Antinociceptive activity of ethanolic neem leaf extract (Azadirachta indica) through opioid pathway in adult rats

INTRODUCTION:
Pain is an unpleasant though very important protective mechanism [1]. It is usually associated with actual or potential tissue damage and is primary reason for seeking medical attention [2]. Opioids are one of the most efficacious analgesics for moderate to severe pain [3]. But they possess strong addictive potential and various other side effects like respiratory depression, drowsiness etc [4]. Therefore, analgesic drugs which lack these side effects as an alternative are need of time [1].

Azadirachta Indica (neem) is used in Indian culture in traditional medicine and earlier studies have showed that it has various active substances with medicinal properties [5]. It has been reported as hypoglycemic, hypolipidemic, anti-inflammatory, apoptotic and antineurotoxic agent [6-8]. So natural products can be explored as an important source of new chemical substance with therapeutic application [9]. Fewer studies has been done to explore its antinociceptive potential [6,10,11]. To further confirm this property of ethanolic neem leaf extract (ENLE) through opioidergic system, this study was planned.

SUBJECTS AND METHODS:
Plant material
The fresh matured leaves of A. indica were collected locally from their natural habitat in month of August-September 2018.

The leaves were identified by a pharmacognosy expert and voucher specimen was retained in museum of the department for further reference.

Preparation of plant extract and reference drugs
The ethanolic extract was prepared by procedure described by Chattopadhyay (1998). A. indica leaves were shade dried and powdered. Powder was mixed with 70% ethyl alcohol and left at room temperature for 36 hrs. It was stirred intermittently and filtered. The filtrate was concentrated under reduced pressure (bath temp 50°C) and dried in vacuum desiccator. The residue obtained was stored in storage vial and refrigerated until used for experiment. The doses of ENLE of 50 mg/kg and 100 mg/kg were prepared by suspending extracts in normal saline.

Experimental Animals
In this study, adult rats weighing 150-250g of either sex were used. The animals were kept under standard condition at 24±2°C with 12 h light and 12h day cycle. Food and water were available ad libitum. The study was approved by Institutional Animal Ethics Committee.

RESULTS:
Evaluation of antinociceptive activity
Hot plate test: The adult rats were taken randomly in test and reference group. In this test, morphine and naloxone were used as reference drugs. The animals were placed on heated surface. The paws of rats are very sensitive to hot surface at 55±1°C. A cut off period of 15s was set to avoid any damage to paws. The responses were withdrawal of paw, licking of paw or jumping. The reaction time was noted with help of stop watch and measured every 15min at interval of 0, 15, 30, 45, 60, 75 and 90 min.

Rats were divided into four groups of six animals each. In Group I, animals received normal saline (10ml/kg, s.c.) as negative control while Group II was treated with morphine (1mg/kg, s.c.) served as positive control. In test group (Group III and Group IV) rats were treated with two different doses of ENLE (50mg/kg and 100 mg/kg, p.o.) respectively. To study the effect of naloxone pre-treatment, rats were divided into five groups of six animals each. Group I received naloxone (1 mg/kg, i.p.) alone. Group II and Group III received naloxone (1 mg/kg, i.p.) 30 min prior to control (10ml/kg, s.c.) and morphine (1 mg/kg, s.c.) respectively. Group IV and Group V received naloxone (1 mg/kg, i.p.) 30 min prior to ENLE (50 mg/kg and 100 mg/kg, p.o.) respectively.

Drugs
Normal saline and morphine sulphate were used s.c. while naloxone was used i.p. ENLE was dissolved in normal saline to desired concentration and used p.o. The dose of drugs used were selected on basis of previous studies [5,6].

Evaluation of antinociceptive activity
Heat plate test: The adult rats were taken randomly in test and reference group. In this test, morphine and naloxone were used as reference drugs. The animals were placed on heated surface. The paws of rats are very sensitive to hot surface at 55±1°C. A cut off period of 15s was set to avoid any damage to paws. The responses were withdrawal of paw, licking of paw or jumping. The reaction time was noted with help of stop watch and measured every 15min at interval of 0, 15, 30, 45, 60, 75 and 90 min.

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Statistical Analysis:
Statistical analysis was done by using One way analysis of variance (ANOVA) followed by Least significant difference test for multiple comparison to assess the significant differences between the groups. All the values were expressed as mean ± SEM. Value of p<0.05 and p<0.01 were considered significant.

