EVALUATION OF ANTINOCICEPTIVE AND ANTIINFLAMMATORY ACTIVITIES OF METHANOLIC EXTRACT OF ALPINIA GALANGA RHIZOMES IN ANIMAL MODELS

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ABSTRACT: Alpinia galanga Linn. (Zingiberaceae) presents many chemical constituents of the volatile oil extracted from the rhizome. The rhizome of Alpinia galanga is used by people in many regions for relieving toothache, abdominal pain, muscular swelling and rheumatism. This study was performed to determine the antinociceptive and anti-inflammatory activities of a methanolic extract of Alpinia galanga. Analgesic activity was evaluated by acetic acid induced writhing responses, tail flick and hot plate methods in mice. In analgesic activity the A. galanga extract significantly reduced the writhes and increased the tail withdrawal time and paw jumping in albino mice. The anti-inflammatory activity was evaluated by carrageenan induced edema in wistar albino rats. It was found that the extract inhibits the paw edema significantly (p<0.05) at both 250 mg/kg and 500 mg/kg dose levels when compared with control. The positive control drugs for analgesic and anti-inflammatory studies were employed as morphine and indomethacin, respectively. The present findings indicate the antinociceptive and anti-inflammatory effect of A. galanga extract in animals.

INTRODUCTION: Traditional medicinal herbs are being used extensively in various part of the world, including India, to treat various types of ailments as an alternative to modern medicine. The major importance of these herbal medicines has less toxicity compared to synthetic ones. The extracts are exhaustively used to treat various disorders, although there is relatively little knowledge about their mode of action. Alpinia galanga or galanga or kulanjan in hindi, (family- Zingiberaceae), is a well-known plant in India, Sri Lanka, Malaysia, Indonesia, and Thailand.

A. galanga has been extensively used for treatment of various disorders including hypertension, rheumatism, diabetes, anti-ulcer, asthma, immunomodulation, inflammation and antimicrobial. It is also a good natural antioxidant and various researchers have studied the active constituents isolated from A. galanga.

The phenolic compounds like flavonoids and phenolic acids are copiously found in this plant. The main constituents are acetoxychavicol acetate (ACA: 76.49%) obtained from the rhizome and seed extracts. Along the major Phytoconstituents some minor phytoconstituents are also found such as p-coumaryldiacetate (7.96%), palmitic acid (3.19%), acetoxyeugenol acetate (3.06%), 9-octadecenoic acid (2.28%), Eugenol, ß-bisabolene, ß-farnesene and sesquiphellandrene. Inflammation is part of the complex biological
response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. As many as phytoconstituents are found in *A. galanga* were found very useful in different disorders. It is used in polyherbal preparations as analgesics available over the counter. The anti-inflammatory and analgesic effects of *A. galanga* have been studied in rheumatoid conditions. From the literature survey it may revealed that still there is lacking scientific data on analgesic effect of *A. galanga*. This study is conducted to evaluate the analgesic and anti-inflammatory effect in experimental animals using methanol extract.

**FIG.1: PHYTOCONSTITUENTS OF A. GALANGA.**

**MATERIALS AND METHODS:**

**Plant material and extract preparation:**

Rhizomes of *A. galanga* were purchased from local market and plant material was identified and authenticated by Dr. K C Bhatt, at National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi. The shaded dried rhizomes (250 g) were extracted by methanol in Soxhlet apparatus. The methanol extract was evaporated under reduced pressure and temperature less than 50 ºC to obtain a dark brown viscous mass. The extract (8.5% yield of original) was then stored at temperature of 2–4 ºC for phytochemical and pharmacological evaluations.

**Drugs & chemicals:**

The methanolic extract of *A. galanga* at doses of 250 and 500 mg/kg was prepared by suspending this remainder in the 2% gum acacia and given to the animals orally. The reference drugs used in this study were indomethacin (10 mg/kg), morphine sulphate (5 mg/kg) and naloxone (2 mg/kg) and administered orally by dissolving in 0.9% normal saline.

**Animals:**

Adult rats of either sex, weighing 200–250 g and albino mice of either sex, weighing 20–25 g were obtained from the animal house of R. V. Northland Institute, Dadri, Greater Noida, G B Nagar, UP, India. They were housed in an artificial regulated room on a 12 hr light: 12 hr dark cycle with 25 ± 2 ºC and had water at libidum. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC, Reg. No.1149/ac/07/CPCSEA) of R V Northland Institute, Greater Noida, G B Nagar, UP and experiments were conducted according to the CPCSEA, India guidelines on the use and care of experimental animals.

