, 6.20 g) and ethanol (EtOH, 19.87 g) extracts. The solvents were removed employed a rotary evaporator (Heidolph Laborota 4000) and after were fully dried in a vacuum oven (ShelLab) at 25ºC. The extracts were kept in the dark at room temperature for further use.

Antioxidant activity

DPPH radical-scavenging activity

The percent of inhibition the DPPH radical-scavenging activity was obtained with the method of Brand-Williams modified by Miliauskas (Brand-Williams *et al*., 1995; Miliauskas *et al*., 2004). In this assay for each extract were prepared solutions with a concentration of 33 mg / mL in MeOH. After to 100 µL of these solutions was added 2.9 mL of DPPH solution (9 × 10-5 M in MeOH), for the blank sample only was used 100 µL of MeOH. After, the samples were incubated at 37°C for 30 minutes in a water bath. The absorbance of the samples (AE) and of the blank (AB) were measured with a UV-Vis spectrophotometer (Varian Model Cary-100) at 517 nm, all determinations were made by triplicate. The antioxidant activity was calculated using the next equation:

$$\% \text{ inhibition} = [(AB - AE) / AB] \times 100$$

Total phenolic content

The concentration of total phenols was determined according to Spanos and Wrosltad (1990). The experiments were performed in triplicate by mixing 50 µL of each extract (33 mg/mL in MeOH), 2.5 mL of Folin-Ciocalteu reagent (1/10 dilution in water) and 2 mL of Na_2CO_3 (7.5%, w/v), then incubated for 15 minutes at 45°C. Absorbance was measured using a UV-Vis spectrophotometer (Varies Model Cary-100) at 765 nm. Total phenol content is calculated as GAE gallic acid equivalent (mg/L) using a gallic acid standard (range 20 to 1000 mg/L).

$$\text{Absorbance} = 0.001\ [\text{GAE (mg / L)}] + 0.0754$$

FRAP (Ferric Reducing/Antioxidant Power)

In this assay was employed the method proposed by Benzie and Strain method in 1996. In this technique, the FRAP reagent is prepared mixing 25 mL of sodium acetate buffer solution (3.1 g of sodium acetate $3H_2O$ and 16 mL of acetic acid in 1 L, pH 3.6), 2.5 mL of TPTZ (10 mM in HCl 40 mM) and 2.5 mL of $FeCl_3 \cdot 6H_2O$ (20 mM); after was incubated for 5 min at 37ºC. 2.7 mL of FRAP solution were transferred into an amber vial, was added 150 µL of an aqueous solution of different extracts (33 mg/mL in MeOH) and 150 µL of distilled water. The experiments were performed in triplicate; the absorbance was measured using a UV-Vis spectrophotometer (Varies Model Cary-100) at 593 nm. The same blank was prepared with 300 µL of distilled water to 2.7 mL of FRAP solution. The results were expressed in mmol de Fe^{2+} /L, employed a calibration curve of $FeSO_4$ (100-1000 mmol/L).

$$\text{Absorbance} = 0.00109\ [\text{ mmol } Fe^{2+} / \text{L}] + 0.10715$$

In vivo assays the animals

By the anti-inflammatory and acute toxicity assays were used male CD1 mice (20 - 25 g), by the determination of hypoglycemic and cardioprotective effects male Wistar rats (250 - 300 g) were used. The animals were maintained under standard laboratory conditions (25 ºC, 12 h dark/12 h light, 50 % relative humidity) according to the Mexican Official Norm (Norma Oficial Mexicana, 1999) NOM-062-ZOO-1999, the food and water were provided *ad libitum*.

Anti-inflammatory activity

TPA induced acute ear edema in mice

The ear edema formation was induced by the topical application of the 2.5 µg of TPA dissolved in 25 µL of acetone 30 min before to applied the all treatments; after 30 min, the extract or indomethacin to doses of 2 mg/ear dissolved in 50 µL of acetone was applied by the same route. The control group of inflammation only received on the right ear TPA and on the left ear acetone. After 6 h of TPA application, the mice were sacrificed by cervical dislocation and a 6 mm diameter section of the both ears were cut and weighed. Anti-inflammatory inhibition activity of the extract or indomethacin was determinated with the weight difference between the right and the left ear section compared to inflammation control group (Young & De Young, 1989; Meingassner *et al*., 1997).

