Anti-inflammatory Activity of Muntingia calabura Fruits

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ABSTRACT

Muntingia calabura, the sole species in the genus Muntingia, is a flowering plant, that belongs to Elaeocarpaceae family. This is a fast growing fruit tree. It is a pioneer species that thrives in poor soil, able to tolerate acidic and alkaline conditions and drought. The fruits are commonly called Jamaican cherry and are red in colour. The flowers are used as an antiseptic and to treat abdominal cramps and spasms. It is also taken to relieve headaches and colds. Muntingia calabura fruits possess antioxidant property. However, their anti-inflammatory activity has not been investigated so far. The aim of this study, therefore, was to evaluate the anti-inflammatory activity from the fruits of M. calabura. The methanolic fruit extracts (100, 200 and 300 mg/kg i.p.) reduced the Carrageenan-induced edema of the hind paw of adult male Wistar Albino rats in 3 hours. The activity was compared with that of the standard drug indomethacin. Acute toxicity was investigated and the results indicated no abnormalities in the behaviour and lethality by the extract up to 1000 mg/kg. These results indicate the fruit extract of M. calabura possess potent anti-inflammatory activity. Therefore, these pharmacological results clearly support traditional folkloric application of M. calabura fruits in the control and/or pain, inflammatory illness as well as an antioxidant agent.

Key words: muntingia calabura fruits; elaeocarpaceae, anti-inflammatory, antioxidant activity

INTRODUCTION

Plants are potent biochemical factories and have been components of phytomedicine. Since time immemorial man is able to obtain from them a wondrous assortment of industrial chemicals. Plant-based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc., that is, any part of the plant may contain active components.\[1\]

The protective effect of fruits and vegetables has generally been attributed to their antioxidant constituents, including vitamin C (ascorbic acid), Vitamin E (tocopherol), carotenoids, glutathione, flavonoids and phenolic acids, as well as other unidentified compounds.\[2\]

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 mechanism or action is known.\[3\] Many herbal preparations are being prescribed widely for the treatment of inflammatory conditions.\[6\] 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 health care in developing countries.

Plant secondary metabolites have provided an important source of drugs since ancient times and now around half of the practical drugs used are derived from natural sources.\[8\]

Some research has shown that flavonoid compounds are present in various plants; exert beneficial effects on human health such as cardiovascular protection, anti-cancer activity, antinociceptive activity and anti inflammatory effects.\[6\]

It is known that there are links between the inflammatory and nociceptive, oxidative and cancer processes. The ability to inhibit any of the processes will definitely lead to the inhibition of the others.\[4\]

Water soluble extract from leaves of M. calabura produced potent antinociceptive and anti-inflammatory activities. The preliminary phytochemical analysis performed in M. calabura leaves showed the presence of flavonoids, chalcones, terpenoids and phenolic compounds. The constituents responsible for the analgesic and anti-inflammatory effects of M. calabura have not yet been elucidated.\[10\]
It was scientifically proved that *M. calabura* leaves possess anti-inflammatory and antipyretic activity.[11] Plant secondary metabolites have provided an important source of drugs from ancient times and now around half of the practical drugs used are derived from natural sources.[3]

**MATERIALS AND METHODS**

**Plant materials**

*Muntingia calabura* plant belongs to the family Elaeocarpaceae. The fruits of *M. calabura* were collected from the surrounding areas of Erode district, Tamilnadu, India and the plant was identified, authenticated and deposited (Voucher number: BSI/SC/5/23/09-10/Tech-132) at Tamil Nadu Agricultural University, Coimbatore, Tamilnadu.

**Preparation of methanolic extract**

The fruits of *M. calabura* were freshly collected and extracted with methanol; the extract was completely dried in vacuum, stored in refrigerator at 4°C and protected from sunlight for further use.

**Antioxidant activity**

DPPH radical scavenging assay

The effect of fruit extracts on DPPH (1,1-diphenyl-picryl hydrazine) radical was determined.[11] Different concentrations of the extracts (500, 400, 300, 200, 100 µg/ml) were prepared and subjected to antioxidant tests. To 1 ml of each of the extracts, 5 ml of 0.1mM methanol solution of DPPH was added, vortexes, followed by incubation at 27 °C for 20 min. The control was prepared without any extract and absorbance of the sample was measured at 517 nm using UV/VIS Spectrophotometer (ELICO) using methanol to set 0. The ability to scavenge DPPH radical was calculated by the following equation:

\[
\% \text{ inhibition} = 100 \times \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}
\]

**Pharmacological tests**

**General animal preparation**

Experiments were performed on healthy male Wistar Albino rats (120-150 g), procured from the small animals breeding station, Mannuthy, Kerala, India. They were housed in polypropylene cages (38 × 23 × 10 cm) with not more than six animals per cage and maintained at standard environmental conditions (14/10 hrs dar/light cycles; temp 25 ± 2 °C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M’s Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. All rats were acclimatized to the environment for two weeks prior to experimental use. Animals were fasted overnight before the experimental schedule, but had free access to water *ad libitum*. All animal procedures were performed in accordance with Institutional Animal Ethic Committee (IAEC) guidelines, after getting the approval of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) in Karpagam University, Coimbatore. (CPCSEA No: 739/03/abc/CPCSEA).