**Experimental design:**

**Acetic acid-induced writhing responses in mice:**

The method previously described by Koster et al., was used to evaluate the antinociceptive activity. The extract at doses of 250 and 500 mg/kg was administered orally 60 min before intraperitoneal injection with 0.6% acetic acid in 0.9% normal saline (10 ml/kg body weight) to induce the characteristic writhings. The mice were observed and counted for the number of abdominal constrictions and stretchings in a period of 0–20 min. The responses in the treated groups were compared with those of animals in the control group. The percentage of inhibition of the number of writhings was calculated.

**Tail flick test in mice:**

The tail flick analgesic activity was performed by analgesiometer with slight modifications. *A. galanga* extract (250 and 500 mg/kg) was administered orally 60 min. before the evaluation of analgesic activity. Morphine sulfate (5 mg/kg) was administered intraperitoneally before the 15 min. of evaluation. Basal reaction time of all the albino mice to radiant heat was recorded by placing
the tail (1.5 cm measured from the root of the tail) on the radiant heat source. The cut-off reaction is fixed at 15 sec to avoid tissue damage. The tail withdrawal (flicking action) from the heat source i.e. reaction time was recorded at 30 min, 1 hr, 2 hr, 3 hr and 4 hr. Control group received normal saline solution (5 ml/kg, i.p.).

Hot plate test in mice:
The hot plate test was used as previously described by Woolfe and MacDonald\textsuperscript{14}. The animals in the control group received normal saline (10 ml/kg, p.o.) while the reference groups were treated with morphine sulphate (5 mg/kg, i.p.) and naloxone (2 mg/kg, i.p.). The animals in the test groups were treated with different doses (250 and 500 mg/kg, p.o.) of \textit{A. galanga} extract. In the remaining groups, the animals received naloxone (2 mg/kg, i.p.) 10 min before morphine (5 mg/kg, i.p.) or the extract (250 and 500 mg/kg, p.o.). After 60 min of treatment with all test drugs (except only 15 min for morphine and 10 min for naloxone), mice were placed on a hot plate maintained at 55±1 °C.

The latency of nociceptive response (reaction time) of each mouse that was identified by the time for licking and flicking of a hind limb or jumping was recorded. The reaction time was measured at 30 min., 1 hr, 2 hr, 3 hr and 4 hr. The cut-off time of observation was 45 seconds. Only mice that showed a nociceptive responses within 15 seconds were used in the experiments.

Carrageenan induced paw edema in rats:
Twenty four rats were divided in to four groups of six rats in each group. 0.1ml of 1% Carrageenan in physiological saline injected subcutaneously in to sub-planter region of right hind paw to induce edema\textsuperscript{13, 15}. The test groups (drug treated groups) of rats were given orally 250 & 500 mg/kg of methanolic extract of plant one hour before carrageenan injection. The vehicle treated control group of rats was given the same volume of 1% CMC as in test groups. Another standard group or reference group of rats was treated with 10 mg/kg of indomethacin orally one hour before carrageenan injection. The paw volume was measured initially at 30, 60, 120, 180 and 240 hours after Carrageenan injection by mercury displacement method using a plethysmograph.

The percentage inhibition of edema (paw volume) calculated for each group with respect to its vehicle treated control group. The anti-inflammatory activity was calculated by using the relation –
\[
\% \text{ inhibition of inflammation} = \left( \frac{V_c - V_t}{V_c} \right) \times 100
\]

Vc represents change in paw volume in the corresponding vehicle treated control group

Vt represents change in the drug extract treated groups.

Statistical Analysis:
The data obtained were analyzed using SigmaStat software (3.5) and expressed as a mean ± SEM. The statistically significant differences between groups were calculated by using one way ANOVA, followed by Dunnet’s test. The \( p < 0.05 \) selected as the level of statistical significance.

RESULTS:
Effect of \textit{A. galanga} (AG) extract on the writhing response in mice:
The AG extract administered orally at different doses (250, and 500 mg/kg) caused a significant inhibition (23.67% and 47.06%, respectively) compared to the control of the writhing responses induced by acetic acid (0.6% v/v, i.p.). The decrease in the number of writhing was dose-dependent. Indomethacin (10mg/kg, p.o.) produced a 54.21% reduction compared to the control (Table 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of writhings</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (10 ml/kg, p.o.)</td>
<td>-</td>
<td>53.56 ± 2.38</td>
<td>-</td>
</tr>
<tr>
<td>AG 250</td>
<td>250</td>
<td>40.88 ± 2.05*</td>
<td>23.67</td>
</tr>
<tr>
<td>AG 500</td>
<td>500</td>
<td>28.35 ± 1.85**</td>
<td>47.06</td>
</tr>
<tr>
<td>INDO 10</td>
<td>10</td>
<td>24.52 ± 1.75**</td>
<td>54.21</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. * \( p < 0.05 \), **\( p < 0.01 \) when compared to control.
Effect of *A. galanga* extract on tail flick test in mice: The tail-flick latency response was significantly increased after administration of 250 and 500 mg/kg, p.o. of AG compared to the control (*p* < 0.05). Morphine sulfate (5mg/kg, i.p.) significantly increased (*p* < 0.01) the tail-flick latency at 30 min after treatment. However, after naloxone pretreatment the effect of morphine or AG at a dose of 500 mg/kg, p.o., the tail-flick latency response was significantly decreased (*p* < 0.01) (Table 2).

