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ANTI-INFLAMMATORY ACTIVITY OF THE ETHNOLIC EXTRACT OF THE LEAVES OF Manihot esculenta crantz (YUCA) IN AN EXPERIMENTAL MODEL OF ACUTE INFLAMMATION

ACTIVIDAD ANTIINFLAMATORIA DEL EXTRACTO ETANÓLICO DE LAS HOJAS DE Manihot esculenta Crantz (YUCA) EN UN MODELO EXPERIMENTAL DE INFLAMACIÓN AGUDA

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ABSTRACT

Introduction: Manihot esculenta Crantz (yucca) leaves have been used around the world and over time in order to decrease the anti-inflammatory response. **Objective:** To evaluate the anti-inflammatory activity of the ethanolic extract of the Manihot esculenta Crantz leaf in rats. **Methods:** An experimental study was conducted. The study population included 60 albino rats sp. Rattus novergicus, distributed in 4 groups of 15 rats each. A homogeneous solution of ethanolic extract of Manihot esculenta Crantz leaves was used for intraperitoneal administration. Carrageenan was used as an inflammatory inducer that was administered intradermally; for the measurement of plantar edema, the Digital Water Plethysmometer (LE7500) was used. Tween 80 / water solution (1:10) was administered to the negative control group at a dose of 1mL./100g., To the betamethasone positive control group 4mg / Kg and to treatment groups 1 and 2 Manihot esculenta Crantz, 2 mg / kg and 4 mg / kg, respectively. The 1-tail ANOVA test and the Tukey post hoc test were used for comparisons between the groups. **Results:** In 37.67% of treatment group 2 a reduction in edema was observed 3 hours after administering Manihot esculenta Crantz ($p < 0.05$). In both administration groups of Manihot esculenta Crantz there was a nonsignificant trend to reduce plantar edema with values close to significance. **Conclusion:** The Manihot esculenta Crantz ethanolic extract at a 4 mg / kg dose probably have anti-inflammatory activity in this animal model of acute inflamation.

Key words: Manihot esculenta Crantz; Inflammation; Edema (source: MeSH NLM).

RESUMEN

Introducción: Las hojas de Manihot esculenta Crantz (yuca) han sido usadas alrededor del mundo y a lo largo del tiempo con el fin de disminuir la respuesta antiinflamatoria. **Objetivo:** Evaluar la actividad antiinflamatoria del extracto etanólico de la hoja de Manihot esculenta crantz en ratas. **Métodos:** Se realizó un estudio tipo experimental. La población de estudio fueron 60 ratas albinas sp. Rattus novergicus, distribuidas en 4 grupos de 15 ratas cada uno. Se usó una solución homogénea de extracto etanólico de hojas de Manihot esculenta crantz para su administración vía intraperitoneal. Se utilizó carragenina como inductor inflamatorio, que fue administrado por vía intradérmica; para la medición del edema plantar, se hizo uso del Digital Water Plethysmometer (LE7500). Se administró al grupo control negativo solución de tween 80/agua (1:10) a dosis de 1ml/10g., al grupo control positivo betametasona 4mg/Kg y a los grupos de tratamiento 1 y 2 Manihot esculenta crantz, 2 mg/kg y 4 mg/kg, respectivamente. Se utilizó la prueba de ANOVA de 1 cola y la prueba post-hoc de Tukey, para las comparaciones entre los grupos. **Resultados:** En el 37,67% del grupo de tratamiento 2 se observó una reducción del edema a las 3 horas de administrar Manihot esculenta crantz ($p < 0,05$). En otras mediciones se encontró una tendencia no significativa en ambos grupos de administración de Manihot esculenta crantz a la reducción del edema plantar. **Conclusión:** El extracto etanólico de Manihot sculenta a partir de la dosis de 4 mg/kg parece tener actividad antiinflamatoria en la reducción del edema plantar en el modelo animal utilizado.

