SYSTEMIC AND LOCAL ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS LEAF EXTRACT FROM JATROPHA GOSSEPIFOLIA L. (EUPHORBIACEAE)

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ABSTRACT

Objective: Jatropha gossypiifolia L. (Euphorbiaceae), popularly known in Brazil as “pinhão-roxo”, is a medicinal plant largely used in folk medicine as an anti-inflammatory, healing, and antihypertensive drug. It is included in the National List of Medicinal Plants of Interest to Brazilian Public Health System (RENISUS) due to its recognized therapeutic potential. The plant is frequently medicinally used by infusion or decoction. However, phytochemical and pharmacological studies conducted with aqueous extracts are poorly described to the species. The aim of this study was to evaluate the anti-inflammatory activity of the aqueous leaf extract of J. gossypiifolia and characterize its phytochemical constitution.

Methods: A phytochemical screening of the aqueous leaf extract prepared by infusion was performed through chemical reactions and thin layer chromatography (TLC). Systemic and local anti-inflammatory activity (50-200 mg/kg, orally, and 1-5% w/w lipogels, topically) was evaluated in carrageenan-induced paw edema model in rats.

Results: Alkaloids, flavonoids, gums, resins, and sulfur compounds were detected by chemical reactions. TLC analysis suggests that flavonoids could be the major compounds in the extract. In both routes, the extract was effective in reducing paw edema induced by carrageenan.

Conclusion: These results suggest the potential of the aqueous leaf extract of J. gossypiifolia as a source of anti-inflammatory herbal drugs and/or molecules, and seem to justify part of its main popular uses in traditional medicine.

Keywords: Jatropha gossypiifolia, Anti-inflammatory activity, Rat paw edema, Medicinal plants, Pluronic®/ Soybean oil based lipogel

INTRODUCTION

Jatropha gossypiifolia L. is a medicinal plant belonging to Euphorbiaceae popularly known in Brazil as “pinhão-roxo” or worldwide as “ballyache-bush”. This species is widely distributed in countries of tropical, subtropical and dry tropical weather and tropical semi-arid regions of Africa and the Americas [1]. In Brazil, it predominates in the Amazon, Caatinga and Atlantic Forest and is distributed throughout the country in the North, Northeast, Midwest, South and Southeast regions [2]. Several human and veterinary uses in traditional medicine are described for different parts (leaves, stems, roots, seeds and latex) and preparations (infusion, decoction, maceration, among others) based on this plant, by oral or topical use. The most frequent reports regards to its antihypertensive, anti-inflammatory, antiphidoidan, analgesic, antipyretic, antimicrobial, healing, haemostatic, anti-anemic, antioxidant, anti-hemorrhagic, among many other examples [1, 3, 4]. As an anti-inflammatory drug, the plant is commonly used in traditional medicine topically, such as baths and/or dressings or by oral route as tea [5]. However, despite the wide popular use, there are relatively few phytochemical and pharmacological studies with this vegetal species, especially concerning aqueous extracts from its leaves. This is important to be mentioned since the major form of use of the extract is as tea, that is, in fact, an aqueous extract. Regarding its phytochemical constitution, alkaloids, coumarins, flavonoids, lignoids, phenols, saponins, steroids, tannins and terpenoids were already detected in different extracts from different parts of this plant [6]. Among the main activities already studied for this species (including various types of extracts from different parts of the plant), mainly stand out the antihypertensive, antimicrobial, antioxidant, antineoplastic, among others, supporting some of its popular uses [4, 7]. An important feature of J. gossypiifolia species is that, due its important potential medicinal applications, it is included in the National List of Medicinal Plants of Interest to Brazilian Public Health System (Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde Brasileiro – RENISUS), that is a relation published by the Brazilian Health Ministry in February 2009 that includes 71 species of medicinal plants that have the potential to generate pharmaceutical products of interest in Brazilian public health [8]. Therefore, this study was carried out aiming to evaluate the systemic and local anti-inflammatory activity of the aqueous leaf extract of J. gossypiifolia and characterize preliminary its phytochemical constitution.

