Pharmacobotany, Phytochemical Analysis and Anti-inflammatory effect of the Ethanolic Extract of Luffa operculata

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ABSTRACT

Background: Luffa operculata is a vegetable species well known in the traditional peruvian medicine for its many medicinal properties and cosmetic applications. Objective: The aim objective was to determine the pharmacognostic characteristics of L. operculata as well as observing the pharmacological effect, of the ethanol extract of L. operculata fruit on chronic inflammation in rats. Materials and Method: Phytochemical analysis was carried out by using specific chemical reagents for each constituent chemical, the pharmacobotanical study was done with a histological tinction (fruit, steam and leaves), which were stained with Safranin 1% and Toluidine blue 1%; the chronic inflammation was assessed by air bag method in Holztman male rats. Doses of 100, 250 and 500 mg/Kg were tested in order to determine the anti-inflammatory effect, which was demonstrated with histopathological evaluation and lymphocytes reduction. Results: The main findings indicate that the ethanolic extract presented saponins, alkaloids, carbohydrates, terpenes and steroids. The efficiency of lymphocyte reduction per field in the histopathological study of the granuloma was 58.4% with the middle dose of 250 mg/kg (p < 0.0001), which gives a dose-independent anti-inflammatory effect in rats. Conclusion: Luffa operculata presented anti-inflammatory effect at 250 mg/Kg by oral administration in a chronic experimental model of inflammation in rats.

Key words: Pharmacobotany, Saponins, Luffa operculata, Anti-inflammatory.

INTRODUCTION

Nature medicine plays a crucial role in the prevention and treatment of several pathologies (acute and chronic diseases). As of medicinal plants are obtained numerous bioactive phytochemicals, which could be studied as promising drugs passing by preclinical and clinical steps. These compounds generally are tested based on its traditional uses known as ethnopharmacology. Being necessary validate this information, pharmacological studies are carried out in order to give information scientific to population.

Inflammatory process is produced as a response to cell injury and against pathological microorganism (parasites, bacteria, fungi, virus, etc), trauma, or toxins participating as a critical host-defense mechanism. Additionally, acute inflammatory may lead to a chronic inflammation, which carry out a subsequent event since tissue destruction until fibrosis, and necrosis. Several kinds of cells are involved releasing inflammatory mediators such as prostanoids, histamine, nitric oxide, interleukins among other. Otherwise, in acute inflammation, neutrophils are the major cell types whilst mononuclear cells (mostly macrophages, lymphocytes and plasma cells) are involved in chronic inflammation.

Luffa operculata belongs to Cucurbitaceae family, which is a medicinal plant with different popular names: in Brazil buchinha-do-norte or cabacinha, or toxins participating as a critical host-defense activator of transcription 3). At present, in Peru no more scientific information has been found on Luffa operculata. For this reason, this research seeks to determine the pharmacological and phytochemical characteristics of the ethanol extract of the Luffa operculata Cogn fruit. (field soap) as well as observing the effect on chronic inflammation in rats.

MATERIALS AND METHODS

Plant material

Luffa operculata plant was collected, in January 2019 from San José Bajo, Santiago de Cao district,
Ascope Province, La Libertad Department, Peru. The material plant was classified (043-USM-2019) at the National Herbarium of the Universidad Nacional Mayor de San Marcos (UNMSM), Lima, Peru.

**Animals**

An integral of 36 male Holtzman rats (110 ± 20 g) were purchased from Biotério of the Center of Biological products of the National Institute of Health ( Lima, Peru). Animals were acclimatized at room temperature during 15 days before the experiments, they were kept in plastic cages with free access to pelleted food and water with 12 h light/dark cycle. Rats were randomized into six groups of six animals per plastic cages.

**Chemicals**

Carrageenan was purchased from Sigma-Aldrich, USA, ethylenediaminetetraacetic acid, toluidine, safranin, ethanol absolute, glacial acetic acid and trichloro acetic acid (TCA) were obtained of local chemical companies.

**Pharmacobotanical study**

Stems, leaves, flowers and fruits were fixed in FAA (formaldehyde, ethyl alcohol, glacial acetic acid, 10: 50: 5: 35). The samples were processed, at the level of the middle parts of the stem, leaf, female flower and fruit, with the following techniques: transverse and longitudinal freehand cuts of the stem, leaves, flower and fruits, as well as superficial cuts of leaves; all histological sections were rinsed in 50% sodium hypochlorite, washed and then stained in 1% toluidine blue and 1% cresyl violet stains, with mounting in carboxy-glycerin. Photomicrographs at 40, 100 and 400 magnification on a Leica DM50 microscope with built-in camera and the use of Lasez ® software. The final images were processed with Adobe Photoshop CS3 10.0.

