ANTIINFLAMMATORY ACTIVITY OF HEDYOTIS LESCHENAULTIANA DC (RUBIACEAE) IN ACUTE INFLAMMATION

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Received on 15-05-2016
Accepted on 18-06-2016

Abstract:

Aim: The aim of the present study was to evaluate the antiinflammatory activity of ethanol extract of whole plant of Hedyotis leschenaultiana.

Methods: The antiinflammatory activity was evaluated by carrageenan induced paw edema to determine the activity on acute inflammation. The ethanol extract was administrated orally at a dose of 10,200 and 400 mg/Kg body weight to experimental rat. The antiinflammatory activity was compared with standard indomethacin.

Results: Maximum inhibition (74.52%) was obtained at the dose of 400 mg/Kg body weight of H. leschenaultiana whole plant after 3 hours of drug treatment in carrageenan induced paw edema, whereas, indomethacin produced 70.35% of inhibition.

Conclusion: The result of present study confirms marked antiinflammatory activity of H. leschenaultiana. This activity was attributed to 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 9,12-Octadecadienoic acid (Z,Z)-, 9-Octadecenoic acid (Z)-methyl ester, Phytol, Vitamin – E and β-Sitosterol present in the ethanol extract of H. leschenaultiana.

Key Words: Hedyotis leschenaultiana, Indomethacin, acute inflammation

Introduction

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process [1]. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to
harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues.

A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, immune system and various cells within the injured tissue. Prolonged inflammation known as chronic inflammation leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process [2]. Many synthetic drugs are now available in market to treat inflammation and pain, leading to side effects. So, the herbal drugs are of utmost important and there is a need for the production of novel herbal drugs.

The genus *Hedyotis* finds a prominent place in different Indian systems of medicine. The different ethnic communities in India have used different species of *Hedyotis* in the treatment of various ailments [3]. Taking into consideration of the medicinal importance of *Hedyotis*, the ethanol extract of whole plant of *Hedyotis leschenaultiana* were undertaken to evaluate the antiinflammatory activity in carrageenan induced rat paw edema. However, to the best of our knowledge there is no information in the literature about the antiinflammatory activities of an ethanol extract of this plant.

**Materials and Methods**

**Plant Material:**

The whole plant of *Hedyotis leschenaultiana* DC was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. The plant were identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

**Preparation of Plant Extract for Phytochemical Screening and Antiinflammatory Studies**

The whole plant of *H. leschenaultiana* were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *H. leschenaultiana* whole plant was packed in a Soxhlet apparatus and extracted with ethanol.

The extracts were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures [4-6]. The ethanol extracts was concentrated in a rotary evaporator. The concentrated ethanol extracts was used for antiinflammatory studies.
Animals: Adult Wistar albino rats of either sex (150-200g) were used for present investigation. Animals were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12:12h). Rats were feed standard pellet diet (Goldmohur brand, Ms Hindustan Lever Ltd., Mumbai, India) and water ad libitum.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study [7]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 2000 mg/kg body weight.

Carrageenan Induced Hind Paw Oedema

Albino rats of either sex weighing 150-200g were divided into five groups of six animals each. The dosage of the drugs administered to the different groups was as follows, Group I - Control (normal saline 0.5ml/kg), Group II – whole plant extract of *H. leschenaultiana* (150 mg/kg, p.o.), Group III – whole plant extract of *H. leschenaultiana* (200mg/kg, p.o.), Group IV - whole plant extract of *H. leschenaultiana* (400mg/kg, p.o.) and Group V-Indomethacin (10mg/kg). All the drugs were administered orally.

After one hour of the administration of the drugs, 0.1ml of 1% w/v carageenan solution in normal saline was injected into the subplantar tissue of the left hind paw and the right hind paw of the rat was served as the control. The paw volume of the rats were measured in the digital plethysmograph (Ugo Basile, Italy), at the end of 0 min., 60min., 120min., 180min. The percentage increase in paw oedema of the treated groups was compared with that of the control and the inhibitory effect of the drugs were studied.

