Investigation of Analgesic & Anti-Pyretic Potentials of Callicarpa Macrophylla Vahl. Leaves Extracts

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Authors declare no competing interests.

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Investigation of Analgesic & Anti-Pyretic Potentials of Callicarpa Macrophylla Vahl. Leaves Extracts

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Abstract

Leaves of Callicarpa macrophylla, an indigenous plant of India, had been the plant of study for the current research work. Aqueous as well as ethanolic extracts of C. Macrophylla leaves were evaluated for their analgesic as well as anti-pyretic effect using Tail Immersion Model and Brewer’s Yeast Induced Pyrexia Model respectively. Aqueous extract of leaves induced better analgesia and have anti-pyretic potential than ethanolic extract when compared to standard drugs. Combination of analgesia as well as anti-pyretic effect will ascertain its significant role in infection induced fever.

Introduction

Herbal Medicine also called botanical medicine or phytomedicine refers using plant’s seeds, berries, roots, leaves, stems, bark or flowers for medicinal purposes. The use of natural products with therapeutic properties is as ancient as human civilization [1-3] because medicinal plants are capable of synthesizing an overwhelming variety of low molecular weight organic compounds called secondary metabolites, usually with unique and complex structures [4]. India has a rich heritage of traditional medicine and the traditional health care system have been flourishing for many centuries [5]. Callicarpa macrophylla Vahl. (fam-Verbenaceae) is an erect shrub which is globally distributed across India, Nepal, Bhutan, Myanmar, South East Asia, and China. Previous to this study, we had evaluated anti-inflammatory activity of alcoholic and aqueous extracts of its leaves along with the analgesic as well as anti-inflammatory potential of its root extracts [6,7].

Methods

Collection & Authentication of Plant Material:

The drugs were collected from Banaras Hindu University campus, Varanasi and authenticated by Dr. V.K. Joshi, Dean of Faculty of Ayurveda, Institute of Medical Science, B.H.U., Varanasi and also through National Botanical Research Institute (NBRI), Lucknow. A Voucher specimen of all the plants has been preserved in the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad, for further references. The collected leaves were shade dried 15 days and size reduced by laboratory grinder in to coarse powder. The air dried coarse powder is used for preparation of extract. The experimental protocol was approved by the Institutional Animal Ethical Committee of College of Pharmacy, IFTM, Moradabad (857/AC/09/CPCSEA).

Preparation of Extracts:

The ethanolic and aqueous extracts were prepared according to the standard procedure [1, 8,9]. The filtered, extracts were dried in a vacuum evaporator and aqueous & alcoholic extracts were kept in desiccators until further use.

Animals:

Male / female albino rats weighing between 120 to 150 grams, from Animal House, College of Pharmacy, IFTM, Moradabad, were divided in ten groups of six animals each. The animals were kept in polypropylene cages, under standard condition of 12:12 light and dark cycle.

Evaluation of Anti-pyretic Potential Using Brewer’s Yeast Induced Pyrexia Model:

The antipyretic activities of the ethanolic and aqueous extracts were evaluated using Brewer’s yeast-induced pyrexia in rates (Balamurugan et al., 2009; Vogel et al., 2002). Prior to experiments, rates were maintained in separate case for 7 days and with those rate approximately constant rectal temperatures were selected for the study. Pyrexia was induced by injecting 10 ml/kg (s.c) of 15% w/v aqueous suspension of Brewer’s yeast in normal saline below the nape of the neck. Immediately after yeast administration, food was withdrawn. The rectal temperature of each rat was recorded by using a telethermometer immediately before (-18h) and 18h after (0 h) Brewer’s yeast injection. The different groups were treated with the vehicle, ethanolic and aqueous extract (200 and 400 mg/kg), and standard drug, paracetamol (150 mg/kg). Tween 80 (1% v/v) was used as suspending agent. The rectal temperature was then recorded again 30, 60, 120, 180 and 240 min post dosing.
Evaluation of Analgesic Potential Using Tail Immersion Model:
Rats (six per group) were used. Rats was administered orally with vehicle (3ml/kg), pentazocine (30mg/kg), ethanolic, aqueous extract (200 and 400 mg/kg) of leaves and roots. The distal part of tails (3cm) of the animals was immersed in hot water at a temperature of 55±0.5°C. The time taken to withdraw the tail was noted as reaction time with a stopwatch. A cut off time of 10 sec was maintained at 55 ±0.5°C to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45 and 60 min after treatment, respectively.

