ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF AZADIRACHTA INDICA LEAVES ON EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

BACKGROUND
To study the anti-inflammatory action of ethanolic extract of Azadirachta indica (EEAI) leaves on experimental animal models (both acute and chronic).

MATERIALS AND METHODS
The extract of Azadirachta indica leaves was prepared by percolation method using 95% ethanol. Acute oral toxicity test of the extract was performed as per OECD 423 guidelines. Acute inflammation was studied by carrageenan-induced rat paw oedema method and paw volumes were measured plethysmometrically at one hour interval for 4 hours. Activity against chronic inflammation was studied by Freund’s complete adjuvant induced arthritis method. Paw volumes were measured on 1st day, 5th day (injected paws) and on 21st day (non-injected paw). Aspirin 100 mg/kg was taken as the standard drug. A control group is maintained in both the models.

RESULTS
The results were analysed by ANOVA followed by Dunnett’s multiple comparison test. EEAI in doses of 200, 400 and 800 mg/kg produced dose-dependent and significant (p < 0.05) reduction of paw oedema in carrageenan-induced acute inflammation in comparison to control. EEAI also found effective in chronic arthritis model in dose dependent manner.

CONCLUSION
The present study indicates that EEAI has significant anti-inflammatory activity in both the models.

KEYWORDS
Azadirachta Indica, Carrageenan, Adjuvant Induced Arthritis, Anti-Inflammatory Activity.

**Animals**
Healthy albino rats of Wistar strain of either sex weighing 150-200 gm each were procured from the Central Animal House, Assam Medical College and Hospital, Dibrugarh, Assam. The study was conducted in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals) guidelines and the study was approved by the Institutional Animal Ethics Committee (Registration No. 634/02/a/CPCSEA). Rats were kept on standard laboratory diet and water ad libitum.

**Acute Oral Toxicity Study**
Acute toxicity test was performed for the ethanolic extract of Azadirachta indica following OECD 423 guidelines.\(^1\) As no mortality was recorded up to maximum dose of 2000 mg/kg. Hence, 1/10\(^{th}\) of the maximum dose, i.e. 200 mg/kg is selected as minimum dose for the study.

**Anti-Inflammatory Studies**
For each experiment, the animals were divided into 5 groups with 6 animals in each group.
- **Group A (control)** received 3% gum acacia 10 mL/kg p.o.
- **Group B1 (Test)** received EEAI leaf extract 200 mg/kg p.o.
- **Group B2 (Test)** received EEAI leaf extract 400 mg/kg p.o.
- **Group B3 (Test)** received EEAI leaf extract 800 mg/kg p.o.
- **Group C (standard)** received aspirin 100 mg/kg p.o.

**Anti-Inflammatory Study Against Acute Inflammation**
Acute inflammation was produced by subplantar injection of 0.1 mL of freshly prepared 1% carrageenan suspension in normal saline in the right hind paw of the rats.\(^1\) Paw volume was measured plethysmographically as described by Chattopadhyay et al at 0' hour and then at the end of 1st, 2nd, 3rd and 4th after carrageenan injection.\(^10\) The volume of oedema was recorded as the difference between the paw volume at 0' hour and at the end of each hour. The drugs were administered orally in the respective groups one hour before carrageenan injection. The percentage inhibition of the rat paw oedema was calculated after each hour of carrageenan injection up to 4 hours by the following formula.\(^12\)

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\% \text{ inhibition } = \frac{(\text{Control mean} - \text{treated mean})}{\text{Control mean}} \times 100
\]

### Table 1. Anti-Inflammatory Effect of Ethanolic Extract of Azadirachta indica on Carrageenan Induced Rat Paw Oedema at the End of 1st, 2nd, 3rd and 4th hour

