Pharmacological evaluation of Artemisia annua for Antinociceptive Activity in rats

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INTRODUCTION

Pain has been defined by International Association for the Study of Pain as an obnoxious sensory and emotional incident allied with actual or potential tissue damage (Michel YB et al., 2003). Drugs that are currently used for the management of pain are opioids or nonopioids and that for inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects. It was reported that the risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide and Piroxicam etc increased the risk of bleeding in both acute and chronic therapy (Pilotto et al., 2003). There is an estimated market only of analgesics is $ 20 billion and approximately $ 4 billion needed to treat their side effects. Statistics from The Daily Telegraph suggests that 2000 deaths per annum are due to aspirin alone due to high risk of side effects like blood dyscrasia, nervous disorders, vomiting, nausea, bleeding tendency, metabolic disorders and rashes (Ghulamuddin et al., 2011). In contrast, plant origin drugs having very less undesirable effect and represent a large natural source of useful compounds that might serve as lead for the development of novel drugs effects. The drugs of plant origin are gaining increasing popularity and are being investigated for remedies of a number of disorders (Edzard, 1998; Sreemantula, 2005). Spices are dried herbs that have been effectively used in the indigenous systems of medicine in India and also in other countries (Nadkarni, 1976). It is therefore crucial that efforts should be made to bring in new medicinal plants to build up more effective and cheaper drugs. In this study Artemisia annua (Asteraceae) is native to China. Its ancient Chinese name, Qing Hao, literally means “green herb A. annua L. is a source of both essential oil (1.4 – 4.0 %) depending on chemotype, and other substances such as sesquiterpene lactones, flavonoids, poyalkynes and coumarins. The essential oil composition has been studied thoroughly and about 60 components have been identified; camphor, artemisia ketone, germacrene D and 1,8-cineole, are usually the main components. The professed medical uses of artemisia annua include treatment for malaria, infections, fever, inflammation, bleeding, headaches and cancer, particularly some lung and thyroid cancers. The ethanolic extract of aerial parts of Artemisia annua (200mg/kg) was found to be effective against chemical as well as thermal stimuli. So the results are in agreement with the traditional use of plant as analgesic agent.

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MATERIALS AND METHODS

Plant Material
Aerial parts of Artemisia annua Linn. (Asteraceae) were collected from Himalayan region of Ranikhet, Uttarakhand, India, during February 2011.

It was authenticated by Mr. Ravi Kumar, Field Officer, Saffron and Medicinal Plants Garden, Regional Research Institute of Himalayan Flora, Tarikhet (Ranikhet), Almora, (U.K.), India. The aerial parts were air dried under shade at room temperature and powdered in electrical grinder.

Preparation of extracts
The drug powdered was extracted with 95% ethanol by the help of soxhlet apparatus for 72 hrs. The extract was cooled, filtered and Dried on water bath at 40°C.

Formulation
Suspension of extract was prepared by using 2% Tween 80.

Selection of dose
200 mg/kg body weight was selected (Mojarad et al., 2005).

Animals
Experiments were performed on Swiss albino rats (120-150 gm) of either sex. Animals were procured from the animal house of the I.F.T.M.

University, Moradabad and maintain on a natural day night cycle (12 hrs dark: 12 hrs light) at room temperature of 24-26 °C, with free access to standard food pellets and water ad libitum. Animals were acclimatized for at least 10 days before exposure to behavioral experiments.

Experiments were carried out between 10:00-17:00 hrs. The experiment protocol was approved by the institutional animal ethics committee, I.F.T.M. University, Moradabad.

Chemicals
Tween80 – CDH Laboratory, Delhi.
Acetic Acid – Otto Chemie Pvt Ltd, Mumbai.
Sterile Water for injection- Nirlife Health Care, Mumbai.
Diclofenac sodium- Novartis India Ltd.

Experimental Design
Animals were divided into 3 groups of 6 animals each and treated as follows.

Group I: (Control group) was administered 10 ml/kg per oral (p.o.) of vehicle.

Group II: (Test Group) was treated with 200 mg/kg. p.o. of Artemisia annua.

Group III: (Standard group) was treated with 6 mg/kg. p.o. of Diclofenac sodium.

Acetic acid induced Writhing test
Writhing was induced in rat (n = 6) by intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The number of writhing was counted over a 20 min period. Animals were treated through oral route with ethanolic extract of Artemisia annua (200mg/kg) or Diclofenac sodium (6mg/kg) 30 min before injection of acetic acid. The control group received only vehicle (10 ml/kg) (Besra et al., 1996).

Eddy’s Hot Plate Test
The Hot plate test used to evaluates the thermal pain reflexes due to foot pad contact with a heated surface. Rats of either sex weighing 130-140 gm were divided into 3 groups of six animals each. The test drug (200 mg/kg) was given to group II. Animals in standard group were treated with Diclofenac sodium (6 mg/kg) while those in control group were administered 10 ml/kg of vehicle. The animals were placed on the hot plate maintained at constant temperature of 55 °C and reaction time (in seconds) jumping of paw responses was noted. This was repeated at 30, 60, 90, 120 minute time of intervals. A cut off period of 15 sec was observed to avoid damage to the paw (Mahomed et al., 2004).

