Evaluation of anti-inflammatory and antinociceptive activities of the *Austroplenckia populnea* extract in topical formulations

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**INTRODUCTION**

The use of medicinal plants by the world population has contributed to the cure of various diseases and has the potential for many additional applications, compared with the rigor displayed by alternative substances synthesized in laboratory (Cordell and Colvard, 2012; Shaw et al., 2012; Wang et al., 2013). *Austroplenckia populnea* (Reiss.) Lundell is a Brazilian Cerrado plant that is a potential candidate for use in alternative medicine. It is known as “marmelinho do campo, mangabeira brava, mangabarana, Maria mole or vime” and it is used in
Brazilians use folk medicine to treat dysenteries and especially inflammatory disorders, such as rheumatism (Andrade et al., 2006). It belongs to the botanical family Celastraceae, which includes several other plant species that have been widely used in folk medicine and have anti-ulcerogenic, analgesic, anti-inflammatory, antitumor and other properties (Andrade et al., 2006, 2007, 2008). Phytochemical studies have revealed several compounds, among them the pentacyclic triterpenes, which could be responsible for the biological activities of this species, particularly their anti-inflammatory and analgesic activities (Andrade et al., 2006, 2007; De Sousa et al., 2006, 1990).

Pharmaceutical creams are semi-solid preparations that contain one or more medicinal agents dissolved or dispersed in oil/water (O/W) or water/oil (W/O) emulsions. They are primarily used in products for topical application on the skin and in products for rectal or vaginal application (Allen et al., 2010). Various strategies have been proposed to achieve efficient drug delivery. Hydrogels have been chosen for use in the medical and pharmaceutical industries due to their biocompatibility and similarity to natural tissue (Bonacucina et al., 2006).

This study was performed to evaluate the anti-inflammatory and antinociceptive activities of the hydroalcoholic wood extract of A. populnea in semi-solid formulations administered topically in rats.

MATERIALS AND METHODS

Preparations of the raw hydroalcoholic A. populnea extract (RHAPE)

A. populnea was collected in the “Cerrado” area of Brasília, Goiás, Brazil in June 2007. The wood portion was used to prepare the extract and was allowed to dry at room temperature for one week before being pulverized using a knife mill. Subsequently, 1300 g of material was macerated with 5 L of aqueous ethanol (Vetec 96% v/v) for 12 days, also at a room temperature (25±2°C). The crude extract (103 g) was obtained after the evaporation of the solvent under reduced pressure (600 mmHg) at 40°C. A voucher specimen was deposited in the herbarium of the University of Brasília under the number J. Elias de Paula (UB) 3747.

Laboratory animals

Wistar Rattus norvegicus (n = 84) weighing between 230 and 280 g were used. The animals were kept under controlled environmental conditions, with food and water ad libitum, which was withdrawn 12 h before the test. All experimental procedures were approved by the Ethics Committee on Animal Use, Institute of Biology, under protocol number 46510/2010.

Preparation of formulations

The O/W creams were prepared by a phase inversion technique. The technique consisted of weighing the aqueous and oily excipients separately, heating the two phases to 70°C and adding the aqueous phase of the oil with stirring at 300 rpm in a mechanical stirrer (Fisatom, Model 175, São Paulo, Brazil). When the mixture reached 50°C, it was added to the raw extract dissolved in ethyl alcohol (Allen et al., 2010; Lachman et al., 2009). The preparation was cooled to 30°C while being stirred and was then added to a solution of sodium lauryl sulfate (SLS) in distilled water. The final pH value of the O/W cream was verified. The carbopol® 940 was hydrated in cold water 24 h before the preparation of the hydrogels. After hydration, the following were added with stirring at 200 rpm: propylene glycol, glycerin, disodium EDTA, preservatives, RHAPE diluted in ethyl alcohol, SLS diluted in 20 ml of distilled water and triethanolamine. For the last step, the pH of the preparation was verified.

