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Evaluation of anti-inflammatory, anti-nociceptive and toxicological effects of hydro-alcoholic extract of *C. pulverulentus* activity in a murine model

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Abstract

Costus pulverulentus is a plant used in traditional medicine for the treatment of infectious diseases and kidney inflammation. In order to check their antiinflammatory effect inflammation model carrageenan was used, the results showed a percent inhibition of edema at a concentration of 8 mg / kg similar to the effects of indomethacin (88.53 and 79.85%) EHCP while antinociceptive activity in the concentration of 8 mg / kg of EHCP exerted greater activity (57.58%) than that shown by aminopyrine in the late phase of the trial. Toxicological effects were determined by measuring the amount of digestive tissue ulceration, weight gain, behavioral changes, mortality and clinical signs and symptoms during administration of the treatment; treated groups EHCP concentrations or vehicle showed no apparent changes in the toxicological study period. This coupled with the anti-inflammatory and antinociceptive activity shown by the EHCP suggests that *C. pulverulentus* may be a desirable alternative to inflammatory conditions.

Ethnomedicine, carrageenan, side effects, *C. pulverulentus.*

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Introduction

The inflammation is a natural response of the organism to potentially harmful agents, under normal conditions this process is considered beneficial (Gupta et al., 2015, Lin et al., 2015), however, the excessive duration of this, caused by different Pathological conditions generate complications that can induce tissue loss (Komatsu et al., 2013). The drugs of choice to cope with this are non-steroidal anti-inflammatory drugs (NSAIDs), daily use of these and poor diet can lead to gastrointestinal diseases such as gastritis or stomach cancer (Salvatierra et al., 2006, Pérez et al. ., 2002). An alternative for the treatment of these diseases is usually the use of medicinal plants that apparently do not generate side effects, in Huasteca Potosina there is a wide range of medicinal plants, among them is Costus pulverulentus, belonging to the family of Costaceae, The main ethnomedical uses are in the treatment of renal, hypoglycemic, pain and inflammation diseases (Guzmán & Guerrero, 2009; Quintans et al., 2010). Therefore, the present research aimed to evaluate the antiinflammatory, antinociceptive and side effects of a hydroalcoholic extract of C. pulverulentus (EHCP) in order to show the ethnomedical uses referred to.

Methodology

Obtaining the extract

The plant material of C. pulverulentus was collected in the municipality of Aquismón (21 ° 37'5 "N 99 ° 01'51" O), S.L.P., Mexico in July 2015 in the rainy season.

The stems were washed and subjected to a drying process under dark conditions for one week, after finishing this process they were processed in a mechanical mill to obtain fine particles (Mahecha, 2007). To obtain the extract, the percolation technique was used, placing the plant material with 70% ethanol in a separating flask covered with aluminum to avoid exposure to light. The percolation was done by dripping and the extract obtained was evaporated to dryness (Carreón & García, 2010).

Experimental animals

For the determination of the anti-inflammatory and antinociceptive activity of the EHCP, female Wistar rats of 4 to 6 weeks of life with an average weight of 160-180 gr were used. Which were maintained in a control of temperature, humidity, free access to water and food with light / dark cycles of 12 hours each. The duration of the experiment was as short as possible, always considering that the number of animals used was the minimum necessary. Each animal was sacrificed following ethical guidelines for research on pain in experimental animals of the International Pain Association (Zimmerman, 1983).

In the toxicological evaluation 16 female Wistar rats of 4-6 weeks of age with a weight of 160 - 180 gr. Maintained under controlled temperature and humidity conditions with free access to water and food in 12-hour light / dark cycles. The animals were divided into 4 treated groups as follows: one control group with Indomethacin, one group with deionized water and two more with concentrations of 8 or 4 mg / kg EHCP.

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Model of Carrageenan Inflammation

The animals were divided into 4 groups composed of 4 rats each, which were pretreated with oral administration of EHCP at doses of 8 or 4 mg / kg, a positive control group, given Indomethacin (10 mg / kg) And a negative control group, to which only deionized water and DMSO (5: 1, vehicle) were given.

One hour after administering the treatments, inflammation was induced by injection of 1% carrageenan (Sigma, USA) into the sublantar region of the right paw of the animals (Winter et al., 1962).

The inflammatory process was measured with the aid of a digital plethysmometer (UGO BASILE), quantifying the volume of edema induced every hour for six hours. The percentage of the anti-inflammatory effect was calculated by the following equation:

$$\%\ \text{"Inhibition of edema"} = \frac{(\text{Ct-Co})\ \text{control} - (\text{Ct.-Co})\ \text{"Treated"} * 100}{(\text{Ct-Co})\ \text{control}} \quad (1)$$

Where Ct is the paw volume at time t after carrageenan injection and Co is normal leg volume before carrageenan injection.

