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Anti-inflammatory activity of alcoholic extract of leaves of *Holostemma ada-Kodien* Schult in albino rats

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ABSTRACT

The alcoholic extract of *Holostemma ada Kodien* Schult (Family: Asclepiadaceae) was screened for anti-inflammatory activity. The study was performed using two different dose levels (200 mg/kg and 400 mg/kg) in carrageenan induced rat paw edema. The effects were compared with reference standard, Ibuprofen (10 mg/kg). The percentage inhibition of edema at dose 400 mg/kg at 3, 6, 24 hours were 50.00%, 58.57%, 71.87% respectively. This effect was comparable with that of standard drug Ibuprofen.

Keywords: Holostemma ada Kodien Schult, Carrageenan, Anti-inflammatory activity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAID’s) are widely used in treatment of inflammatory diseases such as arthritis, lumbago and rheumatism. These agents exhibit an inhibitory action on the cyclooxygenase that catalyzes the biosynthesis of prostaglandins and thromboxane from arachidonic acid [1,2]. Though these drugs have potent activity, the greatest disadvantage of the presently available drugs lies in their toxic symptoms on gastro intestinal tract and kidney [3]. Therefore, naturally originated agents with little side effects are required to substitute chemical therapeutics [4].

*Holostemma ada-Kodien* is an important medicinal plant belonging to family: Asclepiadaceae and widely distribute in tropical forest in India [5,6]. The plant is used as anti-diabetic [7], rejuvenative, aphrodisiac, expectorant, galactogogue, stimulant, and in ophthalmic disorders [8,9]. There is huge demand for this plant; more than 150 tones is required every year in south Indian pharmacies [10]. The present investigation was undertaken to study the anti-inflammatory activity of *Holostemma ada Kodien* at two different dose levels in carrageenan-induced rat paw edema.
MATERIALS AND METHODS

PLANT MATERIAL

Fresh leaves were collected and authenticated at the Department of Botany, S. V. University, Trurupati (AP), INDIA, by Dr. K. Madva Chetty. A voucher specimen No.HAK1/PRRMC 06-11 was deposited for future reference at the Department of Pharmacognosy, P. Rami Reddy Memorial College of Pharmacy, Tirupati (AP), INDIA.

EXTRACTION

The leaves were shade dried and powdered. The power was passes through 40 mesh sieve, defatted with hexane and extracted with ethanol by soxhlet and water (cold maceration). The ethanolic extract of Holostemma ada Kodien (EEHK) was evaporated and dried under reduced pressure. The percent yield was found to be 15.5% w/w.

PRELIMINARY PHYTOCHEMICAL STUDIES

Preliminary phytochemical analysis was done using the procedures of Khandelwal [11] and Kokate [12]. Preliminary phytochemical studies of EEEKH confirmed the presence of alkaloids, flavonoids, carbohydrates, tannins, steroids and proteins were found.

ANIMALS

Albino wistar rats of either sex weighing (200-250 g) were employed for study. They were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum.

ANTI-INFLAMMATORY ACTIVITY [13-18]

The anti-inflammatory activity was determined in albino rats of either sex according to the method of Winter et al [19]. Animal are divided into four groups of six rats in each group.

Group-1: Served as normal rats treated with 2 ml/kg of 1% NaCMC; Group-2: served standard group received Ibuprofen 10 mg/kg i.p; Group-3: Received EEEKH 200 mg/kg in 1% NaCMC per orally; Group-4: Received EEEKH 400 mg/kg in 1% NaCMC per orally.

One hour after oral administration of the extract, edema was induced to all the groups by injecting 0.1 ml of 1% carrageenan in 0.9% w/v in saline [13, 14] in the sub-plantar region of left hind paw of rats. All the doses were administered orally according to the body weight of the animals. Paw edema volume was measured with plethysmometer just before 0 (before drug challenge) 3rd, 6th and 24th hour after administration of drug. The percent inhibition of inflammation were calculated by using formula -

\[
\text{% of Inflammatory inhibition} = \frac{A - B}{A} \times 100
\]

Where A and B denote mean increase in paw volume of control and drug treated animals, respectively.