Palabras clave: Manihot esculenta Crantz; Inflamación; Edema (fuente: DeCS BIREME).

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INTRODUCTION

Inflammation is a protective response of the body to rid it of the injury-causing agent, which can be pathogenic microorganisms, necrotic cells, and even hypoxia. Inflammation can be acute or chronic depending on the nature of the stimulus and the effectiveness of the initial reaction to remove it. The acute phase begins quickly (in minutes) and lasts for a short time, it is characterized by the exudation of fluid and plasma proteins (edema) and the migration of neutrophils. The chronic phase is characterized by the presence of lymphocytes, macrophages, vascular proliferation, fibrosis and tissue destruction. The inflammation ends when the agent responsible for the damage is eliminated[1].

In order to combat inflammation, mainly due to its unwanted effects such as pain, tumor or others, it is common to use nonsteroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids, which are drugs that can cause adverse reactions, in the case of NSAIDs, it produces effects on gastrointestinal, cardiovascular and renal function and regarding to glucocorticoids, it produces effects on endocrine and metabolic, musculoskeletal, immune, gastrointestinal, ophthalmic and central nervous system functions[2].

Because of this, at the present time the treatments seek to reduce the risks potentiating the benefits and within them we find therapeutic alternatives as the natural products, which among these highlights the use of plants as an alternative or aid in treatments. Such is the case of Manihot esculenta crantz (MEC) "YUCA" also called: Mandioca, Tapioca, Shushu, Muk shue, Cassave, Imanoka, Maniba, Kasaba, Katela boodin, Sweet potato tree and Brazilian arrurruz[3].

The MEC, is a woody shrub of the euphorbiaceae family, crotonoideae subfamily and is widely cultivated in tropical and subtropical countries of Africa, Asia and Latin America, with an estimated production of 276.7 million tonnes. It is classified as the fourth most important crop in the world and a basic food for almost a billion people[3,4]. The study will contribute to the investigation of medicinal plants, because our country has a great diversity of them[5].

Although it contains some toxic substances associated with the high concentration of cyancynic glycosides such as linamarin, lotaustralin and lataustraline[3,8], they vary according to location, cultivars, environmental conditions and soil nutrient state4. The plant stores a number of beneficial compounds such as antioxidants, vitamin A, B1, B2, C, iron, zinc, manganese, magnesium, calcium, phosphorus, potassium, zinc, copper, sodium, anthocyanins (flavonoids), saponins, steroids and glycosides. Previous studies have reported that several plants containing saponin and flavonoids have analgesic, anti-inflammatory, antipyretic, antimicrobial, and anticancer effects[4,6-8].

Because of this, it is used in Nigeria for the treatment of ringworm, tumor, conjunctivitis, sores, abscesses, boils, prostatitis, hawthorn, rheumatism, fever, headache, diarrhea, marasmus, loss of appetite and snake bite[3].

Therefore, in the present experimental study, we evaluated the anti-inflammatory activity of the ethanolic extract of Manihot esculenta crantz leaf (EEHMEC) in rats, using validated experimental models for the induction of acute inflammation.

METHODS

An analytical, experimental and prospective study was carried out.

Preparation of the vegetal material: The selected vegetal material, MEC (YUCA), was collected from the central jungle, sending the sample to its respective taxonomic position, according to the Cronquist classification system (1998) carried out at the Museum of Natural History - The National University of San Marcos (UNMSM).

A complete sample of leaves and stems was obtained, they were left to dry at 40 °c in an oven for 3 days and then at room temperature under shade for 3 days. They were crushed manually, grinding was done with the help of a mortar and a roller.

Then, the vegetal material already in powder and under quality conditions of the sample according to the standards established by pharmacopoea, was placed in an alcoholic solution of 96% macerating for 7 days, then filtered, taken to drying for 10 days at room temperature and humidity obtaining the dry extract and prepared a solution using as diluent tween 80/ water (1:10), achieving a homogeneous solution for administration intraperitoneal (IP).

