Evaluation of Analgesic Activity of Aqueous Extract of Aerial part of *Erythrina indica* in Rodents

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ABSTRACT

The aim of the study was to investigate analgesic, anti-inflammatory and anti-arthritic activities of aqueous extract of aerial parts of *Erythrina indica* (AEEI). AEEI was evaluated for both peripheral and central analgesic activities by acetic acid induced writhing, tail immersion and hot-plate methods. The AEEI exhibited significant analgesic activities on experimental animals and the results were comparable to those observed with standard drugs. The analgesic action of AEEI can be attributed to its active constituents such as alkaloids, flavonoid and tannins which are known to act through inhibition of prostaglandins biosynthesis.

Key words: *Erythrina indica*, analgesic, Eddys Hot-Plate.

INTRODUCTION

Nociception has been defined as an unpleasant though protective sensory experience and causes discomfort while inflammation is a local response of living mammalian vascularized connective tissue to injury due to various exogenous and endogenous stimuli thus, producing defensive reaction to eliminate or limit the spread of injurious agent and subsequent removal of necrotic cells and tissues [1]. Inflammation-associated pain is a major cause of morbidity worldwide and considered as the king of human miseries [2]. Inflammatory component of Rheumatoid Arthritis (RA) is chronic in nature and believed to be of autoimmune origin that is driven primarily by activated T-cells. In addition, it is typified by white blood cells (WBC) infiltration into synovium thereby accelerating cartilage destruction [3]. Current pharmacologic treatment includes steroidal and non-steroidal analgesics and anti-inflammatory agents to alleviate pain and reduce inflammation [4]. Present-day physicians heavily prescribe these synthetic agents in various clinical settings. However, these agents are associated with some serious side effects. For instance, non-
steroidal anti-inflammatory agents (NSAIDs) may produce gastric ulceration and hemorrhage on prolonged oral administration whereas steroidal agents have been documented for producing respiratory depression and constipation. Plant sources are being explored scientifically to find new leads for the treatment of pain and inflammation and formulate them as effective analgesic and anti-inflammatory drugs with minimal side effects.

The *Erythrina indica* (Fabaceae), a quick growing tree reaching about 15 m in height and with a spread of 12 m is distributed widely in deciduous forests throughout India. It also grows in gardens as an ornamental plant and used to support black pepper vine, commonly found in Bengal, Southern India, Sri Lanka, Burma, Malacca and Andaman and Nicobar islands [5].

Previous phytochemical screening of *Erythrina indica* revealed the presence of alkaloids, flavonoids and terpenoids [5-7]. The Erythrina indica was traditionally used in the treatment of diabetes, rheumatic pain, ear and toothache, burns, cuts and wounds and was also used in inflammatory and mental disorders [8]. The plant is reported to have spasmolytic, smooth muscle relaxant, CNS-depressant, antibilious, and expectorant activities.

Based on folklore medicinal usage, aqueous extract of aerial part of *Erythrina indica* (AEEI) was investigated for its analgesic, anti-inflammatory and anti-arthritic properties in small laboratory animals.

**MATERIALS AND METHODS**

**Drugs and chemicals**

The aqueous extract of aerial parts of *Erythrina indica* (AEEI) was obtained as a gift sample from Green Chem Herbal Extracts and Formulations, Bangalore, India. The carrageenan was purchased from SD fine chem, Mumbai. All other chemicals used were of analytical grade.

**Animals**

Healthy albino Wistar rats of either sex, weighing between 150-200 g and Swiss albino mice of either sex weighing between 20-25 g were procured from NIMHANS, Bangalore and Shri Venkateshwara Enterprises, Bangalore were used in these studies. They were housed in institutional animal house in polypropylene cages under controlled conditions of temperature (23±2°C), humidity (55±5%) and 12 h light and 12 h dark cycles, and were fed with free access of food and water. All experimental protocols were approved by Institutional animal ethics committee.

