Research Paper

Cnidoscolus chayamansa Mc Vaugh, an important antioxidant, anti-inflammatory and cardioprotective plant used in Mexico

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A B S T R A C T

Ethnopharmacological relevance: Cnidoscolus chayamansa Mc Vaugh (Euphorbiaceae) is commonly known as ‘chaya’ in Central America. In South East Mexico, because of its high nutritional values, it is an important part of the diet of many indigenous communities. Chaya is also used as a traditional remedy for the treatment of diabetes, rheumatism, gastrointestinal disorders and inflammation-related diseases. Although Cnidoscolus chayamansa is one of most used and valued medicinal plants, only few studies on documenting its pharmacological properties can be found.

Materials and methods: Dried leaves of Cnidoscolus chayamansa were subjected to a successive maceration using Hex, EtOAc and EtOH. The antioxidant activities of the extracts were tested using the DPPH radical scavenging, Ferric reducing/antioxidant power and total phenolic content assays. To determine the anti-inflammatory activity, the TPA-induced mouse ear edema and the carrageenan-induced mouse paw edema assays were used. The cardioprotective effects of the EtOH extract was determined using the ischemia/reperfusion (I/R) rat model. Finally, the acute toxicity was determined using Lorke’s method.

Results: The results showed a similar anti-inflammatory activity (≥ 30%) for all extracts but only the EtOAc extract showed relevant activity when applied intraperitoneally. When tested for their antioxidant activity none of the extracts showed a significant activity suggesting that the anti-inflammatory activity is not related to a direct free radical scavenging of the extracts. Additionally, the EtOH extract showed a strong cardioprotective effect at 500 mg/kg when given orally. Both the EtOAc and the EtOH extract have a LD50 > 5 g/kg, confirming their safety in acute oral administration.

Conclusions: All these results are relevant for a better understanding of the therapeutic used of Cnidoscolus chayamansa in the Mexican traditional medicine and highlights its cardioprotective potential.

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1. Introduction

Cnidoscolus chayamansa Mc Vaugh (Euphorbiaceae) is commonly known as ‘chaya’ in Central America. In South East Mexico, it is mainly consumed because of its high nutritional values. Cnidoscolus chayamansa is also used as a traditional remedy for the treatment of diabetes, rheumatism, gastrointestinal disorders and inflammation (Koltermann et al., 1984; Kuti and Torres, 1996). The hypocholesterolemic effect of aqueous extracts of Cnidoscolus chayamansa has previously been documented. Additionally, infusions of this plant have shown therapeutic effects in the treatment of noninsulin-dependent diabetes mellitus (Kuti and Kuti, 1999; Loarca-Pina et al., 2010). Phytochemically, plants belonging to the Cnidoscolus genus are known to have coumarins and glycosidic flavonoids in their aerial parts (Yuan et al., 2007). In Cnidoscolus chayamansa, cyanogenic glycosides, together with the flavonoids dihydromyricetin, astragalin and kaempferol-3-rutinoside have been reported (González-Laredo et al., 2010).

Medicinal plants have proved to be an important source in the search for new drugs, mainly because of the diversity of pharmacological properties and chemical structures (Balunas and Kinghorn, 2005; Adeneye et al., 2006). In the present study, the lipids profile,
and the antioxidant, anti-inflammatory and cardioprotective activity of leaves of *Cnidoscolus chayamansa* were analyzed in order to understand the value of this plant in Mexican traditional medicine.

2. Material and methods

2.1. Chemicals

All chemicals used in this study were analytical grade. Methanol (MeOH), ferric chloride, acetone, sodium carbonate, sodium acetate, acetic acid, ethanol (EtOH), chloroform (CHCl₃), ethyl acetate (EtOAc), sodium picate, acetic anhydride, Grignard reagent, sulphuric acid, potassium hydroxide, potassium iodine, basic bismuth salts and cobalt chloride were purchased from Merck Co (Germany). 2,2-dipheophosphoric acid, potassium hydroxide, potassium iodine, basic bismuth salts and cobalt chloride were purchased from Merck Co (Germany). 2,2-dipheophosphoric acid, potassium hydroxide, potassium iodine, basic bismuth salts and cobalt chloride were purchased from Merck Co (Germany). 2,2-dipheophosphoric acid, potassium hydroxide, potassium iodine, basic bismuth salts and cobalt chloride were purchased from Merck Co (Germany). 2,2-dipheophosphoric acid, potassium hydroxide, potassium iodine, basic bismuth salts and cobalt chloride were purchased from Merck Co (Germany).