MATERIALS AND METHODS

Plant material

Leaves of Jatropha gossypiifolia L. (Euphorbiaceae) were collected in Natal (city of Rio Grande do Norte State, Brazil), in October 2009, at coordinates 5.822715°S, 35.257307°W, and identified by MsC. Alan de Araújo Roveg. A voucher specimen was deposited in the Herbarium "Parque das Dunas" (Bioscience Center of Federal University of Rio Grande do Norte) (number of deposit: UFRN 12561). The collection of the plant material was conducted under authorization of Brazilian Authorization and Biodiversity Information System (SISBIO) (process number 35017) and Brazilian Access Authorization and Dispatch Component of Genetic Patrimony (Process 010844/2013-9). The leaves were dried at room temperature, triturated and stored in hermetically sealed bottles away from light and humidity until use for extract preparation.

Extract preparation

Dried leaves were submitted to infusion (10% w/v, plant: solvent) for 15 min at temperature around 95°C to obtain the aqueous leaf extract of J. gossypiifolia (yield: 7.35% relative to dry plant). For in vivo experiments, the extract was freeze-dried and dissolved in water at adequate concentrations for each dose.
Lipogels preparation

Pluronic® (poloxamer) and soybean oil based lipogels were prepared for indomethacin and extract veiculation, as follows. Ethanol (10% v/v) and soybean oil (2.5% v/v) was mixed under sonication for 5 min, producing the “A phase”. Drug or freeze-dried extract was incorporated in 10% (w/v) polyethylene glycol and mixed with “A phase”, until perfect homogenization, under sonication for 5 min, producing the “B phase”. 1% (w/v) hydroxypropylmethylcellulose was added to “B phase”, under sonication for 5 min, producing the “C phase”. In another beaker, 20% (w/v) Pluronic® was dispersed in cold water, under vigorous agitation and ice bath, producing the “D phase”. Finally, “C” and “D phases” were mixed under frequent agitation until gel formation, followed by 5 min sonication to produce the lipogels.

Phytochemical analysis

The extract was characterized by phytochemical screening reactions [9] and thin layer chromatography (TLC) [10] in order to obtain its qualitative profile. Chemical reactions were conducted to investigate the presence of the main secondary metabolites (alkaloids, carotenoids, coumarins, flavonoids, gums, lactones, phenols, quinines, resins, saponins, steroids and/or terpenoids, sulfur compounds and tannins). The results were observed by the formation of precipitate and/or color development, according to the specific class reaction [9]. In TLC analysis, aluminum pre-coated sheets with silica gel F254 (Merck®) as adsorbent was used. The following mobile phases were employed: (1) ethyl acetate: formic acid: water (8:1:1, v/v/v), (2) ethyl acetate: formic acid: acetic acid: water (7:0.5:0.5, v/v/v/v), (3) chloroform: glacial acetic acid: methanol: water (4:3:2:12.8, v/v/v/v), (4) ethyl acetate: formic acid: water (90:0.5:0.5, v/v/v) and (5) chloroform: water (7:2.5:0.5, v/v/v). The chromatograms were analyzed under 254 and 365 nm UV light and then sprayed with specific chromogenic agents according to the class of compounds investigated (sulfuric vanillin + heating, natural reagent A, ferric chloride, Dragendorff reagent and Liebermann-Burchard). The retention factors (Rf) color and behavior of the spots were recorded for further comparison with chromatographic profiles of reference substances in the specialized literature in the area [10]. Standard samples (Sigma® Aldrich) of flavonoids (isoorientin, isouquer cetin, isovitexin, orientin, rutin and vitexin) were employed.

Animals

Male albino Wistar rats [150-200 g, 6-8 weeks-old], maintained under standard environmental conditions and fed with standard food and water ad libitum were used. All the procedures requiring animals were performed in agreement with institutional and international guidelines of animal care and were approved by the Ethics Committee on Animal Use from Federal University of Rio Grande do Norte (protocol 028/2009). On the day of the experiment, the animals were placed in the experimental room with at least one hour prior to tests for acclimation. At the end of the experiments, the animals were euthanized by sodium thiopental overdose by intraperitoneal route.

Evaluation of systemic anti-inflammatory activity

The systemic activity of extract was evaluated against carrageenan-induced paw edema in rats [11]. Groups of two hours fasted animals (n=5/group) were treated orally (p.o.) with 0.9% saline solution (10 mL/kg), indomethacin (10 mg/kg) or extract (50, 100 and 200 mg/kg) one hour before intraplantar injection of 100 µL of 1% kappa-carrageenan (Sigma® Aldrich) at the right paw of each animal (1 mg/paw). 100 µL of 0.9% saline solution were injected at the same time in the left paw, as a control. Increase in paw thickness was measured with a digital caliper for a period of four hours after inflammation induction. Edema was expressed as percentage of the difference between the left and the right paw thickness.