Tail Immersion test
Prior to the analgesic experiments, the animals were screened for a sensitivity test by immersing the tip of tail (5 cm) gently in hot water (55°C).Within a few seconds, the rats reacts by withdrawing tail. The reaction time is recorded by stopwatch. The reaction time was determined periodically after oral administration of vehicle (10ml/kg), ethanolic extract of Artemisia annua (200 mg/kg) and Diclofenac sodium (6mg/kg) to groups I, II and III respectively at 30, 60, 90, 120 min. The cut off time of tail immersion was taken 15 seconds (Vogel et al., 1997).

Statistical Analysis
The observations were expressed as mean ± S.D. The difference in response to test drugs was determined by one-way analysis of variance followed by Duncan’s test. P<0.05 was considered significant.

RESULTS AND DISCUSSION

Writhing test
Oral administration of the ethanolic extract of aerial part of Artemisia annua (200mg/kg) more significantly (p<0.01) reduced the number of writhing induced by acetic acid in rat when compared to the control group (Table1). The activity was found to be comparable to the standard drug Diclofenac Sodium (6 mg/kg). A dose dependant and significant response was found in Acetic acid induced writhing test demonstrating the analgesic activity possessed by the test drug. Drugs exert response against chemical stimuli have been reported to produce effect as non selective COX inhibitors (Tripathi 2004). Pain which is caused by releasing endogenous substance, which then excites pain nerve
endings, the abdominal constrictions are related to the sensitization of nociceptive receptors to prostaglandins (Vinegar et al., 1969). The test drug demonstrated significant effect against chemical nociceptive stimuli. Therefore, it can be concluded that it could be effective against chemical nociceptive stimuli and possesses Aspirin like properties (Burke et al., 2006).

Eddy's Hot Plate Test

The animals were placed on the hot plate maintained at constant temperature of 55°C and reaction time of animals (paw licking and jumping) was noted at 30, 60, 90 and 120 minute intervals. When the mean of various groups were observed at 30, 60, 90 and 120 minute intervals, it was observed that 60 and 90 minutes after administration of the test drug showed significant effect compared to control group but was found to be comparable to standard drug up to 120 minutes and results are given in the Table 2.

Table 1: Analgesic effect of ethanolic extract of Artemisia annua on rats by writhing method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Writhing in 20 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group (10ml/kg p.o.)</td>
<td>10.16±0.60</td>
</tr>
<tr>
<td>Test Group (200mg/kg p.o.)</td>
<td>6.00±0.36**</td>
</tr>
<tr>
<td>Standard group (6mg/kg p.o.)</td>
<td>4.16±0.30**</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunnet’s test, (n=6), Data are significantly different from the control at ** (p<0.01),*(p<0.05), *(non-significant) compared to control group.

Table 2: Analgesic effect of ethanolic extract of Artemisia annua on rats by “Eddy’s Hot Plate”.

<table>
<thead>
<tr>
<th>Reaction times in minutes</th>
<th>Groups</th>
<th>After 30Min</th>
<th>After 60Min</th>
<th>After 90Min</th>
<th>After 120Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group (10 ml/kg p.o.)</td>
<td>4.66±</td>
<td>4.50±</td>
<td>4.83±</td>
<td>4.16±</td>
<td></td>
</tr>
<tr>
<td>Test group (200 ml/kg p.o.)</td>
<td>6.41±</td>
<td>9.66±</td>
<td>8.11±</td>
<td>3.83±</td>
<td></td>
</tr>
<tr>
<td>Standard group (6mg/kg p.o.)</td>
<td>9.33±</td>
<td>11.00±</td>
<td>10.16±</td>
<td>8.16±</td>
<td></td>
</tr>
<tr>
<td>Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett’s test, (n=6), Data are significantly different from the control at ** (p&lt;0.01),*(p&lt;0.05), *(non-significant) compared to control group.</td>
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</table>

Table 3: Analgesic effect of ethanolic extract of Artimissia annua on rats by using “Tail immersion method”.

<table>
<thead>
<tr>
<th>Reaction times in minutes</th>
<th>Groups</th>
<th>After 30Min</th>
<th>After 60Min</th>
<th>After 90Min</th>
<th>After 120Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Group (10ml/kg p.o.)</td>
<td>2.66±</td>
<td>2.66±</td>
<td>2.88±</td>
<td>2.83±</td>
<td></td>
</tr>
<tr>
<td>Test group (200 mg/kg p.o.)</td>
<td>4.00±</td>
<td>8.00±</td>
<td>7.50±</td>
<td>3.58±</td>
<td></td>
</tr>
<tr>
<td>Standard group (6mg/kg p.o.)</td>
<td>9.66±</td>
<td>12.50±</td>
<td>9.00±</td>
<td>13.00±</td>
<td></td>
</tr>
<tr>
<td>Data are expressed as Mean±S.E.M. Test employed ANOVA one way followed by Dunett’s test, (n=6), Data are significantly different from the control at ** (p&lt;0.01),*(p&lt;0.05), *(non-significant) compared to control group.</td>
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CONCLUSION

On the basis of these findings it may be stated that the ethanolic extract of aerial parts of Artemisia annua has analgesic effect. It was found to be effective against chemical stimuli better than that of thermal stimuli. So the results are in agreement with the traditional use of plant as analgesic agent.

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REFERENCES


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