Lecithin-based organogel for an industrialized butter from *Platonia insignis* Mart. seeds and its anti-inflammatory potential: Formulation and preclinical studies

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**Abstract:** *Platonia insignis* Mart. (Clusiaceae) is a medicinal plant from Brazilian Amazon rainforest. In traditional medical practices, seeds oil extracted from “bacuri” is used in the treatment of wounds, eczema, herpes or other skin diseases. In view of the therapeutic potential of seeds butter from “bacuri” (BBI), the present study aims to propose a topical formulation using an industrially obtained BBI (F-BBI) and its anti-inflammatory pre-clinical assessment in the carrageenan-induced paw edema in rats. The results of this study suggest that BBI are able to act in different phases of inflammatory response depending on the administration route. The proposed F-BBI are able to act in all phases of inflammatory response. This report suggests a new application for *Platonia insignis* seeds extract as a promising topical anti-inflammatory agent, and encourage further studies in order to elucidate mechanisms involved in this effect.

**Keywords:** Bacuri; Clusiaceae; Edema; Formulation; Inflammation.

1. Introduction

Medicinal plants have represented an important source for the discovery of new therapeutic agents for human and veterinary diseases. Many studies have investigated the promising use of plants for health purposes, considering most people have broad experience in the use of traditional plant-derived products (drug and supplements). In this context, extensive studies have been performed to discover new natural compounds having anti-inflammatory activities. Inflammation is the most common aspect of tissue pathology which often leads to chronic diseases and has always taken a central role in medical practice [1], [2].

In recent years, a number of publications from natural resources such as medicinal plants have grown due to the search for alternative methods of treatment of tropical diseases and inflammation [3]–[5]. Diseases associated with chronic inflammatory pathology has been the most deaths worldwide. The huge number of deaths are associated with symptoms that are not perceived since the onset of the disease. Most anti-inflammatory drugs are ineffective in protecting the long time these diseases because they can interfere in different systemic pathway [4]. Recent studies with several phytochemicals have shown that isolated compounds are able to induce anti-inflammatory activity by inhibiting nuclear factor kappa B (NF-kB), activator protein (AP-1), signal transducers and activators of transcription (STAT) [4], [6]. In this sense, numerous plant species have been shown as sources of bioactive compounds which may act as alternative method to conventional treatments. These alternatives aim to reduce side effects and toxicity, as well as to reduce the cost of medicines [5], [7].

*Platonia insignis* Mart. (Clusiaceae) is a medicinal plant in northern Brazil. Products derived from this plant have shown hypotensive effects [8] and antiparasitic against helminths [9]. The fruits are known locally
as "bacuri" and have significant commercial relevance [10]. In popular culture, seeds butter from "bacuri" is manufactured by the population from the oil extracted from the seeds containing around 65% fatty acids and derived compounds. It is used indiscriminately in folk medicine for its healing action and anti-inflammatory used in wounds, treatment eczema, herpes or other skin diseases, especially in Latin American countries [8], [10], [11]. The oil obtained from the seeds of fruit of P. insignis has great potential for being used in the popular medicine as an anti-inflammatory agent and cicatrizing [12].

In view of the therapeutic potential of seeds butter from "bacuri" (BBI), the present study aims to propose a topical formulation using an industrially obtained BBI and its anti-inflammatory pre-clinical assessment in the carrageenan-induced paw edema in rats.

2. Materials and Methods

2.1. Preparation and quality control of topical formulation

The seeds butter from "bacuri" (Platonia insignis Mart.) was purchased from Amazon Oil Industry and Trade Company Ltd. (Ananindeua, PA, Brazil; Lot. MBA-006/10). The matrix formulation was prepared by using: granulated lecithin, isopropyl palmitate, sorbic acid, Poloxamer 407, Potassium Sorbate and distilled water. The oil phase was prepared containing granulated lecithin and isopropyl palmitate (1:1) with 20% poloxamer 407.

Then, two topical formulations were prepared: 1) 5.0% BBI in matrix formulation, called “F-BBI”; and 2) only matrix formulation without BBI, called “Vehicle”. In F-BBI, butter bacuri was mixed the oil phase in a water bath and stirred continuously. For the formation of organogel was added to the aqueous gel poloxamer. The formulation was kept under constant agitation for 30 min using a mechanical stirrer Ultra Turrax at 8000 rpm.