2.2. Plant collection

*Cnidoscolus chayamansa* was collected in Veracruz City, Veracruz State, Mexico in March 2009. Plants were identified by Luis Hermann Bojorquez Galván and a voucher specimen (CIB9023) was deposited at the herbarium of the Centro de Investigaciones Biológicas, Universidad Veracruzana.

2.3. Preparation of plant extracts

Approximately, 135 g of dried leaves were extracted successively by maceration using solvents of ascending polarity at room temperature 25 °C. The extraction was carried out successively using hexane (Hex, 5.68 g), ethyl acetate (EtOAc, 5.27 g) and ethanol (EtOH, 9.44 g) and kept in darkness at room temperature for further use. Solvents were removed using a rotary evaporator (Heidolph LABOROTA 4000) and extracts fully dried in a vacuum oven (Shellab) at 25 °C.

2.4. Qualitative phytochemical analysis (QPA) and fatty acid profiling

QPA of the plant extracts was carried out using standard qualitative methods (color test and/or Thin Layer Chromatography, TLC) to detect the presence of sterols, terpenoids, coumarins, flavonoids, lignans and alkaloids (Domínguez, 1973; Ceke et al., 2006). Additionally, the fatty acid analysis of the hexane extract by GC–MS was carried out. Briefly, twenty-five mg of the hexane extract was added to 2.5 mL of BF₃-MeOH (14% v/v) and heated/stirred for 20 min at 80 °C; followed by extraction with hexane (x2) in order to recover the methyl esters derivatives. The extract was collected and solvent removed under low-pressure conditions; the dried extract was reconstituted in 1 mL hexane for GC/MS analysis. The initial temperature of 150 °C was maintained for 5 min, then raised to 210 °C at the rate of 30 °C/min, 213 °C at the rate of 1 °C/min and 225 °C at the rate of 20 °C/min and kept at 225 °C for 40 min. (Carvalho and Malcata, 2005). The atherogenic and thrombotic indexes of the hexane extract were determined according to Ulbricht and Southgate (1991).

2.5. Antioxidant activity

2.5.1. DPPH radical-scavenging activity

The radical-scavenging activity was determined according to Brand-Williams et al. (1995) method and modified by Miliauskas et al. (2004). Briefly, 2.9 mL of freshly prepared DPPH solution 9 × 10⁻³ M in MeOH were placed in an amber vial followed by the addition of 100 μL of plant extracts 33 mg/mL dissolved in MeOH (for the blank, 100 μL of MeOH were added instead of the sample). After mixing, samples were incubated for 30 min at 37 °C in a water bath. Absorbance values of samples (A₀) and blank (A₉) were measured at 517 nm using a UV-vis spectrophotometer (Varian Model Cary-100). Experiments were run in triplicate and the activity was calculated using the following formula:

% inhibition = [(A₀–A₉)/A₀] × 100

2.5.2. Total phenolic content

The total phenolic concentration was determined using the Folin–Ciocalteu reagent according to Spanos and Wroslad (1990). Fifty μL of each sample, 2.5 mL 1/10 dilution of Folin–Ciocalteu’s reagent and 2 mL of Na₂CO₃ (7.5%, w/v) were mixed and incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a UV–vis spectrophotometer. The experiments were carried out in triplicates Results were expressed as gallic acid equivalent GAE (mg/L) using a standard gallic acid graph (range 20 to 1000 mg/L).

Absorbance = 0.001[GAE(mg/L)] + 0.0754

2.5.3. FRAP “ferric reducing/antioxidant power”

The reductive power of samples was measured using the Benzie and Strain (1996). FRAP reagent was prepared by mixing 25 mL of sodium acetate buffer solution (3.1 g of sodium acetate 3H₂O and 16 mL of acetic acid in 1 L, pH 3.6), 2.5 mL of TPTZ (10 mM) in HCl 40 mM, 2.5 mL of FeCl₃·6H₂O (20 mM); then incubated at 37 °C for 5 min. About 2.7 mL of FRAP solution were transferred into an amber vial and added 150 μL mL of an aqueous solution of plant extract (33 mg/mL) and 150 μL of distilled water. The absorbance was measured at 593 nm for triplicate. The blank of the experiment was prepared by adding 300 μL of distilled water to 2.7 mL of FRAP solution. The results were expressed in mmol de Fe²⁺/L, based on a calibration curve obtained from different concentrations of FeSO₄ (100–1000 mmol/L).