Acute toxicity (LD_{50})

Before 12 h the oral administration intragastric route of the plant extracts (dose 2000 mg/Kg) or vehicle (tween 80-water 1:9), n = 3, the animals were fasted. During 14 days, the animals were observed daily and registered deaths and behavioral changes. At the end of the experiment, mice were sacrificed by cervical dislocation, and internal organs (liver, lungs, heart, spleen and kidney) were excised, weighed and macroscopically analyzed (Lorke 1983; OECD, 2016).

Experimental diabetes induced by streptozotocin

The diabetes was induced by intraperitoneal (ip) injection of 65 mg/Kg of STZ, according to literature (Gupta *et al*., 2004; Milani *et al*., 2005; Okutan *et al*., 2005; Yazdanparast *et al*., 2007).

The doses of STZ were prepared immediately before administration, dissolving the respective amount in a 0.1 M Cit Na buffer pH 4.5. Once STZ was administered, the animals were given a 20 % glucose solution for the next 24 hours in order to avoid death due to the hypoglycemia that occurs during this period. To determine glucose levels, an incision was made at the apex of the tail to obtain a drop of blood and an accu-chek active kit glucometer

from Roche laboratories was used.

Glucose levels were evaluated at 0 h (n = 5, $\bar{X}$ = 68.4 mg/dL), 48 h (n = 5, $\bar{X}$ = 113.5 mg/dL), 72 h (n = 5, $\bar{X}$ = 158.5 mg/dL), 5 days (n = 5, $\bar{X}$ = 273 mg/dL). Animals presenting glycemia between 125 mg/dL to 273 mg/dL were included in the study.

Cardioprotective activity

Study protocol

Rats were randomly divided into seven groups of five animals each. Group I (negative control group), Group II (positive control group), Group III was subjected to 1 h of ischemia/4 h of reperfusion in the group of diabetic rats, Groups IV and V was subjected to 1 h of ischemia/4 h of reperfusion in rats group of in the absence/presence of experimental diabetes and treated with 300 mg/Kg of EEtOH from *M. helleri* by oral gavage, finally groups VI and VII was subjected to 1 h of ischemia/4 h of reperfusion in rats groups to which only EtOH and Cit Na were administered as vehicle, respectively.

In vivo studies of ischemia/reperfusion (I/R) surgical preparation

After the study, the rats were anesthetized with pentobarbital (0.1 mL/100 g, ip). Once the anesthetic plane was achieved, a tracheostomy was performed to supply oxygen enriched air using a positive pressure ventilator (Ugo Basile, Model 7025) (Guo *et al*., 1994). Was regulated the temperature at 37°C during the surgical preparation and recovery period using a heating plate and a rectal thermometer. A left thoracotomy was performed to visualize the heart, and the left arterial descending coronary artery (LAD) was subsequently identified and ligated with 6-0 surgical silk, the occlusion of blood flow was maintained for 1 h; subsequently, the myocardium was reperfused for 4 h. At the end of this procedure and under the anesthetic plane, the heart was removed, removing the major vessels. The ventricles were separated and frozen at -20°C for 2 h, followed by this, they were cut into 2 mm thick sections. The sections were incubated in the 1% TTC solution for 20 minutes at 37°C, the sections were fixed with 4% p-formaldehyde (pH 7.4) and then mounted on glass plates for digitization using the software Image J 1.30 (NIH, USA), for the analysis we used to blinded evaluation. Heart damage is calculated using the ratio of the infant area to the total area of the left ventricle (IA/TA) (O´Brien *et al*., 2016; Downey, 2020).

Statistical analysis

The results of the inflammation experiment were processed by a one-way analysis of variance (ANOVA) followed by post hoc Student Newman Keuls and the I/R experiments were analyzed followed by post hoc Tukey's test, (n= 5). In both analyses, a value of $p<0.05$ was considered statistically significant. The results for the antioxidant activity were reported as mean ± SD (Standard Deviation) and the results from the animals experiments as mean ± SEM (Standard Error of Mean).