**Grouping of animals**

For acetic acid induced writhing and hot plate methods the Swiss albino mice were divided into five groups of 6 animals in each group. Group 1 serve as control and group 2 was pretreated with standard diclofenac sodium 15 mg/kg and group 3 to 5 were pretreated with 100, 200 and 400 mg/kg body weight p.o. of AEEI respectively.

For tail immersion, carrageenan induced inflammation and cotton wool granuloma method, the Albino wistar rats were divided into five groups of six animals each. Group 1 served as control group, group 2 was treated with standard diclofenac sodium 15 mg/kg and group 3 to 5 were treated with 100, 200 and 400 mg/kg body weight p.o. of AEEI respectively.

**Acute oral toxicity study**
Acute oral toxicity study for the test extract (AEEI) was carried out using OECD guideline 425 [9].

**Analgesic activity**

**Acetic acid induced abdominal constrictions in mice:** Writhing was produced according to the method of Koster et al. (1959) [10]. The 0.1 ml of 0.6% i.p. injection of acetic acid resulted in constriction of abdominal muscle together with a stretching of hind limbs. All the drugs were administered orally, 30 min prior to acetic acid injection. The numbers of writhing movements were counted for 20 min, from the time immediately after acetic acid injection [11-12]. The percentage protection of abdominal contraction was also calculated as follows:

\[
\text{Percentage protection} = \left(\frac{\text{Writhings in control} - \text{Writhings in test}}{\text{Writhings in control}}\right) \times 100.
\]

**Hot plate method in mice:** The animals were placed on Eddy’s hot plate kept at temperature 55±0.5°C. A cut off period of 15 sec was observed to avoid damage to the paws. The delay in response time (Jumping and hind paw licking response) of animals was recorded at 0, 30, 60, 90 and 120 min after oral administration of drugs [13]. Percentage protection against thermal pain was also calculated by applying the formula:

\[
\text{Percentage protection} = \left(\frac{\text{Ta} - \text{Tb}}{\text{Tb}}\right) \times 100
\]

Where, \( \text{Ta} \) – Mean reaction time of test and \( \text{Tb} \) – Mean reaction time of control

**Tail immersion method:** In this method the animals were placed into individual restraining cages leaving the tail hanging out free. The animals are allowed to adapt to the cages for 30 min before testing. In this method the lower 5 cm portion of tail was marked and this part of tail was immersed in a hot water of 55±0.5°C.

The pain threshold (time required for withdrawn of tail from hot water) was measured at 0, 1, 2, 3 and 4 h. A maximum immersion time of 15 sec was maintained to prevent thermal injury to the animal [12,14]. Percentage increase in reaction time was also calculated as follows: \( \text{PI} = \left(\frac{\text{Rt}-\text{Rc}}{\text{Rt}}\right)\times 100 \), where \( \text{Rt} \) is reaction time of test or standard group and \( \text{Rc} \) is reaction time of control group.

**Statistical analysis**

Values are expressed in mean±SD for six animals in each group. \( P \) value was calculated using ANOVA followed by Tukey’s post hoc test for multiple comparisons. Values of \( P<0.05 \) were considered significant in all cases.

**RESULTS**

**Acute oral toxicity study**

The AEEI was found to be safe up to dose of 2000 mg/kg b.w. without produce any mortality and other toxic effects.

**Analgesic activity**

**Acetic acid induced abdominal constrictions:** AEEI significantly reduces the numbers of abdominal contractions produced by acetic acid compared to control group. Maximum inhibition of writhing response was observed with AEEI-400 mg/kg, which was comparable to standard diclofenac sodium mg/kg (Table 1).