Absorbance = 0.00109[mmolFe²⁺/L] + 0.10715

2.6. In vivo assays animals

Male CD1 mice (20–25 g) were used for the anti-inflammatory and acute toxicity assays. For the ischemia-reperfusion assay male Wistar rats (250–300 g) were used. All animals were maintained under standard laboratory conditions (25 °C, 12-h dark/12-h light, 50% relative humidity) according to the Norma Oficial Mexicana (Mexican Official Norm) NOM-062-ZOO-1999. Food and water were provided ad libitum.

2.7. Anti-inflammatory activity

2.7.1. TPA induced ear edema in mice

In this experiment, TPA and indomethacin were applied topically. In the test group, animals (n=7) were initially treated with 2.5 μg of TPA dissolved in 25 μL of acetone in the right ear. The left ear received only acetone. After 30 min, the sample or indomethacin (2 mg/ear dissolved in 50 μL of acetone) was applied. Mice were sacrificed by cervical dislocation 6 h after TPA treatment and a 6 mm diameter section of the right and left ears were cut and weighted. Anti-inflammatory inhibition activity was calculated according to the weight difference between the right and the left ear section compared to the control group. (Young and De
2.7.2. Carrageenan induced edema in mice

In this assay, edema was induced by subcutaneous injection of 20 μL of 1% carrageenan in saline solution. Following the administration of carrageenan the size of the edema was measured at t=1, 3, 5 and 7 h using a micrometer. t=0 corresponded to a measurement before the administration of carrageenan (Winter et al., 1962; Beloeil et al., 2005). The test groups were injected intraperitoneally (i.p.) indomethacin (5 mg/kg) or plant extract 30 min before administration of carrageenan. The control group only received the vehicle. Animals were observed daily for 14 days registering deaths and behavioral changes. At the end of the experiment, mice were sacrificed by clavicle dislocation, and selected organs were excised, weighed and macroscopically examined (Lorke, 1983).

2.8. Acute toxicity (LD₅₀)

Animals were fasted 12 h before the oral administration via a gastric catheter of the plant extracts (5 g/kg) or vehicle (tween 80-water 1:9). The control group only received the vehicle. Animals were observed daily for 14 days registering deaths and behavioral changes. At the end of the experiment, mice were sacrificed by clavicle dislocation, and selected organs were excised, weighed and macroscopically examined (Lorke, 1983).

2.9. Cardioprotective activity

2.9.1. Study protocol

Rats were randomly divided in four groups of five animals each. Group-I (control group), Group-II (negative control group) was treated with 500 mg/kg ETOH extract of Cnidoscolus chayamansa. Group-III was subjected to 1 h ischemia/4 h reperfusion (I/R) in presence of L-NAME (20 mg/kg), Group-IV was treated with 500 mg/kg EtOH extract of Cnidoscolus chayamansa.

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

Rats were anaesthetized with pentobarbital (0.1 mL/100 g, i.p.). The neck was extended and opened with a ventral midline incision a d a tracheotomy was performed; rats were ventilated with oxygen-enriched air using a positive pressure ventilator (UGO BASILE, MODEL 7025) (Guo et al., 1994). Body temperature of rats was maintained using an intratracheal probe during the surgical preparation and recuperation period (Viniegra et al., 2002). A left thoracotomy was performed and the pericardium was carefully opened to expose the heart. The left arterial descending coronary artery (LAD) was ligated using 6-0 silk, blood flow occlusion was kept for 1 h; later, and myocardium was reperfused for 4 h. At the end of this procedure, after sacrificing the animal, the heart was excised from the thorax and the greater vessels were removed. The ventricles were separated and frozen at −20 °C for 2 h and sliced in 2 mm thick sections. The sections were incubated in 1% TTC solution for 20 min at 37 °C, followed by submersion in 4% p-formaldehyde (pH 7.4) then, placed on glass slides with 2 mm separation between sections (Downey, 2003). Images were captured and analyzed using Image J 1.30 software (NIH, USA) on a blind condition. Cardiac damage was calculated using the ratio between the infarcted area and the total area of left ventricle (IA/TA).

2.10. Statistical analysis

The results for the antioxidant activity were reported as mean ± S.D. and the results from animal experiments were reported as mean ± S.E.M. Significance for the inflammation experiments was determined by one-way analysis of variance (ANOVA) followed by post hoc Student Newman–Keuls. Significance for the (I/R) experiments was determined by one-way ANOVA, followed by post hoc Turkey’s test (n=5). In both cases a value of P < 0.05 was considered statistically significant.