Stability test

The samples of the formulations were subjected to accelerated stability testing for 180 days. The formulations were fractioned in duplication of 20 g, being conditioned inside plastic pots, after 24 h. The samples were stored at temperatures of 4±2°C, 40±2°C/75% relative humidity (RH) and room temperature (25±2°C) for a predetermined number of days (1, 15, 30, 60, 90, 120, 150 and 180). Centrifugation was carried out with duplicated samples of 10 g of each cream at 1575 g for 30 min in a centrifuge (Cenribio, Model 80-2B, Belo Horizonte, Brazil). The pH of the preparations stored at ambient temperature was verified by employing a digital potentiometer (Gehaka, Model 1500, São Paulo, Brazil). The freeze-thaw test was carried out with duplicated samples of 30 g of gel for 24 h at -5°C and 24 h at 45°C for 12 days, for a total of 6 cycles. The color, odor and physical appearance were evaluated during stability testing (Allen et al., 2010).

Paw edema test induced by carrageenan

One hour before applying the carrageenan, 0.2 g of each treatment (4.0% cream, 8.0% cream, 4.0% gel, and 8.0% gel) was rubbed 50 times on right paw of each animal to help the penetration of formulation through the skin. A preparation was applied in the same way for the dorsal surface of the right paw of each animal by gently rubbing with a finger 50 times. Rats from the control group received only gel base and cream base (without extract). Methyl salicylate ointment was applied in the same manner as in the reference group. Seven groups of 6 animals were used.

Formalin test

One hour prior to formalin administration, 0.2 g of treatments (4.0% cream, 8.0% cream, 4.0% gel, and 8.0% gel) was administered to the dorsal surface of the right paw of each animal by gently rubbing with a finger 50 times. Rats from the control group received only gel base and cream base (without extract). Methyl salicylate ointment was applied in the same manner as in the reference group. Seven groups of 6 animals were used.
Table 1. Effect of topical administration of O/W creams with RHAPE on the paw edema test induced by carrageenan (n = 6/treatment).

<table>
<thead>
<tr>
<th>Treatments/Paw volume (ml) at time after carrageenan injection</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cream base</td>
<td>1.52±0.17</td>
<td>1.54±0.25</td>
<td>1.72±0.21</td>
<td>1.70±0.12</td>
<td>1.66±0.25</td>
<td>1.56±0.32</td>
</tr>
<tr>
<td>4.0% Cream</td>
<td>1.05±0.11*</td>
<td>1.19±0.07*</td>
<td>1.35±0.21*</td>
<td>1.10±0.11*</td>
<td>1.41±0.21*</td>
<td>1.47±0.08*</td>
</tr>
<tr>
<td>8.0% Cream</td>
<td>1.07±0.09*</td>
<td>1.24±0.14*</td>
<td>1.39±0.16*</td>
<td>1.30±0.07*</td>
<td>1.60±0.26</td>
<td>1.62±0.23</td>
</tr>
<tr>
<td>Piroxicam gel 0.5%</td>
<td>1.35±0.22</td>
<td>1.33±0.20*</td>
<td>1.48±0.18*</td>
<td>1.49±0.11*</td>
<td>1.46±0.13</td>
<td>1.52±0.14</td>
</tr>
</tbody>
</table>

*P < 0.05 represents a significant difference compared to the control (Cream base).

Table 2. Effect of topical administration of gels with RHAPE on the paw edema test induced by carrageenan (n = 6/treatment).

<table>
<thead>
<tr>
<th>Treatments/Paw volume (ml) at time after carrageenan injection</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel base</td>
<td>1.63±0.17</td>
<td>1.88±0.13</td>
<td>2.05±0.30</td>
<td>2.1±0.22</td>
<td>2.1±0.41</td>
<td>2.1±0.30</td>
</tr>
<tr>
<td>4.0% Gel</td>
<td>1.11±0.29*</td>
<td>1.13±0.21*</td>
<td>1.26±0.20*</td>
<td>1.49±0.26*</td>
<td>1.52±0.26*</td>
<td>1.49±0.28*</td>
</tr>
<tr>
<td>8.0% Gel</td>
<td>1.05±0.14*</td>
<td>1.34±0.17*</td>
<td>1.29±0.18*</td>
<td>1.43±0.27*</td>
<td>1.36±0.21*</td>
<td>1.45±0.17*</td>
</tr>
<tr>
<td>Piroxicam gel 0.5%</td>
<td>1.35±0.22*</td>
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<td>1.49±0.11*</td>
<td>1.46±0.13*</td>
<td>1.52±0.14*</td>
</tr>
</tbody>
</table>

*P < 0.05 represents a significant difference compared to the control.