Adult male Wister rats taken for the study were divided into 6 groups; each group containing 6 animals and each group was treated as follows:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Control rats (normal saline only)</td>
</tr>
<tr>
<td>Group 2</td>
<td>Inflammation by carrageenan</td>
</tr>
<tr>
<td>Group 3</td>
<td>Standard drug</td>
</tr>
<tr>
<td>Group 4</td>
<td>100 mg/kg fruit extract + carageenan</td>
</tr>
<tr>
<td>Group 5</td>
<td>200 mg/kg fruit extract + carageenan</td>
</tr>
<tr>
<td>Group 6</td>
<td>300 mg/kg fruit extract + carageenan</td>
</tr>
</tbody>
</table>

**Preparation of test agents**

The methanol extract of *M. calabura* fruit was dissolved in isotonic normal saline (0.9% w/v) to make a stock solution with a concentration of 100 mg/ml. Three different doses at 100, 200, 300 mg/kg were injected into the animals. Additional test agents used in this study included Carrageenan and indomethacin. All chemicals and the extract were administered orally. All drugs and the extract were freshly prepared before use and dissolved in isotonic normal saline (0.9% w/v), which served as the vehicle and volume control for all agents. The only exception was Carrageenan, which was 0.5% CMC in distilled water served as the vehicle control.

**Anti-inflammatory activity**

The anti-inflammatory activity of the fruit sample was investigated in Carrageenan-induced inflammatory model. Acute inflammation was induced in rats.[13] The control group was administered with the saline solution only, while the third group was treated with indomethacin (10 mg/kg p.o.). The fourth, fifth and sixth groups were administered with the fruit extract (100, 200 and 300 mg/kg/day p.o.) respectively. One hour after the administration of fruit extract, the standard indomethacin acute inflammation was produced. Acute inflammatory edema was induced by subplantar injection of 0.1 ml 1% Carrageenan in the right hind paw of each rat in all the groups except the control group. The thickness (mm) of the paw was measured immediately and at 30 mins interval for four hrs after the Carrageenan injection, by using vernier calliper.[14] The percentage of inhibition of edema was calculated for each dose using the following formula:

\[
\% \text{ Inhibition} = \left(1 - \frac{PV_T}{PV_C}\right) \times 100
\]

\(PV_T\) = Paw volume in drug treated group of rats

\(PV_C\) = Paw volume in control group of rats
DISCUSSION

The fruit extract demonstrated H-donor activity. With regard to the estimated IC_{50} value, the extracts of *M. calabura* displayed significant DPPH radical quenching property. The DPPH assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidant property of various natural products.\[^{[1]}\]

Herbal products are consumed in traditional medical systems as functional/recreational food supplements or as medicines in many countries. In recent years, evidence has accumulated to suggest that complementary medicine for treatment of various diseases is another more popular choice.\[^{[15]}\] Many plant extracts of botanical medicinal herbs have been shown to relieve disease’s symptoms comparable to those obtained from allopathic medicines. Furthermore, chemical therapeutics are often associated with severe adverse effects. Therefore, safer compounds of natural products with fewer side effects are needed. In this study, demonstrations have produced novel observations for the first time that the fruit extracts of *M. calabura* possess anti-inflammatory effects in Carrageenan-induced hind paw acute inflammation. In most instances, however the

Statistical analysis

All the values were expressed as mean ± SD. The data were assessed using one way analysis of variance (ANOVA) followed by student’s t’ test. Statistical significance was accepted at \( p<0.05 \).

RESULTS

In our study, the fruits of *M. calabura* were evaluated for its antioxidant activity using DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The IC\(_{50}\) value was obtained for the tested assay, which showed that the lower the IC\(_{50}\) value, the higher was the antioxidant activity (Table 1).

Percentage inhibition of edema, volume of methanol and standard drugs were calculated after every hour for five hours. There was a significant and dose dependent anti-inflammatory activity of methanolic extract in the Carrageenan-induced rat paw edema model. Results of the effect of *M. calabura* fruit extract in Carrageenan-induced edema in test rats are shown in Figure 1 and Plate 1. Edema was greatly suppressed irrespective of the dose level of extract used and was comparable to the standard indomethacin treatment.

The methanol extract of *M. calabura* fruit at the dose levels of 100, 200 and 300 mg/kg caused a dose dependent inhibition of localized swelling caused by Carrageenan at 4 hrs (Table 2). The significant anti-inflammatory effect was dose dependent with 24.36% reduction observed for 100 mg/kg and 44.14% seen for 200 mg/kg dose and 62.43% observed for 300 mg/kg dose. Further, the protection induced by 300 mg/kg was also found to be as potent as indomethacin (80.48%) in reducing paw edema.