RESULTS:
Effect of ENLE on hot plate test
The results of hot plate test are reported in (Table 1 & 2). Both dose of ENLE (50 mg/kg and 100 mg/kg) shows dose dependent increase in mean latency time (p<0.01) as compared to control. Morphine (1mg/kg) shows significant increase (p<0.01) in mean latency time throughout whole observation period when compared with control and ENLE (50 mg/kg). It also shows significant difference in mean latency time (p<0.01 and p<0.05) when compared to ENLE (100mg/kg) (Table 1).
Table 1: Effect of Ethanolic Neem Leaf Extract (ENLE) on reaction time in rats in Hot plate method.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency of reaction time(sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>10 ml/kg, s.c.</td>
<td>3.91±0.04</td>
</tr>
<tr>
<td>Morphine</td>
<td>1, s.c.</td>
<td>4.08±0.07</td>
</tr>
<tr>
<td>ENLE</td>
<td>50, p.o.</td>
<td>3.77±0.07</td>
</tr>
<tr>
<td>ENLE</td>
<td>100, p.o.</td>
<td>3.80±0.23</td>
</tr>
</tbody>
</table>

n=6; The observations are mean±SEM.

*p<0.01 compared to control; *p<0.05 compared to control; #p<0.01 compared to ENLE (50mg/kg).

bp<0.05 compared to ENLE(50mg/kg); cp<0.01 compared to ENLE(100mg/kg).

Effect of Naloxone pre treatment

After naloxone pre treatment, both morphine and ENLE (50 mg/kg and 100 mg/kg) shows no significant difference in increase in mean latency time as both were antagonized by naloxone (Table 2).

Table 2: Effect of Naloxone pre treatment on reaction time in rats in hot plate method.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Latency of reaction time(sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Naloxone</td>
<td>1, i.p.</td>
<td>3.57±0.10</td>
</tr>
<tr>
<td>Naloxone+control</td>
<td>1, i.p.; 10ml/kg, s.c.</td>
<td>3.58±0.09</td>
</tr>
<tr>
<td>Naloxone+morphine</td>
<td>1, i.p.; 1, s.c.</td>
<td>3.45±0.04</td>
</tr>
<tr>
<td>Naloxone+ENLE</td>
<td>1, i.p.; 50, p.o.</td>
<td>3.63±0.05</td>
</tr>
<tr>
<td>Naloxone+ENLE</td>
<td>1, i.p.; 100, s.c.</td>
<td>3.66±0.06</td>
</tr>
</tbody>
</table>

n=6; The observations are mean±SEM.

DISCUSSION:

The dose administered in this study i.e. 50 mg/kg and 100 mg/kg is based on previous studies [5,6]. The dose used in this study is several times lower than oral LD50 for ENLE, which is around 4.75/kg in acute toxicity test [7]. The hot plate test is one of the suitable tests for determining the difference between centrally and peripherally acting analgesics. In this test, high intensity phasic stimuli are given. The pain induced in hot plate method is very specific for centrally mediated activity [4]. Thus this test is very useful in elucidating centrally mediated antinociceptive response which concentrates on changes above the level of spinal cord [12]. The results obtained in this study shows that ENLE has dose dependent antinociceptive activity and this activity is probably centrally mediated as depicted through hot plate test. The hot plate test is also selective for opioid like compounds [13]. So, it can be concluded that ENLE shows its effect through opioid pathway. To determine the involvement of opioid receptors, pre treatment with naloxone was done. Naloxone is non selective antagonist at μ, δ opioid receptors [14]. In naloxone pre treatment, the antinociceptive activity of ENLE was inhibited which suggests that it acts by blocking opioid receptors. The antinociceptive activity of ENLE may be due to presence of bioactive constituents in it. Thus, the findings of this study will be helpful for further phytochemical and pharmacodynamic investigations to explore its antinociceptive potential further.

CONCLUSION:

The results obtained from this study suggest that there is presence of antinociceptive activity in ENLE which may be centrally mediated through opioid pathway. Further studies would be helpful to explore this potential for benefit of human being.

REFERENCES:

8. Muneem AA (2014) Azadirachta indica attenuates cisplatin-induced neurotoxicity in...