RESULTS

Antioxidant Activity of Microsechium helleri

In Table No. 1, DPPH and FRAP tests were carried out to evaluate the antioxidant activities in extracts of *M. helleri*. These determinations are spectrophotometric-based methods and are widely accepted for measuring antioxidant activity in plant extracts *in vitro* (Dominguez-Ortiz *et al*., 2009). In addition, the total polyphenol content was also determined. The three extracts had the same radical scavenging capacity of DPPH (around to 90%), but the $ECHCl_3$ showed the highest ferric reducing ability (175.25 ± 11.66 μmol Fe^{2+}/L). $ECHCl_3$ extract also showed the highest concentration of phenolic content (21.25 ± 3.20 meqAG/gM).

Anti-inflammatory effect

Table No. 2 shows the anti-inflammatory activity results of the extracts of *M. helleri* in the ear edema induced model with TPA. We found that $ECHCl_3$ had the highest anti-edematous effect in the TPA-induced model than the other extracts showing a 54.90% edema inhibition. This result was similar to the inhibitory effect obtained with indomethacin (58.26%). All the *M. helleri* extracts showed a statistically significant difference compared to the control group.

The oral administration of *M. helleri* extract did not cause lethality in the animals or alterations in their behavior, based on macroscopic observations, body weight and organ morphology. The determination of LD_{50} was greater than 2000 mg/Kg (data not shown).

Determination of the hypoglycemic effect of the ethanolic extract of Microsechium helleri

For the determination of the hypoglycemic effect of ethanol extract of *M. helleri* (EtOH), serum glucose was quantified in rats with prior fasting of 8 - 12 h in all groups (n = 5). In the group with treatment [STZ + EEtOH at doses of 300 mg/Kg (oral administration)], serum glucose was determined 2 h after the extract was administered (Table No. 3). As expected, rats that received STZ presented high levels of glucose in the blood when comparing to control group. Interestingly, EEtOH administration resulted in a significant reduction in glucose levels in diabetic rats.

Table No. 1
Antioxidant activity of *Microsechium helleri* extracts

Extract	DPPH	Ferric reducing power (FRAP)	Total phenolics
	Inhibition %	(µmol Fe^{2+} / L)	(meqAG / gM)
EHex	92.04 ± 7.32	143.35 ± 15.14	2.28 ± 1.14
$ECHCl_3$	90.13 ± 9.00	175.25 ± 11.66	21.25 ± 3.20
EEtOH	92.11 ± 8.67	16.35 ± 1.51	3.80 ± 2.50
Ascorbic acid	100	NE	NE

Results are shown as mean ± standard deviation of the radical scavenging effect of DPPH. Extracts were tested at 33 µg/mL and ascorbic acid. Total phenolic content is expressed in milliequivalents of galic acid per gram of extract (meqAG/gM). Ferric reduction power is expressed in µmol of ferric ions reduced per liter (µmol Fe^{2+}/L). NE not evaluated

Table No. 2
Anti-inflammatory effect of *Microsechium helleri* in TPA induced ear acute edema in mice

Treatment	Dose (mg/ear)	Edema inhibition (mg)	% inhibition
Control		10.78 ± 0.52	0
EHex	2	6.05 ± 0.30*	43.80
$ECHCl_3$	2	4.86 ± 0.57*	54.91
EEtOH	2	7.86 ± 0.56*	27.09
Indomethacin	2	4.50 ± 0.86*	58.26

Data represent the mean ± SEM. The application was topical. One-way ANOVA, *post hoc* Student-Newman-Keuls, *p<0.05. n=6

Table No. 3
Hypoglycemic effect of *Microsechium helleri*

Extract	Glucose Control (mg/dL)	Glucose STZ (mg/dL)	Glucose STZ + EEtOH (mg/dL)
Ethanol	96.00 ± 1.15	342.66 ± 28.75***	101.00 ± 19.86**

Comparison of the STZ glucose versus glucose control groups, and the STZ glucose group versus the STZ glucose + EtOH extract (*M. helleri* ethanolic extract) groups. The results were analyzed with the Tukey-Kramer test and they were expressed as the mean ± SEM (n = 3), ***p<0.001, (p<0.01)**

Cardioprotective effects of Microsechium helleri

M. helleri was orally administered to assess its cardioprotective properties. Figure No. 1 shows the cardioprotective activity evaluated in male Wistar rats administered either with ethanol extract of *M. helleri* (EtOH) or vehicle after being subjected to coronary ischemia/reperfusion (I/R). As expected, I/R resulted in infarcted tissue, the area affected was 36.74% compared with the control the animals. Interestingly, the administration of the EtOH extract after I/R resulted in a significant reduction of the infarcted area. Next, we induced I/R in diabetic rats (I/R + STZ), this group showed an increased infarcted area compared to the I/R group. Of note, EtOH administration resulted in a decreased infarcted area in I/R-diabetic rats (I/R + STZ + EtOH) compared to the I/R + STZ group. Importantly the administration of either extract vehicle (EtOH) or STZ vehicle (Cit Na), did not affect the infarcted area caused by I/R.