**Tail immersion:** As shown in Table 1, the mean reaction time in standard and AEEI (100, 200 and 400mg/kg) treated animals was significantly increased, and the increase in reaction time by AEEI at higher of 400mg/kg treated group was found to be comparable to that of standard diclofenac sodium mg/kg. A dose dependent analgesic activity was observed with AEEI.
**Hot plate method:** The results of hot-plate test indicated a significant increase in reaction time at 2 and 3 h with 200 and 400 mg/kg AEEI, whereas the standard diclofenac sodium 15 mg/kg significantly increased the reaction time at 1 and 2 h (Table 2). The AEEI was found to dose dependently cause prolongation of hot plate latency.

**DISCUSSION AND CONCLUSION**

The pain is a major cause of morbidity of the working force throughout the world. This has been called the king of human miseries. Inflammation is predominantly mediated by arachidonic acid metabolites.

The results of AEEI indicates analgesic activity in both peripheral and central analgesic models, in a dose dependent manner. The abdominal contraction response induced by acetic acid, is a sensitive procedure to establish peripherally acting analgesics, it cause increase in concentration of PGE2 and PGF2α in the peritoneal fluid, which stimulate peripheral nociceptive neurons [15,16]. Thermal induced nociception indicates narcotic involvement. The tail immersion and hotplate methods have found to be suitable for evaluation of centrally acting analgesics. The nociceptors seem to be sensitized by sensory nerves [15,17]. The involvement of endogenous substances such as PGs may be minimized in this model. The ability of the extracts to prolong the reaction latency to thermally induced pain (Hot plate test) in mice further suggests central analgesic activity. Thermal nociceptive tests are sensitive to opioid (μ) receptors [18].

The literature survey revealed the presence of alkaloids, flavones-glycosides, isoflavones, β-sitosterol and triterpenoids compounds in the *Erythrina indica*. Flavonoids are known to inhibit enzyme prostaglandin synthesis, arachidonic acid metabolizing enzyme such as phospholipase-A2, COX, LOX and nitric oxide producing enzyme [19,20]. The inhibition of these enzymes by flavonoids decreases the production of inflammatory mediators. In the light of the above findings, we presume that the presence of alkaloids, flavonoids and triterpenoids may contribute for analgesic activity [21] of aqueous extract of aerial part of *Erythrina indica*.

The results of our studies suggest that AEEI may be a good natural source for the treatment of nociception or may be useful in the development of new analgesic and anti-inflammatory therapies.

**ACKNOWLEDGMENT**

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**REFERENCES**


ANNEXURE

Table 1: Analgesic effect of AEEI by acetic acid induced writhing and tail immersion methods

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of writhes in 20 min.</th>
<th>Reaction time at different time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>35.33±5.42</td>
<td>2.02±0.2</td>
</tr>
<tr>
<td>Diclofenac-15mg/kg</td>
<td>7.83±1.47***</td>
<td>2.08±0.15</td>
</tr>
<tr>
<td>AEEI-100 mg/kg</td>
<td>27.66±2.42***</td>
<td>1.99±0.05</td>
</tr>
<tr>
<td>AEEI-200 mg/kg</td>
<td>19.66±3.01****</td>
<td>2.20±0.21</td>
</tr>
<tr>
<td>AEEI-400 mg/kg</td>
<td>12.66±2.80****</td>
<td>2.18±0.23</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6), values in square brackets indicate percentage inhibition of writhing reflexes, values in parenthesis indicate percentage increase in reaction time. a*P<0.05, a**P<0.01, a***P<0.001 significant when compared to control group, ‘b’ nonsignificant when compared to standard group.

Table 2: Effect of AEEI on hot plate latency in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean latency time (sec.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>2.13±0.21</td>
</tr>
<tr>
<td>Diclofenac 15 mg/kg</td>
<td>4.10±0.17***</td>
</tr>
<tr>
<td>AEEI-100 mg/kg</td>
<td>2.36±0.31</td>
</tr>
<tr>
<td>AEEI-200 mg/kg</td>
<td>2.36±0.27</td>
</tr>
<tr>
<td>AEEI-400 mg/kg</td>
<td>3.16±0.19***</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=6), ***P<0.001 significant as compared to control.