<table>
<thead>
<tr>
<th>Conc in μg/ml</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>200</td>
<td>65</td>
</tr>
<tr>
<td>300</td>
<td>78</td>
</tr>
<tr>
<td>400</td>
<td>85</td>
</tr>
<tr>
<td>500</td>
<td>94</td>
</tr>
</tbody>
</table>

\(^{[5]}\) IC\(_{50}\) value 90 μg/ml

Table 1: The percentage inhibition on DPPH radical by *M. calabura* fruit extract

Figure 1: The anti-inflammatory effect of *M. calabura* fruit extract on paw edema in Wistar Albino rats
effects of the extracts were significant and dose dependent. The observed anti-inflammatory effects of *M. calabura* fruit extracts could be due to the presence of biologically active chemical constituents in the extracts.

*M. calabura* has been used widely in both tropical America and Southeast Asia.\[16,17\]. In East Asia, flowers are used for the treatment of headaches and incipient cold or as tranquillizers, anti-spasmodics and antidyspeptics. Recently, ethyl acetate soluble extracts from the leaves of *M. calabura* and its major constituent, flavonoids, have been reported to have chemopreventive effects.\[18\] In order to evaluate anti-inflammatory effects of *M. calabura* fruit extract on the acute inflammation process, the rat paw edema model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial paw thickness (mm)</th>
<th>Paw thickness after 4 hr (mm)</th>
<th>Difference in paw thickness (mm)</th>
<th>Inhibition percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.58 ± 0.28</td>
<td>4.58 ± 0.28</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Induced</td>
<td>5.15 ± 0.33</td>
<td>4.33 ± 0.27</td>
<td>0.82</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>4.64 ± 0.37</td>
<td>4.48 ± 0.29</td>
<td>0.16</td>
<td>80.48</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>5.01 ± 0.41</td>
<td>4.41 ± 0.32</td>
<td>0.7</td>
<td>24.36</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>4.90 ± 0.25</td>
<td>4.36 ± 0.17</td>
<td>0.54</td>
<td>44.14</td>
</tr>
<tr>
<td>300 mg/kg</td>
<td>4.95 ± 0.34</td>
<td>4.56 ± 0.23</td>
<td>0.39</td>
<td>62.43</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six samples in each group
was used. In this experimental model, the Carrageenan-induced edema at 4 hrs was significantly inhibited by the fruit extract. The significant anti-inflammatory effect was dose dependent. This data supports the hypothesis of the effect of M. calabura fruit on the inflammation mediators in inflammatory processes. It is evident that Carrageenan is a sulphated mucopolysaccharide obtained from the seaweed (Rhodophyacea), perhaps the most commonly used to induce acute inflammation producing a maximal edematous in 3 to 5 hrs.[19] While the Carrageenan-induced edema model is typically associated with the activation of the cyclooxygenase pathway and is a multi-mediated phenomenon with the release of various inflammation mediators.

The inhibition of Carrageenan-induced inflammation in rats is an established model for evaluating anti-inflammatory drugs, which has been frequently used to access anti-edematous effect of nature products. Similar results were obtained from the aqueous fruit pulp extract of Hunteria umbellate[20] and Ammonium subulatum fruit extract.[21]

One of the mechanisms that could be used to explain the association between the anti-inflammatory and anti-oxidant activities is the reaction caused by ROS (Reactive oxygen species). ROS which is a type of inflammatory stimulus, has been shown to cause the release of nitric oxide (NO), a compound known to modulate a great number of physiological functions including the peripheral and central nociceptive processing within the body.[22,23] It is suggested that the blocking of ROS will cause a decrease in NO synthesis, which in turn will lead to the anti-inflammatory, anticancer and anti-oxidant activities.[24,25]

The edema induced in the rat paw by the injection of 1% Carrageenan is brought about by autocoids, histamine and 5-hydroxytryptamine (5-HT) during the first one hour, after which kinnins act, to increase the vascular permeability upto two and a half hours. The maximum inflammation is seen approximately three hours post the Carrageenan injection, after which it begins to decline. Following that the prostaglandins act from two and a half hours to six hours, which results in the migration of leucocytes into the inflamed site.[26,27] The pharmacological properties of safflower have been evaluated for antitumor, sedative,[28] antimicrobial,[29] anti-inflammatory and analgesic effects.[30]

M. calabura shows a significant inhibition of inflammation, which is comparable to the standard drug indomethacin. In summary, our results demonstrated that the fruit extracts of M. calabura possess antioxidant activity and anti-inflammatory activities, similar to those observed for non-steroid drugs such as indomethacin. These findings provide scientific supporting evidence for the therapeutic uses of M. calabura fruits in folk medicine. Further studies are required to identify the actual chemical components that are present in the crude extracts of this plant which are responsible for anti-inflammatory activity.

REFERENCES
23. Machelska H, Labuz D, Przewlocki R, Przewlocka B. Inhibition of nitric oxide synthase enhances antinociception mediated by mu, delta and