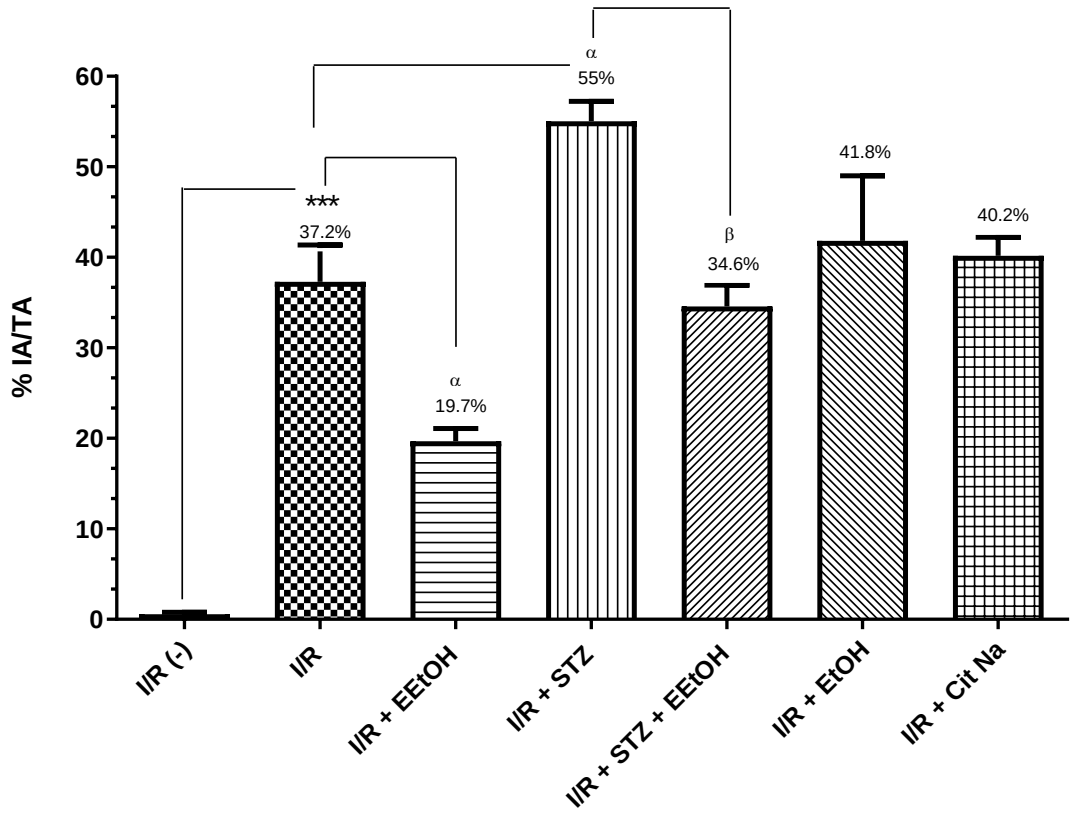

Figure No. 1
Infarcted area/total area (% IA/TA) in groups of the animals in the absence and presence of EtOH of *M. helleri*. The data correspond to the mean ± SEM, n = 5. One-way ANOVA and Tukey's post test were used to establish statistically significant differences when: $^{\alpha, \beta}$ $p<0.05$, *** $p<0.001$

