ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS FRUIT PULP EXTRACT OF BURSERA PENICILLATA IN ACUTE AND CHRONIC INFLAMMATION

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ABSTRACT

The anti-inflammatory effect of the aqueous fruit pulp extract of Bursera penicillata was evaluated using the carrageenan- and dextran-induced rat paw edema, xylene-induced ear edema and formalin-induced arthritis inflammation tests. Oral administration of the extract produced significant (p < 0.05) antiedematogenic effect with a dose of 500 mg/kg throughout the period of the experiment in the dextran induced paw edema and at the 3 h in the carrageenan model. The extract (250 and 500 mg/kg) exhibited a dose-related and significant (p < 0.01) inhibition of xylene induced ear edema and the effect was similar to that produced by dexamethasone (1 mg/kg). In the chronic inflammation (formalin induced arthritis) the extract did not show any significant anti-inflammatory activity. Oral acute toxicity assays did not show any mortality at 15 g/kg of the plant extract. The results indicate that the aqueous extract of Bursera penicillata possesses acute inflammatory activity which may be mediated by either inhibition or by blocking the release of prostaglandins and histamine, thus supporting the usage of the plant in traditional medicine treatment of inflammation.

KEYWORDS: anti-inflammatory, arthritis, edema.

INTRODUCTION

Various herbal medicines derived from plant extracts are being used in the treatment of a wide variety of clinical diseases, though relatively little knowledge about their mechanisms of action is known. [1] Many herbal preparations are being prescribed widely for the treatment of inflammatory conditions. [2] There is a need for research and developmental work in herbal
medicine because apart from the social and economic benefits, it has become a persistent aspect of present day healthcare in developing countries.

The attention of pharmacologist throughout the world has been focused on finding out safer and potent anti-inflammatory drug. The natural products today symbolize safety in contrast to the synthetic drugs that are regarded as unsafe to humans and environment. So, people are returning to the natural products with the hope of safety and security. However, so far there is no systematic study on anti-inflammatory activity has been reported in the literature. Hence the present study focuses on evaluating the anti-inflammatory activity of aqueous fruit pulp extract of Bursera penicillata.

To our knowledge more report on the effect of this plant on experimental information. This study was therefore undertaken to evaluate the effect of ethanol extract of the aqueous fruit pulp extract of Bursera penicillata on anti-inflammatory activity in carrageenan induced rat paw edema.

### Plant Material and Extraction

The ripe fruits of Bursera penicillata were collected from Osmania University, Hyderabad, Telangana, the month of October, 2013. The seeds were removed from the ripe fruits and the fruit pulp was sun-dried to a constant weight over a 14-day period. The dried fruit pulp was then powdered using a mechanical grinder. The powdered fruit pulp (500 g) was boiled in 2 L of distilled water for 30 min. The material was then filtered, concentrated to dryness under reduced temperature and pressure in a vacuum evaporator (yield = 38%). The dried extract was stored in air-tight clean glass container at 4°C until use.

### Phytochemical Screening

The aqueous extract of H. umbellata was subjected to various tests in order to determine the classes of the various chemical constituents present in the extract, by using standard methods. [11]

### Animals

Experiments were performed using either Swiss albino mice (17-23 g) or Wistar rats (150-190 g). The animals were obtained from the Laboratory Animal Centre, National Laboratory for Animal Science, India. The animals were fed with standard rodent cubes obtained from National Institute of Nutrition and had free access to tap water. All animals were fasted.
overnight before the beginning of each experiment. Animals were exposed to natural lighting conditions and were handled according to standard experimental protocols approved by the Faculty of Pharmacy Animal Ethics Committee.

**Drugs and Chemicals**

λ-Carrageenan, dextran-A, indomethacin and diphenhydramine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A). Xylene and formaldehyde were obtained from BDH Chemicals, UK, while dexamethasone was obtained from Vardhman Exports, India.

**Acute Toxicity Study**

Overnight-fasted Swiss albino mice (17-23 g) of either sex were used for the study. The animals were divided into five groups of five animals each. Groups A to D received orally 1, 5, 10 and 15 g/kg of the extract, respectively, while the control (group E), received distilled water (3 mL/kg) by the same route. General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 h were observed for any signs of delayed toxicity for two weeks.