The formulations were submitted to the following stability tests: 24-hour cycles at 45 ± 2°C and 24 hours at -5 ± 2°C for 12 days; 90 days temperature 37 ± 2°C with exposure to light radiation. After the end of the process, the samples were evaluated by the parameters: appearance and homogeneity, pH, spreadability, and viscosity [22]-[24]. The results were analyzed by unpaired t test and expressed as mean ± SD. The analysis of significance was considered for values p<0.05. All analyses were performed using GraphPad™ Prism software, version 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

2.2. Evaluation of anti-inflammatory effect after oral BBI or topical F-BBI in carrageenan-induced paw edema in rats

Female Wistar rats (200-250 g) were kept under controlled conditions (24 ± 1°C, 12-h light/dark cycle) with food and water ad libitum. They were fasted for 18 h and then acclimatized to the test environment for 2 h prior to each experiment. Following experimental procedures, animals were euthanized by sodium thiopental (100 mg/kg, i.p.). All experimental protocols were approved by the Ethics Committee for Animal Research at the Federal University of Piauí (CEEA-UFPF 044/10).

Two different sets of experiments were performed for assessment of anti-inflammatory effects of BBI and F-BBI administered by oral and topical routes, respectively. For BBI, the animals were orally treated with BBI (50, 100, 200 or 400 mg/kg), vehicle, or indomethacin (5 mg/kg). For F-BBI, left hind paws were topically treated with F-BBI, vehicle or 0.1% topical dexamethasone at proportion of 1 mg/g of formulation/body weight). The formation of edema was measured after intraplantar administration of carrageenan (1%, 0.1 mL/paw, i.pl.) into left hind paws of rats every one hour for 6 hours. The difference in paw volume at the end time (Tf) and basal time (Ti) was determined for each animal using a pachymeter [25]. The results were analyzed by analysis of variance (ANOVA) followed by Tukey’s test and expressed as mean ± SEM. The analysis of significance was considered for values p<0.05. All analyses were performed using GraphPad™ Prism software, version 5.0 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results and Discussion

The present study is the first report of anti-inflammatory effect of seeds butter from P. insignis (BBI), an economically important edible species from Brazilian Amazon rainforest. Furthermore, the proposed lecithin-based formulation was able to improve BBI-induced anti-inflammatory effect similarly to topical 0.1% dexamethasone, a commercial anti-inflammatory medicine. Concerning the development of the F-BBI formulation, as show in Table 1, it was produced in bench scale batch and shown to be reproducible. In the
initial screening, the emulsion formulations did not show instability and BBI was compatible with the vehicle used in F-BBI. The formulations had glossy and creamy appearance, yellowish color, characteristic odor and remained this form after conducting this test. There were stable will spin without any hint of instability of physical or chemical nature. The formulations seemed to be stable after centrifugation. Besides, only the viscosity and spreadability FBI was changed at the end of the cycle.

Table 1. Parameters of control assessment of lecithin-based topical formulations with seeds butter from P. insignis (F-BBI).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>VEHICLE</th>
<th>T0</th>
<th>Tc</th>
<th>T0</th>
<th>Tc</th>
<th>F-BBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance and homogeneity</td>
<td>Yellow, homogenous, opaque</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yellow, homogenous, opaque</td>
</tr>
<tr>
<td>pH</td>
<td>5.36 ± 0.04</td>
<td>5.56 ± 0.03</td>
<td>5.36 ± 0.04</td>
<td>5.28 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spreadability (mm²)</td>
<td>1586.66 ± 46.18</td>
<td>1500 ± 171.87</td>
<td>1736.33 ± 63.72</td>
<td>2521.66 ± 134.751</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apparent viscosity (Pa.s)</td>
<td>19.40 ± 0.00</td>
<td>19.40 ± 0.04</td>
<td>19.41 ± 0.05</td>
<td>10.30 ± 0.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend: T0: Time before cycle; Tc: time after cycle. Data were expressed as mean ± SD (n=3). Statistical significance was expressed as \*p<0.05 when compared with BBI_T0. Unpaired t test.

Inflammation is the characteristic response of mammalian tissue to injury. It is related with the succession of changes which occur in a living tissue when it is injured. According to its duration, inflammation could be described as acute, sub-acute or chronic [13]. Development of edema induced by carrageenan, the inflammatory agent used in our experiments, is commonly associated with the early exsudative stage of inflammation, one of the important processes of inflammatory pathology. The carrageenan-induced inflammation in rat paws have long been accepted as useful pharmacological tools for the investigation of new anti-inflammatory drugs, and therefore represents a classical model of acute inflammation widely applied for evaluation of activity of potential drug candidates, then allowing the identification of promising compounds to treat inflammatory disorders [14,15]. The inflammatory process is differentiated by the type of tissue involved, phlogistic agent and inflammatory mediator release. In this case, while the carrageenan mechanism involves the release of prostanoids cyclooxygenase pathway, the mechanism of inflammatory croton oil, involves the activation of phospholipase A2 in different tissues, promotes the release of various inflammatory mediators involved in both the formation of edema as in chemotaxis [16].