Preparation of the animal material: We used a sample of 60 albino rats sp. Rattus novergicus, aged one and a half months, weighing approximately 150 g. Acquired at the National Center for Biological Products of the National Institute of Health - Ministry of Health (MINSA), randomly distributed into four groups: negative control group, positive control group and two treatment groups, of 15 rats each. They were

kept at room temperature and humidity with free access to air, balanced food and water AD libitum for 8 days for their acclimatization in the Laboratory of Pharmacology of the Faculty of Medicine of the Ricardo Palma University.

Preparation of the pharmacological experimental model: Biological material was administered intraperitoneally to four experimental groups: 1. Negative control group (CN): tween solution 80/ water (1:10) at a dose of 1ml/10g. , 2. Positive control group (CP): betamethasone 4mg/ kg. 3. Treatment group 1 (GT1): EEHMEC solution at a dose of 2 mg/kg and treatment group 2 (GT2) EEHMEC solution at a dose of 4 mg/kg.

The experimental model begins with the taking of basal data such as the measurement of the volume of the right hind leg in the Digital Water Plethysmometer (LE7500) continuing with the administration of solutions to the four experimental groups: CN, CP, GT1 and GT2, after 30 minutes they were performed induction of inflammation. The induction of inflammation was performed by carrageenan-induced plantar edema test, intradermal administration of 0.1 mL carrageenan 1% into the plantar aponeurosis of the right hind leg of the rat.

After the induction of inflammation, the volume of the right hind leg was measured at 30 minutes, 1, 2, 3, 4, 5 and 24 hours, always using the Digital Water Plethysmometer (LE7500) to obtain the results.

The results obtained were placed in a database made in Microsoft Excel 2010 and processed in the statistical program SPSS 22. One-way ANOVA and Tukey's post hoc test were used for comparisons between groups. We worked at a statistical significance of $p<0.05$ and a confidence interval of 95%.

RESULTS

The EEMEC at a dose of 4mg/kg produced a significant reduction ($p<0.05$) of edema at 3 hours by 37.67% compared to the negative control, and the positive control also produced a significant reduction ($p<0.05$) of edema at 4 and 5 hours of administration. There were no statistically significant differences in the remaining hours with respect to the negative control, as shown in table 1.

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Table 1. Differences in the increase of carrageenan-induced plantar edema according to the administration of different treatments.

Treatment	Dose (mg/ kg)	Time (h)						
		0,5	1	2	3	4	5	24
Negative Control: physiological serum	10 ml/ kg	0.25 ± 0.11	0.34 ± 0.25	0.90 ± 0.21	1.46 ± 0.26	1.22 ± 0.42	1.16 ± 0.37	0.66 ± 0.33
Positive control: betamethasone	4 mg/kg	0.38 ± 0.23 (-52%)**	0.42 ± 0.17 (-23.52%)	0.53 ± 0.24 (41.11%)	0.96 ± 0.25 (34.24%)	0.62 ± 0.22* (49.18%)	0.61 ± 0.22* (47.41%)	0.38 ± 0.19 (42.42%)
Treatment: solution of the alcoholic extract of Manihot esculenta crantz	2 mg/kg	0.20 ± 0.28 (20%)	0.49 ± 0.32 (-44.11%)	1.00 ± 0.58 (-11.11%)	0.97 ± 0.42 (33.56%)	0.90 ± 0.36 (26.22%)	1.02 ± 0.34 (12.06%)	0.49 ± 0.29 (25.75%)
	4 mg/kg	0.13 ± 0.19 (48%)	0.55 ± 0.54 (-61.76%)	0.84 ± 0.44 (6.66%)	0.91 ± 0.51* (37.67%)	0.81 ± 0.48 (33.60%)	0.79 ± 0.45 (31.89%)	0.42 ± 0.34 (36.36%)

*The reduction of edema is statistically significant with $p< 0.05$ compared to the control.
**The values in brackets are the calculated percentage of edema inhibition relative to the control.