**Anti-Inflammatory Activity**

**Carrageenan Induced Paw Edema**

Male Wistar rats (150-190 g) were divided into four groups of five animals each. The test groups received 250 and 500 g/kg, p. o. of the extract. The reference group received indomethacin (10 mg/kg, p. o.) while the control group received 3 mL/kg of distilled water. After 1 h, 0.1 mL, 1% w/v carrageenan suspension in normal saline was injected into the subplantar tissue of the right hind paw. The paw thickness was measured at hourly interval for 5 h using a vernier caliper.

**Dextran Induced Paw Edema**

Male Wistar rats (150-190 g) were divided into four groups of five animals each. The different groups of animals received extract (250 and 500mg/kg) or diphenhydramine (60 mg/kg) or distilled water (3 ml/kg) orally. The animals were treated 1 h before injection of 0.1 mL of 1.5% w/v dextran in normal saline into the subplantar tissue of the right hind paw. Paw thickness was measured using vernier calipers at 0, 1, 2, 3, 4, and 5 h.

**Xylene Induced Ear Edema**

Swiss albino mice were divided into four groups of five animals each. Animals were treated
orally with the extract (250 and 500 mg/kg), dexamethasone (1 mg/kg) and distilled water (3 mL/kg). Thirty minutes later, edema was induced in each mouse group by applying a drop of xylene to the inner surface of the right ear. After 15 min, the animals were sacrificed under ether anesthesia and both ears cut off, sized and weighed. The anti-inflammatory activity was expressed as the percent-age inhibition of edema in the treated mice in comparison with the control mice.

Formalin Induced Arthritis Inflammation

Male Wistar rats (150-190 g) were divided into four groups of five animals each. Inflammation was produced by subaponeurotic injection of 0.1 mL of 2 % w/v formalin in normal saline in the right hind paw of the rats on the first and third day. The extract (250 and 500 mg/kg) and distilled water (3 mL/kg) were administered orally once a day for 10 days. Indomethacin (5 mg/kg) given orally, was used as standard. The rat paw thickness was measured daily for 10 days using vernier calipers. The percent-age inhibition of the mean increase in the paw edema of each group was calculated on the tenth day and compared with the control.

Statistical Analysis

Data were expressed as the mean ± SEM. The data were analyzed using one way analysis of variance (ANOVA) followed by Turkeys test. Differences between two means were detected using the Students t-test. Data were considered different at significance level of $p < 0.05$.

RESULTS

Acute toxicity studies showed that all the doses (1, 5, 10, and 15 g/kg) of the *Bursera penicillata* extract used for the study were non-toxic. The preliminary phytochemical screening of the aqueous fruit pulp extract of *H. umbellata* revealed the presence of carbohydrates, alkaloids, reducing sugars, saponins, tannins, steroidal components and flavonoids, further corroborating previous reports. In the carrageenan-induced paw edema (Fig. 1), the aqueous fruit pulp extract of *H. umbellata* (500 mg/kg) significantly ($p < 0.05$) inhibited paw edema at the 3rd h compared with the control animals.
Figure 1. Effect of aqueous extract of *Bursera penicillata* on carrageenan-induced paw edema in rats. *p < 0.05, as compared to the control (n = 5 for each group)*

Figure 2. Effect of aqueous extract of *Bursera penicillata* on dextran-induced paw edema in rats. *p < 0.05, **p < 0.01 as compared to the control (n = 5 for each group)*

Which received distilled water. This effect was comparatively less than indomethacin. In Figure 2, the extract (500 mg/kg) produced a significant (*p <0.05*) inhibitory effect on the dextran induced paw edema sustained throughout the period of the experiment. The extractís inhibitory effect was greater than the positive control, diphenhydramine at the 4th and 5th h of the experiment but there was no significant difference (*p > 0.05*). On xylene-induced ear edema (Table 1), the extract at doses of 250 and 500 mg/kg showed significant percentage inhibitions of 62.47 and 69.70%, respectively. The inhibitory effect at a dose of 500mg/kg was comparable to that of examethasone, with an inhibition of 74.56%. In Table 2, the aqueous extract produced no significant (*p > 0.05*) inhibitory effect in formalin-induced arthritis at doses of 250 and 500 mg/kg.