Data analysis and statistics:
The values were expressed as mean ± standard error mean (SEM). Statistical analysis of the data was carried out by two way ANOVA followed by bonferroni test to determine the significant between two groups p<0.05 was considered significant.

Results & Discussion
A significant reduction of the painful sensation due to tail immersion in warm water was observed followed oral administration of the ethanolic and aqueous extract at dose of 200, 400mg/kg of leaves of C. macrophylla Vahl. The effect was found to the dose dependent. In this model, higher dose of the aqueous extract (400mg/kg) at an interval of 60 min has exhibited better analgesic activity than the standard drug. Representation of result of analgesic activity was shown in Table 1. Several flavonoids isolated from medicinal plant have been discovered to possess significant analgesic effects[10-12]. The analgesic activity of ethanolic, aqueous extract of leaves and roots of C. macrophylla Vahl. may be due to the presence of flavonoid compounds.

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 18 h of administration to rats. Treatments with ethanolic, aqueous extract of leaves roots at doses of 200 mg/kg, and 400mg/kg decreased the rectal temperature in a dose-dependent manner. The antipyretic effect started from the first hour and was maintained for 4h, after administration of ethanolic, aqueous extract. The result obtained from paracetamol and ethanolic, aqueous extract treated rats were compared with control group, produced significant antipyretic activity (p< 0.05) yeast induced elevated rectal temperature in rats , represented by Table 2. In general, non-steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus [13]. Therefore, the antipyretic activity of ethanolic, aqueous extracts of C. macrophylla Vahl. is probably by inhibition of prostaglandin synthesis in hypothalamus. Further, ethanolic, aqueous extract of C. macrophylla Vahl. was found to contain carbohydrates, steroids, flavonoids and tannins, through preliminary phytochemical screening. The antipyretic activity may be due to one/more group of above phytoconstituents.

Acknowledgement(s)

Authors would like to express our gratitude to the Management of IFTM University, India for providing us facilaities to execute this research program.

References


### Illustrations

#### Illustration 1

Table 1 Effect of ethanolic, aqueous extract of C. macrophylla Vahl. leaves on pain using tail immersion test in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Average tail withdrawing time (s)</th>
<th>0 min</th>
<th>15 min</th>
<th>30 min</th>
<th>45 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td></td>
<td>4.11 ± 0.20</td>
<td>4.25 ± 0.19</td>
<td>4.20 ± 0.22</td>
<td>4.32 ± 0.09</td>
<td>4.28 ± 0.18</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>30</td>
<td></td>
<td>4.52 ± 0.20</td>
<td>6.66 ± 0.28*</td>
<td>8.63 ± 0.16*</td>
<td>8.72 ± 0.48*</td>
<td>9.48 ± 0.19*</td>
</tr>
<tr>
<td>LEE</td>
<td>200</td>
<td></td>
<td>4.63 ± 0.26</td>
<td>4.89 ± 0.35</td>
<td>5.32 ± 0.28*</td>
<td>6.71 ± 0.31*</td>
<td>7.42 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td></td>
<td>4.41 ± 0.17</td>
<td>5.58 ± 0.31*</td>
<td>5.92 ± 0.24*</td>
<td>8.20 ± 0.32*</td>
<td>8.38 ± 0.23*</td>
</tr>
<tr>
<td>LAE</td>
<td>200</td>
<td></td>
<td>4.37 ± 0.31</td>
<td>5.36 ± 0.25*</td>
<td>5.96 ± 0.20*</td>
<td>7.65 ± 0.09*</td>
<td>8.30 ± 0.20*</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td></td>
<td>4.56 ± 0.18</td>
<td>6.10 ± 0.36*</td>
<td>6.72 ± 0.36*</td>
<td>8.93 ± 0.18*</td>
<td>9.60 ± 0.19*</td>
</tr>
</tbody>
</table>
Illustration 2

Table 2 Effect of ethanolic, aqueous extract of C. macrophylla Vahl. leaves on Brewer's yeast induced pyrexia in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg</th>
<th>Rectal temperature in °C at various times (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>18h</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>37.12±0.05</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>150</td>
<td>37.07±0.03</td>
</tr>
<tr>
<td>LEE</td>
<td>200</td>
<td>37.15±0.04</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>37.1±0.05</td>
</tr>
<tr>
<td>LAE</td>
<td>200</td>
<td>37.26±0.07</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>33.33±0.03</td>
</tr>
</tbody>
</table>
Reviews