**TABLE 2: EFFECT OF *A. GALANGA* EXTRACT ON TAIL FLICK TEST IN MICE**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>30 min.</th>
<th>1 hr.</th>
<th>2 hr.</th>
<th>3 hr.</th>
<th>4 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>4.36 ± 0.01</td>
<td>4.48 ± 0.02</td>
<td>4.38 ± 0.01</td>
<td>4.36 ± 0.02</td>
<td>4.44 ± 0.01</td>
</tr>
<tr>
<td>AG 250</td>
<td>250</td>
<td>6.06 ± 0.02*</td>
<td>7.56 ± 0.01*</td>
<td>8.50 ± 0.02*</td>
<td>9.05 ± 0.01*</td>
<td>8.86 ± 0.02</td>
</tr>
<tr>
<td>AG 500</td>
<td>500</td>
<td>9.66 ± 0.01*</td>
<td>10.80 ± 0.02**</td>
<td>10.95 ± 0.01**</td>
<td>10.87 ± 0.02**</td>
<td>11.05 ± 0.02**</td>
</tr>
<tr>
<td>Morphine</td>
<td>05</td>
<td>13.51 ± 0.01***</td>
<td>14.50 ± 0.02**</td>
<td>13.57 ± 0.01**</td>
<td>14.52 ± 0.01**</td>
<td>13.75 ± 0.02**</td>
</tr>
<tr>
<td>Naloxone + AG500</td>
<td>2; 5</td>
<td>5.02 ± 0.03</td>
<td>5.11 ± 0.18</td>
<td>6.12 ± 0.04</td>
<td>4.28 ± 0.02</td>
<td>5.26 ± 0.12</td>
</tr>
<tr>
<td>Naloxone + Morphin</td>
<td>2; 500</td>
<td>4.22 ± 0.03</td>
<td>4.85 ± 0.11</td>
<td>5.05 ± 0.03</td>
<td>5.45 ± 0.02</td>
<td>5.04 ± 0.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. *P* < 0.05, **P* < 0.01 when compared to control.

Effect of *A. galanga* extract on hot-plate test in mice: AG extract considerably increased the animal’s reaction time to the heat stimulus at a dose level of 500mg/kg. Morphine sulfate (5mg/kg, i.p.) markedly increased pain latency (*p* < 0.01) in all cases from 30 min with its maximum effect at approximately 1 h after treatment. All results are shown in Table 3. Naloxone (2 mg/kg, i.p.) given before the morphine (5 mg/kg, i.p.) or AG (500mg/kg, p.o.) abolished the latency of the nociceptive responses (Table 3).

**TABLE 3: EFFECT OF *A. GALANGA* EXTRACT ON HOT-PLATE TEST IN MICE**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>30 min.</th>
<th>1 hr.</th>
<th>2 hr.</th>
<th>3 hr.</th>
<th>4 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>9.25 ± 0.23</td>
<td>9.46 ± 0.28</td>
<td>8.89 ± 0.22</td>
<td>9.01 ± 0.27</td>
<td>9.32 ± 0.20</td>
</tr>
<tr>
<td>AG 250</td>
<td>250</td>
<td>10.23 ± 0.18</td>
<td>11.20 ± 0.22</td>
<td>11.58 ± 0.28</td>
<td>12.20 ± 0.35</td>
<td>11.88 ± 0.30</td>
</tr>
<tr>
<td>AG 500</td>
<td>500</td>
<td>11.30 ± 0.25*</td>
<td>14.46 ± 0.19*</td>
<td>15.05 ± 0.24*</td>
<td>15.65 ± 0.29*</td>
<td>14.07 ± 0.26*</td>
</tr>
<tr>
<td>Morphine</td>
<td>05</td>
<td>17.55 ± 0.18**</td>
<td>21.05 ± 0.16**</td>
<td>18.86 ± 0.21**</td>
<td>16.98 ± 0.24**</td>
<td>18.54 ± 0.18**</td>
</tr>
<tr>
<td>Naloxone + Morphine</td>
<td>2; 5</td>
<td>8.87 ± 0.21</td>
<td>9.56 ± 0.18</td>
<td>9.25 ± 0.31</td>
<td>10.02 ± 0.32</td>
<td>10.25 ± 0.22</td>
</tr>
<tr>
<td>Naloxone + AG500</td>
<td>2; 500</td>
<td>9.02 ± 0.22</td>
<td>10.04 ± 0.26</td>
<td>9.89 ± 0.32</td>
<td>9.52 ± 0.28</td>
<td>10.45 ± 0.26</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. *P* < 0.05, **P* < 0.01 when compared to control.