Evaluation of local anti-inflammatory activity

The local activity of the extract was evaluated against carrageenan-induced paw edema in rats, according to described above, with some modifications. Groups of animals (n=5/group) were treated topically with 100 mg of lipogels without drug or extract (blank), indomethacin (2% w/w) or extract (1; 2, and 5% w/w) one hour before inflammation induction and after reapplied periodically by standardized fifty movements of friction, hourly. Increase in paw edema was measured as described above.

Statistical analysis

One-way ANOVA with Dunnett’s post test was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. p values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The phytochemical screening of aqueous leaf extract of Jatropha gossypifolia revealed the presence of alkaloids, flavonoids, gums, phenols, resins, saponins, and sulfur compounds, as could be observed in Table 1. The chromatographic analysis by TLC suggests, preliminarily, that flavonoids could be probably the major compounds in the extract, judging by the size and intensity of spots after the use of the specific reagent (Natural A Reagent) and UV light 365 nm, using the mobile phase ethyl acetate: formic acid: water, 8:1:1, v/v/v. Through the TLC analysis, spots with similar color and retention factor (Rf) to the standards vitexin (green fluorescent, Rf=0.73), orientin (yellow fluorescent, Rf=0.52) and isoorientin (yellow fluorescent, Rf=0.41) were observed in the extract. The presence of flavonoids-C-glycosides, including the ones identified in the present study, was early reported in J. gossypifolia leaves [12, 13]. This is an important finding, since many flavonoids are related to the anti-inflammatory activity of various plants [14, 15].

Table 1: Phytochemical screening of aqueous leaf extract of jatropha gossypifolia

<table>
<thead>
<tr>
<th>Test</th>
<th>Result*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Bouchardt</td>
<td>+</td>
</tr>
<tr>
<td>Dragendorff</td>
<td>+++</td>
</tr>
<tr>
<td>Mayer</td>
<td>++</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Gums</td>
<td>+</td>
</tr>
<tr>
<td>Lactones</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+++</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids and/or terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Sulfur compounds</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
</tbody>
</table>

*Strong presence: ++++, moderate presence: ++, trace: +, negative result: -
The rat paw edema induced by injection of different inflammatory agents is one of the most used models for anti-inflammatory activity evaluation. Among the most related inflammatory inducers in literature, the carrageenan, a complex group of polysaccharides made up of repeating galactose-related monomers, stands out [16, 17]. The mechanism of action is still unknown, but, at least partially, an action on eicosanoids mediators, considering the inflammatory mechanism of carrageenan, could be suggested. Therefore, these results indicate the potential of the aqueous leaf extract of J. gossypiifolia as a source of new anti-inflammatory herbal drug and/or molecules, and seem to justify part of its main popular uses in traditional medicine.

CONCLUSION

The results shown in this work suggest the anti-inflammatory potential of the aqueous leaf extract of the vegetal species Jatropha gossypiifolia. The mechanism of action is still unknown, but, at least partially, an action on eicosanoids mediators, considering the inflammatory mechanism of carrageenan, could be suggested. Therefore, these results indicate the potential of the aqueous leaf extract of J. gossypiifolia as a source of new anti-inflammatory herbal drug and/or molecules, and seem to justify part of its main popular uses in traditional medicine.

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REFERENCES


Table 1: Inhibition* percentage of aqueous leaf extract of Jatropha gossypiifolia on carrageenan-induced paw edema in rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Inhibition (%)</th>
<th>Dose (% w/w lipogels)</th>
<th>Local evaluation</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>16.98</td>
<td>1</td>
<td>5</td>
<td>37.79</td>
</tr>
<tr>
<td>100</td>
<td>55.29</td>
<td>2.5</td>
<td>25</td>
<td>54.10</td>
</tr>
<tr>
<td>200</td>
<td>51.47</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Inhibition percentage calculated based on values of area under curve after four hours of experiment (AUC) of each group (control and treated with extract): [(AUCcontrol − AUCtreated) ÷ AUCcontrol] × 100.