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug</th>
<th>Dose, Oral, Single Dose</th>
<th>1st hr.</th>
<th>2nd hr.</th>
<th>3rd hr.</th>
<th>4th hr.</th>
<th>Mean Increase in Paw Oedema (Mean ± SEM) in mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3% gum acacia</td>
<td>10 mL/kg</td>
<td>0.36 ± 0.04</td>
<td>0.43 ± 0.05</td>
<td>0.46 ± 0.05</td>
<td>0.49 ± 0.05</td>
<td>0.18 ± 0.05 * (20.9%)</td>
</tr>
<tr>
<td>B1</td>
<td>EEAI</td>
<td>200 mg/kg</td>
<td>0.29 ± 0.01* (13.6%)</td>
<td>0.32 ± 0.01* (20.1%)</td>
<td>0.34 ± 0.02* (20.9%)</td>
<td>0.35 ± 0.02* (22.6%)</td>
<td>0.18 ± 0.01* (20.9%)</td>
</tr>
<tr>
<td>B2</td>
<td>EEAI</td>
<td>400 mg/kg</td>
<td>0.23 ± 0.01* (33.2%)</td>
<td>0.26 ± 0.01* (36.3%)</td>
<td>0.23 ± 0.01* (47.3%)</td>
<td>0.21 ± 0.01* (53.5%)</td>
<td>0.18 ± 0.01* (53.5%)</td>
</tr>
<tr>
<td>B3</td>
<td>EEAI</td>
<td>800 mg/kg</td>
<td>0.22 ± 0.01* (37.9%)</td>
<td>0.24 ± 0.01* (41.9%)</td>
<td>0.21 ± 0.01* (52.9%)</td>
<td>0.20 ± 0.01* (58.1%)</td>
<td>0.16 ± 0.01* (58.1%)</td>
</tr>
<tr>
<td>C</td>
<td>Aspirin</td>
<td>100 mg/kg</td>
<td>0.20 ± 0.01* (40.6%)</td>
<td>0.22 ± 0.01* (44.7%)</td>
<td>0.18 ± 0.01* (59.6%)</td>
<td>0.16 ± 0.01* (65.8%)</td>
<td>0.18 ± 0.01* (65.8%)</td>
</tr>
</tbody>
</table>

**One-Way ANOVA**

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>Df</th>
<th>p</th>
<th>1st hr.</th>
<th>2nd hr.</th>
<th>3rd hr.</th>
<th>4th hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12.5</td>
<td>5</td>
<td>&lt;0.05</td>
<td>0.18 ± 0.01* (59.6%)</td>
<td>0.16 ± 0.01* (65.8%)</td>
<td>0.15 ± 0.01* (62.8%)</td>
<td>0.13 ± 0.01* (60.8%)</td>
</tr>
</tbody>
</table>

The data were statistically analysed using ANOVA (One-Way analysis of variance) followed by Dunnett’s multiple comparison test. P values < 0.05 were considered significant.

**Study of Anti-Inflammatory Action in Chronic Inflammation**
The anti-inflammatory activity of EEAI against chronic inflammation was tested by Freund’s adjuvant-induced arthritis method in rats.\(^13\) On 1st day, 0.1 mL of complete Freund’s adjuvant is injected into the subplantar region of the right hind paw of the rats. Treatment with the test and standard drugs to the respective groups was started on the same day and continued for 12 days. The paw volume on both sides was measured plethysmographically on the day of injection. The body weight of the rats was also recorded on the first day. On 5th day, the volume of the injected paw was measured again indicating the primary lesion and the effect of the therapeutic agent on this phase. The severity of the induced adjuvant disease was followed by measurement of the volume in non-injected paw (secondary lesions) with a plethysmometer. Purposely, from day 13 to 21, the animals were not administered with the test drug or the standard. On day 21, the non-injected paw volume and the body weight were determined again indicating the primary lesion and the effect of the therapeutic agent on this phase. The severity of the induced adjuvant disease was followed by measurement of the volume in non-injected paw (secondary lesions) with a plethysmometer. Purposely, from day 13 to 21, the animals were not administered with the test drug or the standard. On day 21, the non-injected paw volume and the body weight were determined again indicating the primary lesion and the effect of the therapeutic agent on this phase.

Statistical Analysis was done using one-way ANOVA followed by Dunnett’s multiple comparison test. P value <0.05 was considered as significant.

1. For primary lesions: The percentage inhibition of paw volume of the injected right paw over control was measured at day 5.
2. For secondary lesions: The percentage inhibition of paw volume of non-injected left paw over control was measured at day 21.

An Arthritis index was calculated as the sum of the scores as indicated above for each animal.\(^13\)

DISCUSSION
Carrageenan–induced hind paw edema in rats is the standard model of acute inflammation. It is a biphasic response. The first phase is mediated through release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin–like substances which peak at 3 hours. The results of the present study suggest that EEA1 in doses of 200, 400 and 800 mg/kg significantly suppressed carrageenan–induced paw edema in rats when compared to control. Maximum anti-inflammatory activity was observed at 3rd and 4th hours of carrageenan injection. The probable cause of anti-inflammatory action EEA1 in acute inflammation might be due to the inhibition of some of the mediators release within 3 hours of carrageenan injection.