DISCUSSION

As of yet, there are no reports on the therapeutic properties of *Microsechium helleri* in Veracruz, Mexico. This is the first study reporting the antioxidant capacity, anti-inflammatory and cardioprotective effects of this species. Among the extracts tested in this study for antioxidant activities, the results showed that *M. helleri* $CHCl_3$ have greater content of phenolic compounds than other extracts, it is known that polyphenolic compounds are the main metabolites responsible for the antioxidant capacity in plant extracts (Jiménez *et al*., 2015). This characteristic could be contributing to the antioxidant protection and the reduction of the expression of

acute inflammatory mediators such as TNF-α and IL-1, since phenolic rich extracts have been found to regulate the production of TNF-α and IL-1 in macrophages (Lazarini, *et al*., 2016). Villa-Ruano *et al*. (2013), reported that *Loeselia mexicana*, *Microsechium helleri*, *S. laevigata*, *I. wolcottiana* and *Physalis philadelphica* showed a moderate anti-lipase activity and almost all of them except *I. wolcottiana* and *L. mexicana* demonstrated an acceptable antioxidant activity. The presence of reactive species and free radicals are known to result in adverse reactions in the organism such as tissue lesions, overstimulation of inflammatory mediators and damage to biomolecules that further increase the symptoms of the inflammatory process (Pala & Gürkan, 2008). In the TPA-induced edema model its reported that some phenolic metabolites as flavonoids and coumarins have anti-inflammatory activity (Güevenc *et al*., 2009).

TPA is a phorbol ester extracted from Croton oil (*Croton tiglium* L.), it is the most potent of all phorbol esters belonging to this species (Wambier *et al*., 2019) and is used to induce ear topical acute inflammation, with characteristics such as vasodilation, platelet aggregation and leukocyte tissue infiltration (García-Rodríguez *et al*., 2011). All these events result from protein kinase C (PKC) activation (Muschietti *et al*., 2001), leading to other enzymatic events, such as mitogen-activated protein kinases (MAPK), increased PLA_2 activity. Hence, an increase of arachidonic acid and its metabolites, such as prostaglandins (PG) and leukotrienes (LT), thromboxanes (TX) and proinflammatory mediators such as NF-κB, TNF-α and IL-6 is observed (Passos *et al*., 2013). The $CHCl_3$ extract of *M. helleri* showed significant edema inhibition compared to the control group, the effect was similar to that observed with indomethacin. The mechanisms of the anti-inflammatory effect of the compounds contained in this extract could be associated with their capacity to regulate both PLA_2 and COX-2 and the metabolites derived from these enzymes (Simpson *et al*., 2010).

We also found a significant cardioprotective effect of EEtOH of *M. helleri* in the groups I/R + EtOH and I/R + STZ + EtOH in the infarct model used. These observations are consistent with numerous experimental studies that have demonstrated cardioprotective effects induced by polyphenols in several animal models of myocardial ischemia/reperfusion. Yamazaki *et al*. (2008), found that polyphenol (-)-epicatechin administration had cardioprotective effects in rats that underwent *in vivo* myocardial ischemia/reperfusion. It has also been shown that the administration of various food-grade polyphenols prevents and/or delays the progression of heart failure, reduces pressure overload, myocarditis and cardiotoxicity induced by chemotherapy in animal models of heart failure (Sung & Dyck, 2015).

Dong *et al*. (2012) demonstrated that the administration of natural polyphenols reduced the area of infarction and the area at risk in isolated hearts undergoing myocardial ischemia/reperfusion. Besides, their observations demonstrated a positive regulatory effect on systolic and diastolic partial pressure in these rats. Lebeau *et al*. (2001), evaluated the inhibitory effect of polyphenols on lipid peroxidation during cardiac reperfusion after global ischemia in isolated rat hearts. They found a significant decrease in the area of infarction and improvement in cardiac function when polyphenols were administered. The therapeutic value of polyphenols on cardiovascular diseases and other pathologies such as cancer and diabetes has been attributed mainly to its antioxidant capacity (Kovalchuk *et al*., 2013; Schumacher *et al*., 2015). Also, polyphenols have vasodilatory, antithrombotic, anti-inflammatory, antiapoptotic (Ruf, 1999; Dell´Agli *et al*., 2004; Schroeter *et al*., 2006) anti-ischemic, antiarrhythmic and antihypertensive effects (Goszcz *et al*., 2017), such characteristics support their cardioprotective function.

On the other hand, patients with diabetes and ischemic heart disease are more likely (about 60%) to suffer sudden death, compared to patients who only have diabetes (Pyörälä, 2000). One of the main causes is that the majority of these patients have a higher prevalence of ventricular dysfunction, carotid atherosclerosis and heart failure, which has been called "diabetic cardiomyopathy" (Fernández *et al*., 2000). For this reason, it was not surprising that the induction of diabetes increased the infarction area in response to ischemia/reperfusion compared to the control group.

