STUDY OF ACUTE TOXICITY AND ANTI INFLAMMATORY ACTIVITY OF Andrographis paniculata Nees (ACANTHACEAE)

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Abstract- The aim of the experiment was to study the anti-inflammatory activity of methanolic extract of Andrographis paniculata Nees on a suitable animal experimental model. This plant has been traditionally used for treatment of respiratory diseases, high blood pressure, skin diseases etc. Anti-inflammatory activity of the aqueous extract of the plant was observed using carrageenan induced rat paw oedema. The test extract at dose 200 mg/kg of rat body weight exhibited 55.10 % inhibition of rat paw oedemas. The methanolic root extract displayed a significant anti-inflammatory activity.

Keywords: Carrageenan, inflammation, methanolic extract, rat paw oedema,

Introduction

Inflammation is the defense mechanism adopted by the body against free radicals, accompanied with symptoms of pain, heat, redness, swelling as well as loss of function at the site of inflammation (Moldoveanu et al., 2009). Nitric oxide synthases (NOSs) are a family of enzymes that are responsible for the synthesis of nitric oxide (NO) from the amino acid L-arginine in the body. The expression of inducible NOS (iNOS) can only be induced by inflammatory stimuli and contribute to the large amount of NO production (Yu et al., 2018). Nitric oxide (NO) is recognized as an important intracellular and intercellular biological active molecule that acts diverse physiological and patho-physiological functions in the body, including cardiac contractility and regulation of vasodilation (Vanhoutte, 2018).

Andrographis paniculata Nees (Acanthaceae), occurring in South India and South East Asia (called as kalmegh and chuanxinlian, respectively), is an important ingredient used in herbal medicinal preparations (including, anti-inflammatory) in both traditional Indian and Chinese medicine (Chao and Lin, 2010; Akbar, 2011). The present study aims at evaluating the anti-inflammatory activity of A. paniculata extract, thereby attempting to verify the textual and orally transmitted claims.

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Materials and methods:
Plant Sample: The root bark of *Andrographis paniculata* Nees was collected in July from Sivasagar district (Assam). The plant material was identified by Dr. Pankaj Chetia, Assistant Professor, Department of Life Sciences, Dibrugarh University, Dibrugarh (Assam, India).

Extraction Methods: The root bark of *Andrographis paniculata* was shade-dried for 3 weeks, mechanically crushed into fine powder and stored in an airtight container for further studies. The powder was introduced into the chamber (extractor) and extracted with methanol and continued till the dark green color of the leaf of the plant became colourless. After extraction, the concentrated solvents of distilled pot are evaporated in rotary evaporator.

Experimental animals: Healthy adult, male albino rats (Wistar Strain) weighing between 100-115 grams were obtained from M/S Chakraborty Enterprise, Kolkata, India. The animals were acclimatized under laboratory condition for 2 weeks before starting the experiments. They were provided standard diet and water *ad libitum* and maintained under standard conditions of temperature (24± 1˚C) and humidity (50%) with an alternating 12 hour light / dark cycles.

Acute toxicity testing: Acute oral toxicity studies was carried out by OECD Guideline # 423 organization for Economics Corporation and Development (OECD, 2001).

Anti-Inflammatory Activity using Carrageenan Induced Paw oedema in Rats:
The anti-inflammatory activities of *Andrographis paniculata* Nees was determined by the λ-carrageenan-induced oedema test in the hind paws of mice (Vinegar et al., 1969).

Experimental Design:
The animals were divided into three groups of two animals in each groups.

Group 1: Disease Control (Rats were treated with 0.05 ml of Carrageenan)

Group 2: Rats were given Aceclofenac (100 mg/kg body weight)

Group 3: Rats were treated with methanolic extract of *Andrographis paniculata* (MEAP) (200 mg/kg body weight).

Results
Acute cytotoxicity: The acute toxicity of methanolic root bark extract of *Andrographis paniculata* was non-lethal even at higher doses up to 2000 mg/kg of body weight. The extract showed that the LD$_{50}$ value more than 2000 mg/kg body weight per oral did not show any mortality (Table 1).
Anti–Inflammatory Assay: The methanolic extract of *Andrographis paniculata* was observed to reduce paw edema by 55.10% on oral administration of 200 mg/kg body weight after 24 hours of carrageenan induction as compared to the Control group. Aceclofenac at 100 mg/kg inhibited the oedema volume by 79.75%. Thus, the standard drug Aceclofenac (100 mg/kg) is more potent than the extract (Table 2; Figure 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(mg/kg)</th>
<th>No of animals</th>
<th>No of animals with sign of toxicity</th>
<th>Mortality</th>
<th>LD$_{50}$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEAP</td>
<td>2000</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>&gt;2000mg/kg</td>
</tr>
</tbody>
</table>

**Table 1:** Determination of LD$_{50}$ value of methanolic extract of *Andrographis paniculata*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Initial paw volume</th>
<th>Mean paw volume after carrageenan induction ± SEM (ml)</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease control (Group I)</td>
<td>Normal</td>
<td>0.48± 0.72</td>
<td>0.60±0.84 0.68±0.21 0.71±0.208 0.77±0.014 0.78±0.007</td>
<td>–</td>
</tr>
<tr>
<td>Aceclofenac (Group II)</td>
<td>100</td>
<td>0.53±0.063</td>
<td>0.57±0.042 0.63±0.021 0.68±0.014 0.75±0.007</td>
<td>79.75</td>
</tr>
<tr>
<td>MEAP (Group III)</td>
<td>200</td>
<td>0.37±0.037</td>
<td>0.56±0.063 0.62±0.056 0.67±0.035 0.75±0.042 0.77±0.035</td>
<td>55.10</td>
</tr>
</tbody>
</table>

**Table 2:** Anti inflammatory activity of methanol extract of *Andrographis paniculata*. Values are expressed as mean ± SEM, (n= 6), P< 0.05, when compared with disease control group (One Way ANOVA followed by Dunnett’s test).

**Fig. 1:** Anti-inflammatory activity by carrageenan induced paw oedema in rats
Discussion

The present study was carried out to study the anti-inflammatory activity of methanolic root bark extract of *Andrographis paniculata* Nees. An ample body of scientific substantiation supports the ethnopharmacological implication of *A. paniculata* and its components with respect to their therapeutic efficacy against inflammatory diseases. Our experiments also clearly supported the anti-inflammatory effects of *A. paniculata* on inflammatory responses *in vitro*. Although, workers have reported that, administration of *A. paniculata* methanolic extract produced complete inhibition of carageenan induced inflammation compared with control models (Sheeja et al., 2006). This alteration in effect may be due to the variation of eco-races of the study plant taxon, which will be evaluated as a part of the present study.

Conclusion

In this study, anti-inflammatory assay was done by Carrageenan Induced Paw oedema in rats. The acute toxicity of methanolic extract of *Andrographis paniculata* was non-lethal even at higher dose of body weight of experimental animal. The methanolic extract also showed potential anti-inflammatory activity, less than that of Aceclofenac, but a significant one to consider for anti-inflammatory drug formulation.

References