Freund’s complete adjuvant-induced arthritis is a widely used model of chronic inflammation. This model is widely utilised because of the strong correlation between the efficacy of therapeutic agents used in this model and in rheumatic arthritis in human. EEA1 in all doses significantly reduced adjuvant induced paw edema. The leaves of Azadirachta indica are rich in flavonoids. Flavonoids have demonstrated significant anti-inflammatory activity. Hence, anti-inflammatory action of EEA1 might be due to the action of flavonoids.

Adjuvant induced arthritis is characterised by increased production of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1 (IL-1) associated with loss of body weight. These cytokines influence the hormones that regulate metabolism and also act directly on the target organs like muscle, liver, gut and brain. Thus, arthritis induced reduction in body weight can be prevented by EEA1 and it might be due to inhibition of TNF-α and interleukin-1.

CONCLUSION
The leaves of Azadirachta indica has showed significant anti-inflammatory activity against both acute and chronic inflammation models at all the tested doses establishing its traditional use in acute inflammatory conditions and rheumatism. Hence, further studies are required to establish and elaborate its molecular mechanism and proper clinical utility.

ACKNOWLEDGEMENTS
We are thankful to Prof. L. R. Saikia, Department of Life Sciences, Dibrugarh University for helping us with taxonomical identification of the plant. We are also thankful to the faculty and laboratory staff members of Pharmacology Department of Assam Medical College and Hospital, Dibrugarh for their support and encouragement.

REFERENCES

\[
\begin{array}{|c|c|c|c|c|c|}
\hline
\text{Group} & \text{Drug} & \text{Dose} & \begin{array}{c}
\text{Mean Increase in Paw Volume (mean±SEM) in ml} \\
\text{(% Inhibition in Parentheses)}
\end{array} & \text{Weight Change on 21st Day} & \text{Arthritis Index} \\
\hline
\text{A} & \text{Control} & \begin{array}{c}
\text{(3% gum acacia)}
\end{array} & \begin{array}{c}
0.91±0.02 \\
(-)
\end{array} & \begin{array}{c}
0.52±0.02 \\
(-)
\end{array} & -28±0.32 & 7.8±0.24 \\
\text{B1} & \text{EEAI} & \begin{array}{c}
200 \\
\text{mg/kg}
\end{array} & \begin{array}{c}
0.71±0.03 a \\
(22%)
\end{array} & \begin{array}{c}
0.20±0.02 a \\
(62.14%)
\end{array} & -15±0.21 a & 5.9±0.16 a \\
\text{B2} & \text{EEAI} & \begin{array}{c}
400 \\
\text{mg/kg}
\end{array} & \begin{array}{c}
0.64±0.12 a \\
(29.80%)
\end{array} & \begin{array}{c}
0.15±0.04 a \\
(71.15%)
\end{array} & -10±0.3 a & 5.1±0.12 a \\
\text{B3} & \text{EEAI} & \begin{array}{c}
800 \\
\text{mg/kg}
\end{array} & \begin{array}{c}
0.58±0.02 a \\
(36.2%)
\end{array} & \begin{array}{c}
0.12±0.02 a \\
(76.92%)
\end{array} & -7±0.31 a & 4.5±0.14 a \\
\text{C} & \text{ASPIRIN} & \begin{array}{c}
100 \\
\text{mg/kg}
\end{array} & \begin{array}{c}
0.56±0.02 a \\
(39.19)
\end{array} & \begin{array}{c}
0.11±0.02 a \\
(80.67%)
\end{array} & -5.0±0.14 a & 5.2±0.10 a \\
\text{One-Way ANOVA} & \text{F} & \text{4.5} & \text{< 0.05} & \text{< 0.05} & \text{< 0.05} & 96.32 \text{< 4.25} & 4.25 \\
\text{df} & \text{4.25} & \text{< 0.05} & \text{< 0.05} & \text{< 0.05} & \text{< 0.05} & 66.80 \text{< 4.25} & 4.25 \\
\hline
\end{array}
\]

\text{n} = 6 rats in each group. (-) indicates no inhibition. *p < 0.05 when compared to control. One-Way ANOVA followed by Dunnett’s multiple comparison test is done.