Review 1

Review Title: Investigation of Analgesic & Anti-Pyretic Potentials of Callicarpa Macrophylla Vahl. Leaves Extracts

Posted by Dr. Pankaj Jain on 20 Jun 2012 05:51:00 PM GMT

What are the main claims of the paper and how important are they?:
Article reported the evaluation of Analgesic and Antipyretic Potentials of indigenous plant Callicarpa macrophylla. This report is really worth for the phytochemist as they can now have reason to isolate the active phytoconstituents for the reported activity.

Yes, claims are novel.

In previous literature, no one have scientifically claim analgesic and antipyretic potential of C. macrophylla leaves.

Yes

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?
No, it is not required here now.

Yes, it is scientifically valid.

No, but further research can be certainly encouraged.

No, but further research can be certainly encouraged.

Rating: 8

Comment:
It would like to encourage all the scientists to work more on the natural products as they are structurally more precise and significant for the specific active site of target receptor.

Competing interests: No

Invited by the author to make a review on this article?: Yes

Have you previously published on this or a similar topic?: No

Experience and credentials in the specific area of science:
We have done some significant studies on natural products.

Review 2

Review Title: Animal study of analgesic & antipyretic plant extract

Posted by Faculty Mr. Krishna Kumar Govindarajan on 05 Jun 2012 05:34:12 AM GMT

What are the main claims of the paper and how important are they?:
Claims:
1. Use of natural plant remedies for common ailments like pain / fever
2. To test the efficacy of the extract of leaves

Importance:
Establishment of plant extracts for common ailments forms the basic step in evaluation

Although use of plant products is encouraging, the pharmacological profile of the extracts need to be established before proceeding further.

yes

Pharmacodynamics of the plant extracts along with the complete list of constituents need to be established to add strength to the study.

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?
NA

yes

Extract analysis to document the constituents and pharmacodynamics would be useful

Extract analysis to document the constituents and pharmacodynamics would be useful

Rating: 5
Comment:
NA

Competing interests: No
Invited by the author to make a review on this article? : No
Have you previously published on this or a similar topic?: No
Experience and credentials in the specific area of science:
Drug potency and quantity evaluation comparing generic and hospital supply in a ICMR project

Review 3

Review Title: Investigation of anlgesic & anti-pyretic potencial of Callicarpa Macrophylla Vahl. leaves extracts

Posted by Lead Faculty Prof. Valcinir Bedin on 05 Jun 2012 12:55:18 AM GMT

What are the main claims of the paper and how important are they?:
To prove the use of native botanical drug. It is very important for developing countries to find this type of medicine.

Yes

Yes. As far as the knowledge in this kind of subject is new, the work is welcome.

I think so.

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?
No, the work was wll conducted

Yes

Of course we are waiting for the following paper, using anima nobili.

Of course we are waiting for the following paper, using anima nobili.

Rating: 8

Comment:
I think we should encourage more papers of this kind, especially in developing countries.

Competing interests: no

Invited by the author to make a review on this article?: No

Have you previously published on this or a similar topic?: No

Experience and credentials in the specific area of science:
I am a medicine professor in a developing country

Review 4

Review Title: Investigation of Analgesic & Antipyretic Potentials of Callicarpa Macrophylla Vahl Leaves Extracts

Posted by Prof. Thomas V Chacko on 04 Jun 2012 03:47:32 PM GMT

What are the main claims of the paper and how important are they?:
Analgesic and antipyretic effect of plant extracts is claimed.

Already many analgesic and antipyretic preparations are available and so the need for testing this or advantages of this over some existing preparations has not been clearly stated.

none

For this to be made clear, statisticat tests of significance needs to be applied

Dose response relationship also needs to be established or clearly stated

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?

none

Yes but the concentration of the active principle in the herbal powder is not established

Above mentioned suggestions if incorporated would improve the paper

Above mentioned suggestions if incorporated would improve the paper

Rating: 3

Comment:

none

Competing interests: None

Invited by the author to make a review on this article?: No

Have you previously published on this or a similar topic?: No

Experience and credentials in the specific area of science:
familiar with epidemiological study designs and with Pharmacognosy

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