In this regard, numerous studies have shown that diabetes mellitus is associated with increased formation of free radicals derived from oxygen and decreased body's antioxidant potential (Gupta *et al*., 2004; Milani *et al*., 2005; Okutan *et al*., 2005). This results in oxidative damage of some components of the cell such as proteins, carbohydrates, lipids and

nucleic acids, a situation that prevails while the hyperglycemic state persists (Lüscher *et al*., 2003).
The EEtOH was selected considering their antioxidant effect and the decrease in the percentage of the infarcted area in the I/R + STZ + EtOH can be attributed to this effect. There are several mechanisms by which the antioxidant action of *M. helleri* polyphenols and coumarins may have mediated the cardioprotective effects shown here. The induction of the synthesis of detoxification enzymes is the most accepted in the literature, for example, superoxide dismutase (SOD), catalase (CAT), glutathione S transferase (GST), glutathione peroxidase (GPx), quinone oxidoreductase 1 (NQO1) (Cao & Li 2004; Ungvari *et al*., 2010; Rodrigo *et al*., 2011). However, this is not the only way by which polyphenols exert their cardioprotective action. Song *et al*. (2010), demonstrated that polyphenols found in green tea provide cardioprotection in isolated hearts subjected to ischemia/reperfusion injury by activating the mitochondrial potassium channels sensitive to ATP (mit K_{ATP}). This effect decreases mitochondrial calcium overload, the production of reactive oxygen species (ROS) and the opening of the transition pore of mitochondrial permeability (PTPm), a reaction that leads to myocardial damage. Ca^{2+} ions activate phospholipases, nucleases and many calcium-dependent proteinases. An increase in intracellular Ca^{2+} causes membrane rupture and subsequent cellular damage, which increases the production of oxygen free radicals from the mitochondrial electron transport chain. Namba *et al*. (1988), reported the calcium-antagonistic activity of several coumarins. The scavenging action of coumarins on Ca^{2+} demonstrated peripheral and coronary vasodilator effects, these observations led to the use of these polyphenols as a treatment for angina pectoris (O'Rourke, 2000; Grover *et al*., 2001; Iranshahi *et al*., 2009; Lecour & Lamont 2011).

Together these mechanisms are likely to contribute to the preservation of the function of the heart. The fact that flavonoids and coumarins from *M. helleri* extracts combine their antioxidant and possibly calcium antagonist properties are of particular value in the protection of tissue damage due to myocardial ischemia/reperfusion.

Coronary heart disease (CHD) is the leading cause of mortality in industrialized countries and myocardial infarction (MI) is the predominant manifestation of said disease (Scarborough *et al*., 2010). Oxidative damage caused by free radicals and increased intracellular calcium (Zuo *et al*., 2019) are the main factors involved in this pathology (Ji & Yang, 2011; Saleem *et al*., 2013). A wide variety of clinical and nutritional epidemiological studies indicate that populations whose diet is rich in polyphenols (flavonoids, anthocyanins and coumarins) are less susceptible to cardiovascular diseases along with their complications and related mortality (Mattera *et al*., 2017; Senoner & Dichtl, 2019). Free radicals are generated by the oxidation reaction and incomplete reduction of an oxygen molecule, which leads to structural and functional damage of biomolecules or cell organelles (Akar *et al*., 2017).

Finally, the present work provides the first results that demonstrate the bioactive effect of *M. helleri* metabolites. We have shown that the topical application of *M. helleri* extracts had anti-inflammatory activity. Also, the oral administration of the extracts had an important cardioprotective effect in normal and diabetic rats, being able to attribute said effect to the presence of bioactive metabolites such as polyphenols (coumarins and flavonoids) identified in $CHCl_3$ and EtOH of *M. helleri*. Thus, the use of *M. helleri* extracts has potential application for reducing the incidence of heart attacks in individuals with diabetic cardiomyopathy.

CONCLUSION

The extracts from *Microsechium helleri* contained polyphenolic metabolites (flavonoids and coumarins) with high antioxidant properties. The administration of the extracts showed anti- inflammatory, hypoglycemic and cardioprotective effects in the animal models of inflammation, diabetes and I/R. The results reported here, offer the first relevant insight to the understanding of the mechanisms involved in these pharmacological activities.

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