The relative potency of the drugs under investigations was calculated based upon the percentage inhibition of the inflammation.

The percentage inhibition of the inflammation was calculated from the formula:

\[
\text{Percentage Inhibition} = \frac{D_o - D_t}{D_o} \times 100
\]
Where $D_0$ was the average inflammation (hind paw oedema) of the control group of rats at a given time; and $D_t$ was the average inflammation of the drug treated (i.e extracts or reference indomethacin) rats at the same time.

**Statistical Analysis:** The data were analyzed using student’s t-test. For the statistical tests $p$ values of less than 0.001, 0.01 and 0.05 was taken as significant.

**Results**

The plant extract did not exhibit any mortality upto the dose level of 2000 mg/Kg. So the extract is safe for long term administration. The phytochemical screening of ethanol extract of whole plant of *H. leschenaultiana* revealed the presence of alkaloid, catechin, coumarin, flavonoid, tannin, saponin, steroid, phenol, glycoside, terpenoid and xanthoprotein.

The antiinflammatory activity of *H. leschenaultiana* whole plant against carrageenan induced paw edema has been shown in Table 1 and the results were comparable to that of reference drug indomethacin. The ethanol extract of *H. leschenaultiana* whole plant showed maximum inhibition of 63.07, 69.97 and 74.92 % at the dose of 100, 200 and 400 mg/Kg body weight respectively after 3 hrs of the extract treatment against carrageenan induced paw oedema (Table 1) whereas, the reference drug produced 70.35% of inhibition at the dose 10 mg/Kg body weight.

**Table 1:** Effect of *H. leschenaultiana* whole plant extract on the Percentage inhibition of Carrageenan induced paw oedema.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Oedema volume (ml)</th>
<th>% Inhibition after 180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose mg/kg</td>
<td>0 min</td>
</tr>
<tr>
<td>CONTROL (Group-I)</td>
<td>Normal saline</td>
<td>22.27±1.16</td>
</tr>
<tr>
<td>HLA extract GroupII</td>
<td>100 mg/kg</td>
<td>30.55±1.86</td>
</tr>
<tr>
<td>GroupIII</td>
<td>200 mg/kg</td>
<td>32.68±1.14</td>
</tr>
<tr>
<td>Group IV</td>
<td>400 mg/kg</td>
<td>28.54±1.59</td>
</tr>
<tr>
<td>Indomethacin (Group-V)</td>
<td>10 mg/kg</td>
<td>27.19±1.84</td>
</tr>
</tbody>
</table>

Each Value is SEM ± 5 individual observations * $p < 0.05$; ** $p<0.01$ *** $p<0.001$, Compared to paw oedema induced control vs drug treated rats.
Discussion

It is evident that, carrageenan induced edema is commonly used as an experimental model for inflammation and is believed to be biphasic. The early phase (first phase – 1 to 2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandin release and mediated by bradykinin, leukotrienes, phytomorphonuclear cells and prostaglandins produced by tissue macrophages [8,9]. Prostaglandin – E2, a powerful vasodilator synergizes with other inflammatory vasodilators such as histamine and bradykinin and contributes to redness and increased blood flow in areas of acute inflammation. Since the extract significantly inhibited paw edema induced by carrageenan in the second phase, this finding suggest a possible inhibition of cyclooxygenase synthesis by the extract, because the carrageenan inflammatory model basically reflects the actions of prostaglandins [10,11]. This effect is similar to that produced by non-steroidal antiinflammatory 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.

Conclusion

The presence of phytochemical constituents in the whole plant extract may contribute to its observed antiinflammatory activity. Many flavonoids and alkaloids have been found to exhibit antiinflammatory effects [12]. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 9,12-Octadecadienoic acid (Z,Z)-, 9-Octadecenoic acid (Z)-, methyl ester, Phytol, Vitamin – E and β-Sitosterol were reported in the ethanol extract of H. leschenaultiana whole plant by GC-MS analysis [13]. These compounds may have the role in antiinflammatory activity. Further study will be carried out to isolate and characterize other antiinflammatory chemical constituents present in the extract of this plant.

References


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