Table 1. Effect of aqueous extract of *Bursera penicillata* on xylene-induced ear edema in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Weight of right ear (mg)</th>
<th>Weight of left ear (mg)</th>
<th>Difference (mg)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(mg/kg)</td>
<td>39.76 ± 0.74</td>
<td>23.72 ± 0.62</td>
<td>16.04 ± 0.54</td>
<td>-</td>
</tr>
<tr>
<td><em>Bursera penicillata</em></td>
<td>3 mL/kg</td>
<td>28.02 ± 0.62</td>
<td>22.00 ± 0.50</td>
<td>6.02 ± 0.33</td>
<td>69.70</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>250</td>
<td>30.26 ± 0.46</td>
<td>26.18 ± 0.50</td>
<td>4.08 ± 0.36</td>
<td>74.56</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>
Table 2. Effect of aqueous extract of *Bursera penicillata* on formalin induced arthritis inflammation in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Paw thickness on day 10 (mm)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3 mL/kg</td>
<td>7.34 ± 0.14</td>
<td>-</td>
</tr>
<tr>
<td><em>Bursera penicillata</em></td>
<td>250</td>
<td>6.92 ± 0.10</td>
<td>5.72</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>7.08 ± 0.28</td>
<td>3.54</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>5</td>
<td>5.44 ± 0.23*</td>
<td>25.89</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM values for five rats in each group. *p < 0.05 as compared to the control.

In the present study, the anti-inflammatory activity of the aqueous fruit extract of *Bursera penicillata* has been evaluated in both acute and chronic inflammatory models. The inhibition of carrageenan induced inflammation in rats is an established model for evaluating anti-inflammatory drugs, which has been used frequently to assess anti-edematous effect of natural products. The development of carrageenan induced edema is biphasic \[17\]; the first phase occurs within one hour of carrageenan inflammation and is attributed to the release of cytoplasmic enzymes, histamine and serotonin, from the mast cells. The second phase (> 1.0 h) is mediated by an increased release of prostaglandins in the inflammatory area and continuity between the two phases is provided by kinins. Since the extract significantly inhibited paw edema induced by carrageenan in the second phase, this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract, because the carrageenan inflammatory model basically reflects the actions of prostaglandins. \[18,19\] This effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme, which catalyses the synthesis of cyclic endoperoxides important in the formation of prostaglandins.

Dextran-induced paw edema has been reported to be mediated mainly by histamine and serotonin released by the mast cells. \[20\] The release of these inflammatory mediators’ results in marked vascular changes: including vasodilatation, increased permeability and an increase of blood flow, eventually leading to an increase in paw size. The extract was found to be more effective in dextran-induced edema than the carrageenan-induced edema, suggesting that the extract may also interfere with histamine release or its activity. The xylene ear edema model permits the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents. \[21\] Histopathologically, severe vasodilatation, edematous changes of skin and infiltration of inflammatory cells are detected as signs of acute...
inflammation after topical application of xylene. In the present study, the increases in ear weight were inhibited in a dose-related manner by the extract, suggesting a likely indication of the antiphlogistic effects of the extract. It is well known that inhibition of edema induced by formalin in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents, as it closely resembles human arthritis. Arthritis induced by formalin is a model used for the evaluation of an agent with probable antiproliferative activity. The results of the for malin tests ruled out a possible effect of the extract on formalin induced cell damage and accordingly, arthritic conditions. The presence of the reported phytochemical constituents in the fruit pulp extract may contribute to its observed anti-inflammatory activity. Many flavonoids and alkaloids have been found to exhibit anti-inflammatory effects. Acute toxicity studies revealed no mortality at a dose of 15 g/kg. Hence, the extract was classified as relatively harmless when administered orally. In conclusion, the aqueous fruit pulp extract of *Bursera penicillata* has been shown to be effective against acute inflammation (carrageenan- and dextran induced paw edema, and xylene-induced ear edema) in a dose related manner but without any significant inhibitory effect on chronic inflammation (formalin induced arthritis). This present study supports the claim in the use of the fruit pulp extract of *Bursera penicillata* in traditional medicine for the treatment of inflammatory conditions.

REFERENCES