Carrageenan induced paw edema: We found that 250 and 500 mg/kg AG extract produced significant (*P*<0.01) antiinflammatory effect after 1 h of administration and lasted until the end of the experiment, with the percentage of antiinflammation produced in the range of 18–50% (Table 4). Indomethacin as expected produced significant anti-inflammatory response.

**TABLE 4: CARRAGEEAN INDUCED PAW EDEMA**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>1 hr.</th>
<th>2 hr.</th>
<th>3 hr.</th>
<th>4 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.38 ± 0.01</td>
<td>-</td>
<td>0.42 ± 0.01</td>
<td>-</td>
</tr>
<tr>
<td>AG 250</td>
<td>250</td>
<td>0.31 ± 0.01*</td>
<td>18.42</td>
<td>0.28 ± 0.02*</td>
<td>33.33</td>
</tr>
<tr>
<td>AG 500</td>
<td>500</td>
<td>0.28 ± 0.02*</td>
<td>26.31</td>
<td>0.27 ± 0.01*</td>
<td>35.71</td>
</tr>
<tr>
<td>INDO 10</td>
<td>10</td>
<td>0.14 ± 0.01*</td>
<td>63.15</td>
<td>0.12 ± 0.02*</td>
<td>71.42</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. *P* < 0.05, when compared to control.
DISCUSSION: Herbal remedies are used frequently in healthy as well as diseased persons. A number of herbs have been developed as anti-inflammatory and analgesics and amongst them a very little has studies for the molecular mode of action. In recent some studies A. galanga has shown a different pharmacological actions, even there are less knowledge and significant results about the anti-inflammatory and analgesic actions. To address this issue we evaluated the analgesic and anti-inflammatory activities of A. galanga. To evaluate for a possible central antinociceptive effect of the methanolic extract, the hot plate and tail-flick tests are used for evaluation of the central pain at the supraspinal and spinal levels, respectively, possibly acting on a descending inhibitory pain pathway.

The tail-flick response is believed to be a spinally mediated reflex and the paw-licking hot plate response is a more complex supraspinally organized behavior. In this study, our results indicated that the extract of A. galanga has antinociceptive effect against both the hot plate and tail-flick tests, therefore the antinociceptive is likely to mediated centrally (spinally and supraspinally). The effectiveness of analgesic agents in the tail-flick pain model is highly correlated with relief of human pain. Moreover, the antinociceptive action of morphine and extract in this study was abolished by naloxone, an opioid receptor antagonist.

The μ receptor has generally been regarded as the receptor type associated with pain relief and has been shown to be potent in regulating thermal pain. Nonanalgesic effects mediated by the μ receptors include respiratory depression, inhibition of intestinal motility and most importantly for therapeutic considerations is its induction of physical dependence. Activation of μ2 opioid subtype leads to spinal analgesia and commonly through constipation adverse effect.

Therefore, taking all these data together we believe that the antinociceptive activity of extract is most likely to be mediated peripherally and centrally and indicates a morphine-like mechanisms by binding with opioid receptors.

CONCLUSION: The A. galanga extract exhibits antinociceptive activity and anti-inflammatory activity. The proposed mechanisms of antinociceptive activity based on the pain models used in this study show that they are likely to be mediated peripherally and centrally (spinally and supraspinally) on the nervous system. In addition, the antinociceptive effect of the extract was abolished by naloxone in the same manner as for morphine both in the hot plate and tail-flick tests, indicating that the extract acts partly through opioid-mediated mechanisms. The anti-inflammatory activity was probably due to inhibition of peripheral inflammatory mediators. Further investigations are anticipated to identify the active components and lead to their further clinical use.

REFERENCES:

of *Alpinia conchigera* rhizomes in various animal models. Pharm Biol 2010; 48:861-8