STATISTICAL ANALYSIS

Results were expressed as mean ± SEM, (n=6). Statistical analysis were performed with one way analysis of variance (ANOVA) followed by Student’s ‘t’ test by using Graph Pad Software.

RESULTS AND DISCUSSION

The percentage inhibition of edema of standard drug Ibuprofen at dose 10 mg/kg at 3, 6, 24 hours were 50.03%, 61.42%, 87.50% respectively. The percentage inhibition of edema at 200 mg/kg of EEEKH at 3, 6, 24 hours were 45.45%, 52.85%, 62.50% respectively. The percentage inhibition of edema at 400 mg/kg of EEEKH at 3, 6, 24 hours were 50.00%, 58.57%, 71.87%, respectively. From these results it was found that the extracts have anti-inflammatory activity in dose dependent manner, but EEEKH at 400 mg/kg showed comparable anti-inflammatory activity with that of standard drug Ibuprofen. The results were shown in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Edema volume (in ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>1</td>
<td>Control (2 ml of 1% NaCMC)</td>
<td>0.8 ± 0.12</td>
</tr>
<tr>
<td>2</td>
<td>Ibuprofen (10 mg/kg)</td>
<td>0.71 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>(50.03)</td>
<td>(61.42)</td>
</tr>
<tr>
<td>3</td>
<td>EEEKH (200 mg/kg)</td>
<td>0.82 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>(45.45)</td>
<td>(52.85)</td>
</tr>
<tr>
<td>4</td>
<td>EEEKH (400 mg/kg)</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>(50.00)</td>
<td>(58.57)</td>
</tr>
</tbody>
</table>

Table 1: Anti-inflammatory effect of EEEKH on Carrageenan-induced rat paw edema

Each value represents the mean ± S.E.M. (n=6), 
*P<0.001, **P<0.005 when compared with control
DISCUSSION

In carrageenan induced rat paw edema model, the anti-inflammatory effect of standard drug Ibuprofen 10 mg/kg, EEHK at two different doses 200 and 400 mg/kg was observed up to 24 hrs after carrageenan challenge. Carrageenan is known inflammatory agent and cause increase in prostaglandins and bradykinins synthesis at various time intervals. Carrageenan induced paw edema has been reported to have more than one phase and the initial has been attributed to the release of histamine and serotonin, the maintenance of edema during the plateau phase is caused by kinin like substance and the second accelerating phase of swelling are due to prostaglandin like substances [20].

Administration of EEHK at 200 mg/kg and 400 mg/kg showed decrease in paw edema volume from 3 to 24 hrs. Interestingly both dose levels of extract exhibited similar pattern in reducing carrageenan induced paw edema from 3rd to 24 hrs.

This observation clearly showed that the effect of various phytoconstituent of both extract might interact with the prostaglandins and leukotrienes spurt. Various researchers reported that Flavonoids inhibit eicosanoids synthesis by inhibiting both cyclooxygenase and lipoygenase activities [21-23], as well as hamper the non-enzymatic peroxidation of polysaturated fatty acids required for the activation of these oxygenases [24]. Quercetin and other flavonoids inhibit leukotrienes synthesis and histamine, prostaglandins release, as well as acts as superoxide scavengers [25, 26]. The extract contain number of chemical constituents like tannins, phenolic compounds, alkaloids, among them Quercetin and β-sitosterol [27] in EEHK were isolated. The presence of ortho-dihydro groups at B-ring and –OH substituent pattern at C5 position of the A-ring could significantly contribute to the anti-inflammatory and antioxidant activities of flavonoids may be responsible for inhibition of the phases of carrageenan induced paw edema, confirms the anti-inflammatory activity of the extract [28, 29].

REFERENCES

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