**Extraction of plant material**

Fruits of *Luffa operculata* (500 g) were cleaned, selected and dried at 40°C, then were pulverized and soaked with 96 % ethanol for 7 days. The liquid extract was filtered with a filter paper Whatman N° 1 and evaporated using a rotavap. The crude ethanolic extract obtained (10 g) was stored until further real study in an amber flask at 4°C.

**Phytochemical screening**

Chemical constituents were confirmed by chemical reaction with specific reagents for each metabolite such as: sterols, terpenoids, alkaloids, carbohydrates, flavonoids, tannins, phenols, glycosides, saponins according to the validated methodology.

**Antinflammatory effect in an experimental model of chronic inflammation**

**Experimental design**

For this, chronic inflammation was induced by the experimental model of Sedwick *et al.* or also known as the air bag method in rats. The animals were acclimatized for a week, between 21 and 25 ºC and 50% humidity, with 12 hours of light and darkness. On the first day the skin was shaved, on the second day 20 mL of air was injected subcutaneously into the intracapsular area of the loin, forming an oval air pocket, on the fourth day 10 mL of air was added, on the fifth day the animals were randomly distributed in 6 groups of 6 rats and treated orally: 1) Physiological saline (PS) 2 mL / kg; 2) Carrageenan (C) + PS 2 mL / kg; 3) C + Dexamethasone 4 mg / kg; 4, 5 and 6) C + Extract 100, 250 and 500 mg / kg respectively. Three hours later, 2 mL of 1% carrageenan dissolved in saline solution was injected, directly in the bag; the animals were sacrificed after 24 hours using 100 mg / kg pentobarbital. An injection of 5 mL of saline solution containing 0.1% ethylenediaminetetraacetic acid (EDTA) was applied to the air bag and then a small incision was made in the wall of the bag, and the contents of the air bag were removed, carefully using a sterile Pasteur pipette. 3 mL of exudate was obtained, to which the total proteins were measured, and a histopathological study of the carrageenan-induced granuloma was carried out.

**Histological analysis**

The histopathological evaluation, according to Devi, was carried out with pieces of skin containing the granulomas, which were gently washed with saline solution, to remove the blood and the debris adhered to the tissue, being fixed in a buffered 10% formaldehyde solution; later, 3 μ thick sections were made with the help of a microtome, and stained with hematoxylin and eosin; After dehydration and cleaning of the slides, microscopic observation was made: qualifying inflammatory infiltrate (absent, mild, moderate, severe); number and percentage of lymphocytes. The slides were examined under a light microscope (Olympus BX51).

**Statistical analysis**

Statistical analysis was carried out using SPSS v. 21. Normality test by the Shapiro-Wilk and one-way analysis of variance (ANOVA) followed by Tukey multiple comparison. A P-value of 0.05 was considered statistically significant. Experimental data are presented as mean ± standard deviation (SD).

**Ethical considerations**

All experimental procedures were considered following international guidelines (CCE Council 86/609) and approved by the ethical committee of the Universidad Nacional Mayor de San Marcos (01414-R-12-UNMSM), animals were euthanized by intravenous injection of pentobarbital (100 mg/kg).

**RESULTS**

**Description of the internal tissues of the flower, fruit and seed**

The female flower is largely pedunculed, with an inferior ovary. In cross section it is shown tetratocular with divided locules and parietal ovules. Abundant pubescence of elongated multicellular trichomes and presence of strongly pedunculated glandular trichomes.

**Determination of phytochemical constituents**

In table 1 is showed the main chemical groups identified by precipitation and color change in the ethanol extract of *Luffa operculata*.

**Table 1: Qualitative phytochemical screening for *Luffa operculata***

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Constituents</th>
<th>Tests Performed</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Terpenoids</td>
<td>Salkowskis Test</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Mayer’s Test</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrates</td>
<td>Molisch’s Test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoids</td>
<td>Shinoda’s Test</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>Gelatin Test</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Phenols</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Saponin</td>
<td>Foam Test</td>
<td>+</td>
</tr>
</tbody>
</table>
Figure 1: Internal structure of the *Luffa operculata* flower. A: detail of the female flower, B and C: general view of the ovary in cross section, D: detail of locules and ovules in the ovary, E: Pubescent epidermis of the ovary with elongated multicellular (tr) and glandular (Tg) trichomes, E: Trichome (Tr). Cuticle (Cu). A. bar 1 cm, B and C. 100x, D and E. 400x.

Figure 2: Internal structure of the fruit and seed of *Luffa operculata*. A: General view of the fruit. B: panoramic view of the external part of the fruit. C: detail of the thalamus and epicarp of the fruit. D: general view of the seed (without the embryo). E: detail of the inner seed coat. Pe, peduncle; Ep, epidermis; Escl, sclereids; Esc, sclerenchyma; Pa, parenchyma; Hv, vascular bundle. A. bar 1 cm, B and D. 100 X. C and E. 400 X.