Nociceptive model for formalin.

The animals were divided into 4 groups composed of 4 rats each, which were pretreated with oral administration of EHCP at a dose of 4 mg / kg body weight or 8 mg / kg, a positive control group administered aminopyrine (2 mg / kg) and a negative control group, to which only the vehicle was administered.

One hour after administration of the treatment, pain production was induced by 3% formalin (Sigma, USA) (Zimmerman, 1983) at doses of 20 μg per gram of weight in the subplantar region of the left paw of experimental animals.

The rats were placed in transparent cages for the purpose of observing their behavior. The time of licking and paw shaking was quantified for 30 minutes over 5 minute periods. The percentage of antinociception was calculated by the following equation (Isiordia, et al., 2010):

$$\%\ \text{antinocicepción} = \frac{\text{"Lamination T" "without" "Drug Lamination T-T} * 100\text{"}}{\text{"Lid Tide" "without" "drug"}} \quad (2)$$

Toxicological evaluation

During the treatment of the animals clinical signs and behavioral changes were recorded, increasing weight from day 1 to day 7.

At the end of the experiment the animals were sacrificed. A sagittal cut in the peritoneal area of the animals showed the presence of ulcerations in the gastrointestinal tract caused by the treatments. The number of ulcers was evaluated on a scale of 0 to 4 crosses, with one cross (0-2 ulcers), two crosses (3-5 ulcers), three crosses (6-10 ulcers) and four crosses (11-20 ulcers) (González & Rodríguez, 2014).

Statistic analysis

The normality criteria were reviewed in the generated data and later the ANOVA test was used to determine differences between the groups, taking as significant a value of $p < 0.05$.

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For the generation of graphs, we used the program Excel and Graph Pad Prism version 6.5.

Results

Figure 1 shows the results of anti-inflammatory activity, as shown, the Indomethacin-treated group had a significantly higher anti-inflammatory effect (84.53%, p <0.0032) than the concentrations of EHCP used during the first 2 hours of treatment. Both EHCP concentrations increase their constant anti-inflammatory effect from the third hour to the end of treatment, whereas the 8 mg / kg EHCP concentration exerts a similar effect to that of the control drug in the sixth hour (79.85%)..

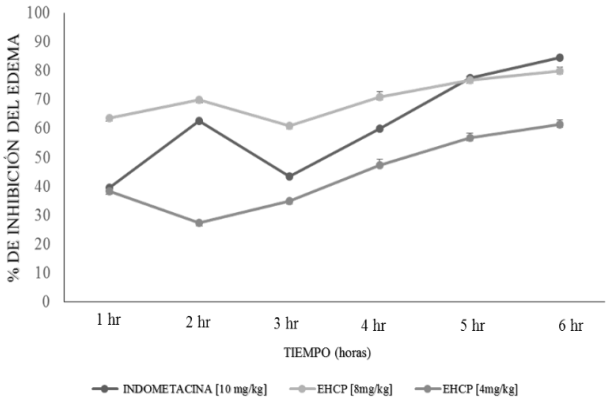

Figure 1 Anti-inflammatory effect of EHCP.

Pain is a symptom widely associated with inflammatory processes and is widely investigated to provide effective therapeutic solutions; The results obtained in the nociceptive model by formalin are shown in figure 2.

The early phase (0-5 minutes) is the result of a stimulus of nociceptive receptors caused by formalin, in this phase the drug aminopyrin showed a percentage of Antinociception of 24.57%, superior to those exerted by EHCP concentrations, although without significant difference between them (p> 0.05). In the late phase (15-30 minutes) the antinociceptive effect exerted the concentration of 8mg / kg of EHCP (57.58%) is higher compared to the antinociceptive effect of aminopyrin (45.89%) however the results do not show significant differences Among them (p> 0.05).

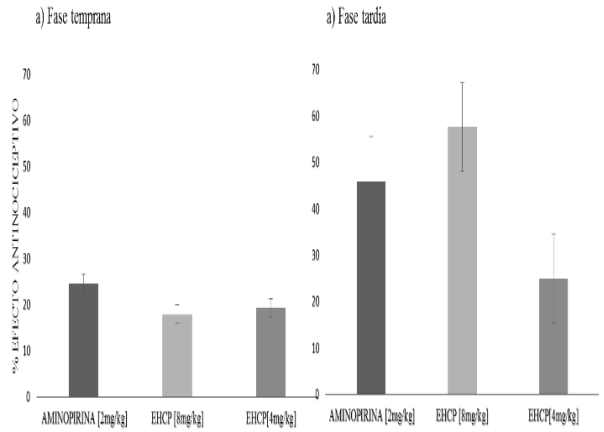

Figure 2 Inhibition of pain mediated by C. pulverulentus. A) Early phase, b) Late phase.