3. Results

3.1. Qualitative phytochemical analysis (QPA) and fatty acid profiling

The results of the QPA using standard qualitative methods are shown in Table 1. Overall, they showed the presence of sterols as major components of the hexane extract; a weak presence for flavonoids and coumarins in the ETOAc extract and a weak presence for flavonoids, coumarins and lignans in the ETOH extract. The fatty acid profiling of the Hex extract of Cnidoscolus chayamansa showed mainly the presence of lauric C12 (31.9%), myristic C14 (11.7%) and palmitic (28.3%), oleic C18:1 (5%) and arachidonic C20:4 (4.80%) acids.

3.2. Antioxidant activity

The results observed for all the antioxidant assays are shown in Table 2. All extracts were tested at three different concentrations, they all showed lower radical scavenging properties compared to the positive control (ascorbic acid). Additionally, the same extracts were tested for their ferric reducing power (FRAP), the ETOAc extract showed the highest activity (387.16 ± 8.01 µmol Fe²⁺/L). The total phenolic content assay showed the highest value for the EtOH extract.
showed by the positive control, indomethacin (50.06%). The results showed a good activity, these were lower compared to that (Table 3) was similar for all extracts of 3.3. Anti-inflammatory activity of Cnidoscolus chayamansa on carrageenan induced acute paw edema.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Extract</th>
<th>Dose (mg/ear)</th>
<th>Edema (mg)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>12.01 ± 0.50</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cnidoscolus chayamansa</td>
<td>Hex 2</td>
<td>8.32 ± 0.30</td>
<td>30.76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ETOAc 2</td>
<td>8.17 ± 0.40</td>
<td>32.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ETOH 2</td>
<td>8.18 ± 0.50</td>
<td>31.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indomethacin</td>
<td>6.00 ± 0.70</td>
<td>50.06</td>
<td></td>
</tr>
</tbody>
</table>

Each group represents the mean ± sem. The application was topical. One way ANOVA, post hoc Student-Newman–Keuls.

* p < 0.05, n = 6.

Table 4 Anti-inflammatory activity of Cnidoscolus chayamansa on carrageenan induced acute paw edema.

<table>
<thead>
<tr>
<th>Edema formation</th>
<th>Paw edema formation mm and (%) inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (h)</td>
<td>Control Indomethacine ETOAc extract ETOH extract</td>
</tr>
<tr>
<td>1</td>
<td>0.48 ± 0.03 0.38 ± 0.05 (20.83) 0.48 ± 0.04 (NE) 0.6 ± 0.06 (NE)</td>
</tr>
<tr>
<td>3</td>
<td>0.43 ± 0.03 0.7 ± 0.04 (36.4) 0.31 ± 0.03 (28.78) 0.37 ± 0.03 (12.90)</td>
</tr>
<tr>
<td>5</td>
<td>1.42 ± 0.1 0.7 ± 0.04 (41.7) 0.99 ± 0.08 (30.29) 1.37 ± 0.08 (3.11)</td>
</tr>
<tr>
<td>7</td>
<td>0.94 ± 0.04 0.7 ± 0.07 (30.0) 0.71 ± 0.06 (24.24) 1.23 ± 0.1 (NE)</td>
</tr>
</tbody>
</table>

Each group represents the mean ± sem. The application was intraperitoneal. The doses were of 5 mg/kg by indomethacin and the 500 mg/kg by the extracts. Values in parenthesis indicate the percent of inhibition edema with respect to control group. One way ANOVA, post hoc Student-Newman–Keuls.

* p < 0.05, n = 8.

(35.70 mEqAG/g). The Hex and ETOAc extracts showed a total phenolic content of 22.35 mEqAG/g and 13.25 mEqAG/g, respectively.

3.3. Anti-inflammatory activity

The inhibition observed in the TPA induced ear edema assay (Table 3) was similar for all extracts of Cnidoscolus chayamansa, Hex (30.76%), ETOAc (32.04%) and ETOH (31.90%). Although they showed a good activity, these were lower compared to that showed by the positive control, indomethacin (50.06%). The results of the carrageenan-induced acute paw edema (Table 4) showed significant anti-inflammatory activity only for the ETOAc extract after 3 h (28.78%) following the application of carrageenan, and until the end of the experiment, after 7 h (24.24%). Although the ETOAc extract showed anti-inflammatory activity in this assay, the effect was lower to that showed by indomethacin.