There is also a tendency to reduce plantar edema with a dose of 4 mg/kg, with values close to significance not only at 3 hours of administration.

DISCUSSION

The results of this study indicate that EEHMEC administered intraperitoneally has significant anti-inflammatory effects at 3 hours of administration being these of short duration. These results were similar to those found by Bokanishow et al. who administered three different doses (100, 250 and 500 mg/kg) EEHMEC orally to rats. Compared with the negative control (physiological serum) found a significant decrease ($p<0.0001$) of plantar edema 5 hours after its administration. The 100 mg/kg dose caused a greater reduction in edema (26.2%) than the other doses and even the positive control (indomethacin). However, there was no significant therapeutic response in the previous hours. Our results show differences with the results found by Bokanimine et al., since the reduction of plantar edema was observed at 3 hours being of short duration[9].

These results are also in agreement with those reported by O. O. Adeyemi et al., who used an aqueous extract of Manihot sculenta leaves at three different doses (100, 200 and 400 mg/kg) orally to rats and used a white thread as an instrument to measure the diameter of the plantar edema in rats. They showed a significant reduction ($p<0.05$) of edema compared to the negative control (physiological serum) from 2 to 24 hours after the application of carrageenan. The maximum therapeutic response was obtained at the highest dose (400 mg/kg) and was higher than that of the positive control (indomethacin). Compared to our research, the anti-inflammatory results differ in latency period and duration, this is attributable to the type of extract but at the same time it is contradictory because it is an aqueous extract compared to ethanolyc extract[10].

In contrast to these previous investigations in which an aqueous extract was used, an ethanolic extract was prepared, because improper processing of MEC leaves leads to high concentrations of cyanide and great loss of protein and vitamin content. In the preparation of an aqueous extract, the leaves of MEC are boiled in water, which eliminates all cyangens, but leads to large losses of proteins, vitamins C, thiamine, riboflavin and nicotinic acid, which are substances that probably play a fundamental role in the therapeutic activity of the extract. In this work, the leaves were crushed so that the enzymes could come into contact and catalyze the hydrolysis of the cyanins, in addition to giving them a rest period of 72 hours at 40°C to allow the enzyme linamirase to hydrolyze linamarin to acetone cyanhydrine and hydroxynitriloliase to hydrolyze cyanhydrine from acetone to hydrogen cyanide, which escapes as a gas. In the preparation of the ethanolic extract, the leaves of MEC were treated rigorously, however, the results obtained with this extract did not achieve the anti-inflammatory effect evidenced in the investigations of Bokaniemi et al. and O. O. Adeyemi et al.[9-12].

The intraperitoneal route of administration was used because it allows rapid and significant absorption of drugs because the peritoneal cavity has a lymphatic circulation that drains directly into both the portal and systemic circulation[13]. Carrageenan is also an inducer of local acute inflammation. Therefore, the maximum anti-inflammatory effect observed in the study was on the late phase of this inflammation, which is characterized by its dependence on calcium and nitric oxide synths[14]. This is why the probable mechanism of action by which the leaves of the ethanolic extract Manihot esculenta crantz have produced their anti-inflammatory effect was mediated by the inhibition in the formation of eicosanoids by acting as an inhibitor of the enzyme nitric oxide synthase during the late phase of acute inflammation by the results obtained in our experimental model as in previous research.

As limitation in this study is the possibility of random error and the consequent risk of spurious associations due to the effect of making multiple comparisons, so studies in other animal models are necessary in order to corroborate our findings.

CONCLUSION

We conclude that the ethanol extract of Manihot esculenta crantz from the dose of 4 mg/kg shows an effect on acute inflammation by reducing plantar edema significantly by 37.67%. Further studies on this topic are recommended, using models of chronic inflammation and higher doses of this plant.

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