The procedures and doses were based on experiments from our research group and other laboratories (Calvo, 2006; Semnani et al., 2004; Silva et al., 2014). After injection of 20 µl of formalin 2.5% into the subplantar region of the right paw, the rat was observed for 30 min, at intervals of 0 to 5 min (neurogenic phase) and 15 to 30 min (inflammatory phase), during which the recorded time interval that passed before licking or biting the right paw began was recorded (Semnani et al., 2004; Silva et al., 2014).

Statistical analysis

All values are expressed as the means ± standard error of mean (SEM) for six animals in each experimental group. A statistical comparison of results was performed using one way analysis of variance (ANOVA), followed by Newman-Keuls post-hoc multiple comparison test. The acceptable level of significance of the tests was P ≤ 0.05. All tests were performed using the Statistical® software package (StatSoft Inc., Tulsa, OK, USA).

RESULTS

The formulations of the O/W cream with the RHAPE were stable at different temperatures during the evaluation period of 180 days. The gel-type formulations were stable at room temperature and 4±2°C, while a temperature of 40±2°C/75% RH caused instability. The average pH over the 180 days period for 4.0% cream was 5.5, while 8.0% cream had an average pH of 5.11. These changes were not statistically significant, as the initial pH values for 4.0 and 8.0% creams were 5.65 and 5.61, respectively. No changes were observed macroscopically or in the centrifugation test of the O/W creams. With regard to the gels, the pH showed no significant change. However, at a temperature of 40±2°C/75% RH, both gels 4.0 and 8.0% showed syneresis from the 90 and 60th days of observation, respectively. In the freeze-thaw test both gels showed syneresis at the end of the test period on the 12th cycle. Even though, gels and hydrogels generally have been considered good candidates for oral, rectal, ocular, dermal and subcutaneous administration (Bonacucina et al., 2006), probably in this present situation there was incompatibility between the hydrogel and extract when temperature changes were applied.

Table 1 shows the results of the paw edema test for the O/W creams following hourly paw volume assessment for 5 h. At the 0, 1, 2 and 3 h time points, there was a reduction in paw volume following administration of 4.0% cream when compared with the cream base. However, the Piroxicam gel significantly reduced edema when compared with the control group (cream base) with 2 and 3 h. At the 3 h time point, the 4.0% cream had significantly reduced swelling by 54.43%, but the 8.0% cream showed negative value, indicating that was not able to inhibit swelling. Therefore, only 4.0% cream showed inhibition of swelling in the 3rd h compared to the Piroxicam gel (12.06%).

Table 2 presents the values of the paw edema test when the gels with RHAPE were administered for 5 h evaluations. The 4.0 and 8.0% gels and Piroxicam gel significantly reduced edema when compared with the control group (gel base) over 5 h. The 4.0% gel was more efficient during the initial period of the treatment until the 2 h assessment when compared with control, with an
inhibition of 87.32% at the 1 h time point and 47.34% at the 2 h time point; however, these values were lower than those from the Piroxicam gel (112.87 and 64.75% inhibition, respectively).

The results obtained in the formalin test (Figures 1 and 2) demonstrated that treatment with 4.0% cream invoked a better antinociceptive and anti-inflammatory response when compared with the methyl salicylate ointment in the late phase of inflammation origin (15 to 30 min). The gels did not show a significant response at any stage.

DISCUSSION

The results of the stability study showed that the cream-type formulations were more stable than the gels. Despite the unfavorable results from the stability tests of the gels, they were evaluated together with the O/W creams for anti-inflammatory and analgesic activities to determine if the pharmaceutical base would affect the pharmacodynamic properties. In this case, we deduced that the pharmaceutical base was important during administration of extract because cream formulation has two phases, oil and water, and the skin has lipophilic and hydrophilic characteristics. So, the cream formulation was better than gel formulation for penetration of extract through the skin.