3.4. Acute toxicity

The LD₅₀ was determined using Lorke’s (1983) method, showing for both the ETOAc extract and ETOH extract an acute toxicity value of > 5 g/kg, when administered at a single dose orally. After 15 days, animals were sacrificed showing no signs of macroscopic damage of internal organs.

3.5. Cardioprotective effects

Cnidoscolus chayamansa is commonly administered orally in Mexican traditional medicine. Hence, the ETOH extract (the most polar and) was selected to evaluate its cardioprotective properties.

Fig. 1 shows the cardioprotective activity evaluated in male Wistar rats administered with either ETOH extract of Cnidoscolus chayamansa or vehicle after being subjected to coronary ischemia/reperfusion (I/R). It can be seen that the difference of infarcted area and total area of left ventricle ratio (IA/TA) between the control group and the negative control is less than 1%. However, when the animals were subjected to coronary ischemia/reperfusion, the damaged area showed a 39% increase, which was then reduced by 10% when animals were treated with the ETOH extract of Cnidoscolus chayamansa. Additionally, the IA/TA ratio was even higher when animals were treated with L-NAME prior to being subjected to coronary I/R, this was reduced by 15% when animals were administered the ETOH extract of Cnidoscolus chayamansa. Overall, there were significant differences between the groups subjected to I/R, I/R + L-NAME vs. I/R + L-NAME + Cnidoscolus chayamansa. L-NAME is a competitive inhibitor of nitric oxide synthase (NOS) (Rees et al., 1989, 1990; Méndez et al., 2006).

4. Discussion

The results observed in the qualitative phytochemical screening are in agreement with previous studies reporting the presence of flavonoids, sterols, coumarins and cyanogenic glycosides in plants belonging to the same genus such as Cnidoscolus texanus and Cnidoscolus aconitifolius (Escañante-Erosa et al., 2004; Yuan et al., 2007). It has been reported that Cnidoscolus chayamansa has higher concentrations of polyphenolic compounds compared to Cnidoscolus aconitifolius. Additionally, the presence of alkaloids as minor components of Cnidoscolus chayamansa has also been reported (González-Laredo et al., 2003). However, we did not find the presence of alkaloids in our study; although, this can be the result of variability among species of the same genus, seasonal
similar mediators cause early in the carrageenan-induced paw edema model has two phases. In the opposite to topical in the TPA-induced edema model. The extract showed a medium anti-inflammatory activity in the results of the three assays. The antioxidant and anti-inflammatory activities of coumarins (e.g. scopoletin) and flavonoids (e.g. quercetin, kaempferol) has been well documented in human diseases where reactive oxygen species are involved (Takahashi and Shibamoto, 2008; Jensen et al., 2008). It is important to note that the results of our QPA showed only a weak positive (+) for the presence of coumarins and flavonoids in both, the EtOAC and EtOH extracts. However, the Hex extract shows a strong positive (+ + +) for the presence of sterols. It is known that sterols and other non-polar compounds can show significant antioxidant activity in several models. Yoshida and Niki (2003) have reported the antioxidant effects for β-sitosterol, phytosterol, campesterol and other plant sterols. Moreover, the role of plant sterols and their mechanisms in the modulation of the antioxidant enzymatic response has been well established (Vivancos and Moreno, 2005; Conforti et al., 2009). Additionally, it is important to mention that there is an ongoing debate regarding the correlation between antioxidant activity and the phenolic content of plant extracts as some studies have shown no correlation between these parameters (Conforti et al., 2009).