Carrageenan triggers an acute inflammation associated with hyperalgesia which is characterized by edema, heightened sensitivity to thermal and mechanical stimuli according to a sequential action of various mediators (histamine, 5-hydroxytryptamine (5-HT), kinins, prostaglandins), including complement system [17]. The edema induced by carrageenan is commonly used as an acute inflammation model [18] that causes a swelling in three phases rich in proteins and neutrophils [18]–[21]. The first time is characterized by increased vascular permeability mediated by histamine and serotonin. The second time, this increase is mediated by kinins. The maximum swelling peak occurs in the third hour after the injection of carrageenan, which is characterized by prostaglandin action on vascular permeability [19], [21]. In the present study, the oral treatment with BBI was able to reduce carrageenan-induced paw edema at 4th hour only in all tested doses only, and at 5th hour for the highest tested doses (400 mg/kg). Indomethacin, the positive control for inhibition of paw edema, was significantly effective in reducing paw edema at all times observed (Table 2).

Interestingly, the F-BBI formulation reduced the development of paw edema induced by carrageenan, maintaining constant response to the end of the 6th hour. Different of the BBI-induced anti-edematogenic effect after oral administration, the topic pre-treatment with F-BBI directly into the paw inhibited edema formation, similar to the topic pre-treatment with dexamethasone acetate 0.1% from 1st to 6th hour (Figure 1). The results suggest that the F-BBI have some action on the inflammatory response mediators. Thus, the results suggest that the F-BBI exhibits topical anti-inflammatory effect with similar response to dexamethasone by topical route.
Table 2. Oral anti-inflammatory effect of seeds butter from *P. insignis* (BBI) on the carrageenan-induced paw edema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.89 ± 0.25</td>
<td>5.16 ± 0.18</td>
<td>5.16 ± 0.07</td>
<td>5.28 ± 0.17</td>
<td>4.74 ± 0.11</td>
<td>4.71 ± 0.21</td>
<td></td>
</tr>
<tr>
<td>BBI (50)</td>
<td>2.29 ± 0.29</td>
<td>4.10 ± 0.45</td>
<td>4.41 ± 0.27</td>
<td>4.15 ± 0.29</td>
<td>4.45 ± 0.27</td>
<td>3.86 ± 0.34</td>
<td></td>
</tr>
<tr>
<td>BBI (100)</td>
<td>3.09 ± 0.30</td>
<td>4.79 ± 0.31</td>
<td>4.46 ± 0.28</td>
<td>3.99 ± 0.27</td>
<td>4.57 ± 0.18</td>
<td>3.94 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>BBI (200)</td>
<td>2.57 ± 0.14</td>
<td>4.42 ± 0.19</td>
<td>4.61 ± 0.21</td>
<td>4.08 ± 0.34</td>
<td>4.48 ± 0.16</td>
<td>3.96 ± 0.23</td>
<td></td>
</tr>
<tr>
<td>BBI (400)</td>
<td>2.69 ± 0.13</td>
<td>4.45 ± 0.17</td>
<td>4.76 ± 0.11</td>
<td>4.08 ± 0.22</td>
<td>4.48 ± 0.16</td>
<td>3.94 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>Indomethacin (5.0)</td>
<td>0.91±0.06***</td>
<td>1.42±0.22***</td>
<td>1.46±0.17***</td>
<td>2.25±0.20***</td>
<td>2.40±0.20***</td>
<td>2.30±0.35***</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SEM (n=6). Statistical significance was expressed as *p<0.05 or ***p<0.001 when compared with vehicle. ANOVA One-way followed by Tukey’s test.

Figure 1. Topical anti-inflammatory effect of lecithin-based formulation of seeds butter from *P. insignis* (F-BBI) on the carrageenan-induced paw edema in rats. Statistical significance was expressed as *p<0.05, **p<0.01 or ***p<0.001 when compared with vehicle. ANOVA One-way followed by Tukey’s test.

4. Conclusions

The present report suggests that BBI are able to act in different phasis of inflammatory response depending on the administration route. The proposed lecithin-based topical formulation of BBI are able to act in all phases of inflammatory response in carrageenan-induced paw edema in rats. This paper suggests a new application for *Platonia insignis* seeds extract as a promising topical anti-inflammatory agent, and encourage further studies in order to elucidate mechanisms involved in this effect.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

1. **BBI** Seeds butter from “bacuri” (*Platonia insignis* Mart.)
2. **F-BBI** Lecithin-based topical formulation with BBI

References

Astragalus sinicus L. in cytokine-induced keratinocytes and skin.

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