Figure 3: Histopathological analysis in the test of granuloma induced by Carrageenan on the skin of the rat. (10X). Inflammatory infiltrate (absent, mild, moderate, severe) - number and percentage of lymphocytes: A:PS 2 mL/Kg (Absent – 8%), B: Carrageenan +PS (Moderate to severe – 40%), C: Carrageenan + Dexamethasone 4 mg/kg (mild – 13%), D: Carrageenan + Extract LO 100 mg /Kg (Moderate – 39%), E: Extract LO 250 mg /Kg (Mild – 22%), F: Extract LO 500 mg /Kg (Moderate – 38%).
Histopathological evaluation in the anti-inflammatory effect of *L. operculata*

As shown in Figures 3 and 4, there was a reduction in the inflammatory infiltrate of the chronic model in rats induced by air bag. Percentage of lymphocytes was higher in the group treated with *L. operculata* at 250 mg/Kg by oral administration. However, animals treated with dexamethasone at 4 mg/Kg had the highest reduction compared with the different treatments carried out with the ethanol extract.

**DISCUSSION**

The Cucurbitaceae family makes up an important group of plant species, mostly developing in tropical climates, with approximately 130 genera and 1,300 species, the vast majority is used for food and medicinal purposes. In Peru, around 27 genera and 110 have been reported. Species and some of them endemics. *L. operculata* is a fruiting and useful vine, with angular branches that can be up to 3 meters high. Its broad, kidney- or heart-shaped leaves have three to five lobes and are between 7.5 and 10 millimeters long. Its fruit has a tuber-like shape, ovoid to ovoid-oblong, pulpy, fibrous and with small flat, dark brown seeds that are between 10 to 12 cm long. Its interior of the fruit is yellow to pale yellow, the lobes of the staminate flowers are close to the ovary, less than 10 centimeters long. The interior of the fruit is a remedy for urethritis and edema. On the other hand, several compounds were isolated of *L. operculata*, which could be responsible for the anti-inflammatory effect such as Opercurins A and B as well as Neocucurbitins A and B present in the methanol extract. Although, we did not isolate the phytocomponents, the phytochemical screening revealed the presence mainly of steroidal saponins but without any reaction to flavonoids.

Inflammation, like the host's response to deleterious stimuli, is mediated by pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6, which are secreted by immune cells recruited to injury sites. Sustained release of inflammatory cytokines and reaction result in chronic inflammation, which is characterized by excessive infiltration of immune cells and is the main cause of many diseases. The mRNA levels of TNF-α, IL-1β and IL-6 can also be suppressed in mice and rat model of multiple diseases by saponins like dioscin, and IL-1 and IL-6 can also be suppressed in mice and rat model of multiple diseases by saponins like dioscin, such as acute liver injury, liver fibrosis, obesity, brain and intestinal I / R injury and inflammatory lesions of kidney, thus attenuating the inflammatory damage, and these histopathological changes have been also showed with the oral treatment of *Lufia operculata* evidenced in Figures 3 and 4, due to its steroid saponin content.

Saponins found in *L. operculata* have been used to understand the mechanism of various biological activities such as dysregulates melatonin and pro-inflammatory cytokines, due to the presence of cucurbitacins. In other study the relationship between the chemical structure and pharmacological activity is strongly linked, for example the activity of propanaxatriol-type saponins from Panax notoginseng is based on the number, length and position of sugar side chains, and the type of glucosyl group in the structure of the molecule affecting its hemolytic activities and adjuvant potentials but have significant effects on the nature of the immune responses. Saponins from *Bupleurum rotundifolium* were active in chronic inflammation, reducing efficiently ear weight and neutrophil influx.

In Brazil, the aqueous extract of *L. operculata* fruit has been used as a remedy for urethritis and edema. On the other hand, several compounds were isolated of *L. operculata*, which could be responsible for the anti-inflammatory effect such as Opercurins A and B as well as Neocucurbitins A and B present in the methanol extract. Although, we did not isolate the phytocomponents, the phytochemical screening revealed the presence mainly of steroidal saponins but without any reaction to flavonoids.

**CONCLUSION**

The ethanolic extract of *L. operculata* showed chronic anti-inflammatory effect against granuloma model induced in rats. Histopathological evaluation showed a reduction of lymphocytes at 250 mg/Kg and saponins present in the ethanol extract could be the bioactive metabolites responsible of the pharmacological effect.

**CONFLICTS OF INTEREST**

The authors have no conflicts of interest.

**REFERENCES**


Arroyo-Sandoval J, et al.: Pharmacobotany, Phytochemical Analysis and Anti-inflammatory effect of the Ethanolic Extract of *Luffa operculata*

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