The evaluated extracts of Cnidoscolus chayamansa showed a low concentration of phenolic compounds which can explain the low anti-inflammatory activity observed in the TPA-induced edema assay. Several flavonoids, coumarins are phenolic metabolites have been reported with anti-inflammatory activity when the TPA-induced edema model has been used (Güevenc et al., 2009). The TPA-induced ear edema is an anti-inflammatory model used for evaluating the capacity of topical agents to inhibit inflammatory mediators resulting from protein kinase C activation (Muschietti et al., 2001). Protein kinase C activation has been known to stimulate phospholipase A2 resulting in the cleavage of arachidonic acid present in cell membranes and the biosynthesis of prostaglandins and leukotrienes involved in the inflammation process (Gorzalczany et al., 2011). So, extracts showing activity in this assay can act via the inhibition of any of these mediators of inflammation. In the carrageenan-induced edema assay the EtOAC extract showed a medium anti-inflammatory activity, it is important to note that in this model the extracts are administered i.p. opposite to topically in the TPA-induced edema model. The carrageenan-induced paw edema model has two phases. In the first phase (90–180 min), the release of histamine, serotonin and similar mediators cause early inflammation. Then, in the second phase (270–360 min), prostaglandins, proteases and lysosome are associated to late inflammation (Orhan et al., 2007). Hence, in our results the activity observed in the EtOAC extract is more likely to be the result of an interaction between phenolic and sterol metabolites, since only this extract showed the presence of both type of compounds. Moreover, the role of plant sterols in the modulation of the antioxidant enzymatic response and their anti-inflammatory activity has been reported (Vivancos and Moreno, 2005; Conforti et al., 2009).

As mentioned earlier, the reason for selecting the EtOH extract of Cnidoscolus chayamansa to evaluate its cardioprotective activity was based in its oral consumption in the Mexican traditional medicine. The results observed are in agreement to those reported by Yamazaki et al., (2008), where flavanols found in fruits, beverages and cocoa have shown cardioprotective activity. Furthermore, the response observed when the EtOH extract of Cnidoscolus chayamansa was administered, suggesting the activation of NOS by the extract. The decrease in bioavailability of NO is a common response involved in several pathological disorders such as hypertension, atherosclerosis, diabetes and the damaged observed in the model used in our cardioprotective assay (Pernow et al., 1994; John and Schneider, 2003; Ferdinandy and Schulz, 2003). In the other hand, the results obtained in the ischemia/reperfusion assay, showed the ability of Cnidoscolus chayamansa to decrease the infarcted area and total area of left ventricle ratio (IA/TA). This suggests the potential of protection against myocardial damage when this extract was given as an oral supplement. The documented presence of coumarins, lignans and flavonoids in Cnidoscolus chayamansa seems to support its beneficial effects, since epidemiologic studies have demonstrated that food and drinks rich in polyphenols are able to reduce the risk of hypertension and coronary diseases (Ghosh and Scheepens, 2009). Additionally, it is important to mention that the EtOH extract showed the highest content of total phenolic compounds. However, we did not observed a correlation between the results of our selected antioxidant assays (radical scavenging activity and ferric reducing ability) and the cardioprotective activity of the EtOH extract of Cnidoscolus chayamansa. This is because some phenolic compounds such as resveratrol and flavonoids can also act via de modulation of both, enzymes involved in oxidative stress protection and other in vivo mechanisms. Hence, we cannot fully discard the role of any potential involvement of the antioxidant activity in both the anti-inflammatory and cardioprotective activities. The cardioprotective activity could be the results of the antioxidant activity and the modulations of other pathways involved in the protection of the heart (Pallás et al., 2010; Procházková et al., 2011). The cardioprotective activity of polar compounds such as saponins has also been reported (He et al., 2012). This is significant since the presence of sterols in the Hex extract could signal the presence of steroid saponins, which have been reported for Cnidoscolus spp (Fan and Zhou (2010)). Additionally, the presence of cupper, manganese, zinc, calcium and magnesium has been documented in Cnidoscolus chayamansa. These, have been known to act as essential cofactors for the proper activity of calcium-dependant NOS (eNOS and nNOS) and other enzymes such as superoxide dismutase (SOD) and vasoactive enzymes, which are relevant to cardiovascular homeostasis (Kuti and Kuti, 1999; Aye, 2012). Finally, based on the results observed in the acute toxicity assay for the tested extracts, the consumption of Cnidoscolus chayamansa as food and medicine can be considered safe and no lethal at a single dose (Lorke, 1983; OECD, 2001).

5. Conclusion

Cnidoscolus chayamansa is a commonly used medicinal plant in Southeast Mexico, it is highly valued for its numerous medicinal and nutritional properties. However, there is a lack of pharmacological studies that can shed light into understanding the therapeutic mechanisms and the safety of medicinal plants consumption. It is clear that Cnidoscolus chayamansa possesses a significant anti-inflammatory and cardioprotective activities that could be due to the presence of sterols, flavonoids, coumarins, saponins and metals. Additionally, Cnidoscolus chayamansa showed no lethal effects in a single dose, when given orally. Finally, this study supports the therapeutic use of this species in the Mexican traditional medicine and highlights its cardioprotective potential.

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References