In order to show the possible adverse effects caused by treatments in animals, the number of ulcers present in the digestive tissue was quantified (figure 3).

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The control group treated with Indomethacin showed a total of 16 ulcers on average, significantly higher ($p < 0.0036$) than vehicle-treated groups (8 ulcers) or EHCP concentrations (8 and 9 ulcers respectively), which also showed Gastrointestinal damage but to a lesser extent than the Indomethacin control group, possibly due to the stress to which the animals were subjected during treatments.

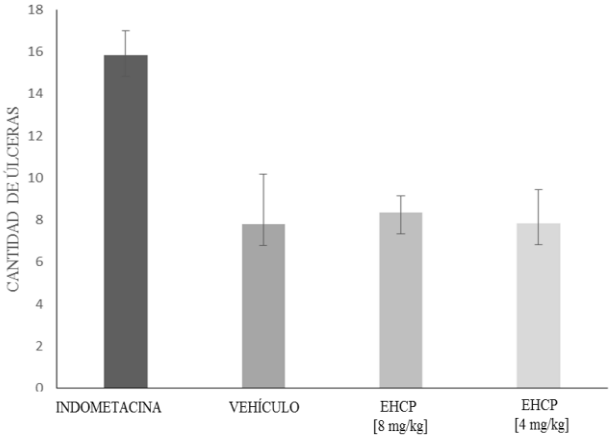

Figure 3 Number of ulcers observed in stomach and intestinal tissue of experimental animals in different groups.

The weight gain obtained for each treated group is shown in figure 4, it is observed that the groups treated with the vehicle or the concentrations of the EHCP maintain a constant weight gain while the group treated with Indomethacin maintains a significant weight gain ($P < 0.0001$) low compared to the other groups, possibly due to drug-induced ulceration.

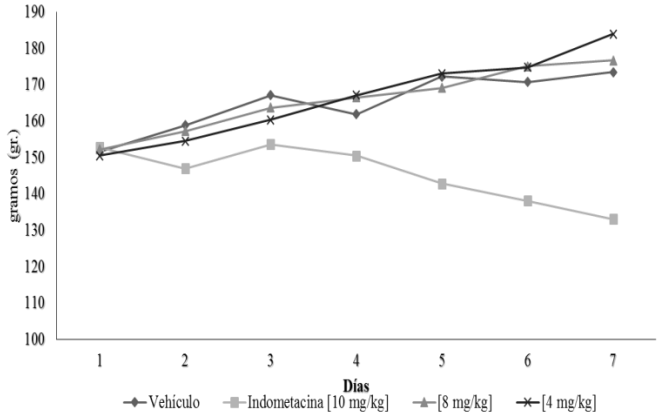

Figure 4 Weight gain in grams of the animals during the treatment period.

Changes in behavior, mortality, clinical signs and symptoms (Table 1) were observed during administration of the treatments. A significant change was observed in the animals treated with Indomethacin, which present aggression, polydipsia, sedation, and irritability until reaching the death of this group, vehicle-treated groups, and EHCP concentrations show no apparent changes.

		GRUPOS DE ESTUDIO			
Parámetros evaluados		Vehículo	Indometacina [10 mg/kg]	EHCP [10mg/kg]	EHCP [4mg/km]
Mortalidad		0/4	4/4	0/4	0/4
Alteraciones neurológicas	Inmovilidad	s/c	++	s/c	s/c
	Sedación	s/c	++	s/c	s/c
	Polidipsia	s/c	++	s/c	s/c
	Agresividad	s/c	++	s/c	s/c
Anormalidad del comportamiento	Irritabilidad	s/c	++	s/c	s/c
	Pasividad	s/c	+	s/c	s/c
Condiciones de piel y mucosas	Piel	s/c	s/c	s/c	s/c
	Mucosas	s/c	s/c	s/c	s/c
	Heces	s/c	consistencia acuosa	s/c	s/c
(S/C) Sin cambios, (+) Cambio leve, (++) Cambio moderado.					

Table 1 Signs and symptoms observed during treatment.

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Conclusions

The EHCP shows anti-inflammatory and antinociceptive effects similar to the drugs used as control, without provoking apparent side effects, so it could be considered as an effective treatment alternative whenever more in-depth studies corroborating the safety of its use by time prolonged.

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