The edema induced by carrageenan occurs in two phases, as various mediators act in sequence to produce an anti-inflammatory response. In the early phase (0 to 1 h), it is possible to observe the release of various chemical mediators such as histamine, serotonin and bradykinin. The inflammatory response is correlated with the subsequent phase (1 to 6 h) and is characterized by increased production of prostaglandins, cyclooxygenase-2 (COX-2) activation, and the release of nitric oxide (Gorzalczany et al., 2011). Carrageenan causes an inflammatory process mediated by prostaglandin, reaching peak levels within 2 to 3 h after application (Arawwawala et al., 2010). Because inflammation mediated by the release of prostaglandin occurs between the 2nd and 3rd hour, it is relevant to assess the possibility of reducing edema with RHAPE treatments.

Inhibition of inflammation with the treatments investigated was significantly observed, as 4.0% cream showed significant reduction at the 3 h time point that was greater than that of Piroxicam gel, as previously mentioned. The fact that the formulation with a lower concentration of extract had a greater anti-inflammatory effect than the formulation with higher concentration indicates that the maximum effect would have been reached by the 4.0% cream. Our hypothesis is that this probably could have been the result of a saturation of likely targets of action of the substances under study.

The formalin test is frequently used because it is an antinociceptive model sensitive and reliable to evaluate various classes of analgesic drugs. The test can also be used to identify the potential mechanism for the antinociceptive effect of a particular analgesic. Centrally acting drugs, such as opioids, inhibit the nociceptive and the inflammatory phases equally. Peripherally acting drugs, such as aspirin, indomethacin and dexamethasone, on the other hand, only inhibit the late stage, because this inflammatory phase can be inhibited by anti-inflammatory agents (Calvo, 2006; Wang et al.,
Figure 2. Effect of RHAPE on the reaction time of licking the formalin-induced paw (n = 6/group) when used as a gels formulation. *P≤0.05 represents a significant difference compared to control animals treated with gel base.

The neurogenic phase (0 to 5 min) is the pain process which activates the channels; the late stage involves the production of nociceptive mediators of inflammation and occurs within 15 min of formalin application (late stage). Substance P and bradykinin act as mediators of the early phase, while histamine, serotonin, prostaglandins and bradykinin are involved in the nociceptive response in the late phase (Nonato et al., 2011). Based on the result of the formalin test, the 4.0% cream has a similar effect as the methyl salicylate, suggesting a significant reduction of production of nociceptive mediators of inflammation involved in the response observed in Figure 1.

The anti-inflammatory response observed with topical administration of RHAPE supports previous research showing an anti-inflammatory effect following oral administration of the hydroalcoholic wood A. populnea extract in rats (Andrade et al., 2007). It was observed that this plant can be an alternative in phytotherapeutical medicine to treated inflammation and pain because our results had similar effects when drugs like piroxicam and methyl salicylate were used as control in paw edema and formalin test, respectively. The inflammation is a complex process, which is frequently associated with pain; so if a medicinal plant has anti-inflammatory and antinociceptive activities, it is essential for study and development of an herbal medicine.

Considering that there are only a few preliminary data reported in the literature regarding the anti-inflammatory properties of A. populnea preparations, these results collaborate to increase interest to study more about this plant and its anti-inflammatory activity using other routes of administration and types of pharmaceutical formulations.

Conclusion

The results of this study serve as a starting point for further investigations related to the effectiveness of topical preparations of RHAPE in treating localized inflammation. The formulations of O/W cream were stable over 180 days; moreover, the in vivo tests with 4.0% cream showed significant anti-inflammatory action relative to the control. It was also observed that the pharmaceutical base influenced the effectiveness of RHAPE, as the cream was superior to the gel base with this extract and at this concentration. Then, we concluded that this herbal extract has activity in pharmaceutical base like O/W cream to topical administration. So, this study contributed with other treatment alternatives in case of inflammation.

Competing interests

The authors declare that they have no competing interests.

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REFERENCES


