Original Research Article

STUDY OF ANTI-INFLAMMATORY, ANTIPYRETIC AND ANALGESIC EFFECTS OF AZadirachta indica LEAVES EXTRACT IN ALBINO RATS

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Abstract: An evergreen tropical tree known as neem (Azadirachta indica) has long been used in traditional medicine to various conditions, including fever, pain and inflammation. The prospective research aims to examine the analgesic, antipyretic and anti-inflammatory properties of the chloroform extract of A. indica. For this investigation, 108 albino rats weighing 160 to 200g each were employed. Carrageenan was used to induce paw edema to test the anti-inflammatory activity. Yeast was used to induce pyrexia to test the anti-pyretic activity. Finally, acetic acid induced abdominal writhing in rats to test the analgesic action. The chloroform extract was injected into the abdominal muscle and intraperitoneal tissue. The largest percentage of anti-inflammatory action that chloroform leaf extract could suppress was 53%. The maximal percentage inhibition of leaf extract against fever in terms of antipyretic efficacy was 90%. The highest percentage of inhibition against pain during analgesic activity was 56%. When compared to the common medications diclofenac and paracetamol substantial outcomes were seen in the anti-inflammatory, antipyretic and analgesic actions at 400 mg/kg, respectively. Fever, pain and inflammation all decreased significantly (p≤ 0.05). As a result, chloroform leaf extract from A. indica can be utilized as a potent medication to treat fever, pain (discomfort) and inflammation, proteins.

Keywords: Azadirachta indica, Anti-inflammatory, antipyretic, analgesic, chloroform, leaf, paracetamol, diclofenac, carrageenan

Introduction

Locally, Azadirachta indica is referred to as neem. It belongs to the Meliaceae subfamily of mahogany trees. The genus Azadirachta, it has one or two species. It is indigenous to Pakistan, Bangladesh, Nepal, Thailand and India. It is thriving in tropical and subtropical areas (Hossain et al., 2013). It is a tree of fast-growing that rarely attains the height of 35 to 40 meters. Despite being evergreen, it may lose most or almost all of its leaves after a severe drought. The Indian tradition has known for thousands of years that neem has medicinal benefits (Owoyale et al., 2019). The adaptable medicinal plant neem is the source of several substances with various chemical structures and biological effects. Neem has several of active phytoconstituents, including steroids, glycosides, alkaloids and tannins. Neem leaves efficiently treat eczema, ringworm and acne have hypoglycemic and anti-inflammatory qualities (Ogidii et al., 2021).

Since dawn, man has been looking for treatments for the most prevalent human sufferings, including pain, fever and inflammation. Chronic inflammation concerns in the pathogenesis of different diseases. A clear association has been established for neurodegenerative diseases, obesity, cardiovascular disease, T2DM, metabolic syndrome and cancer (Alyas et al., 2020). The most common treatments include analgesics, anti-inflammatory, and antipyretic medications. Various inflammatory illnesses are treated using traditional plant medicines all around the world. One such plant is neem (Indian lilac, Azadirachta indica), an inherent of the Indian sub-continent and a highly revered tree for the people in the area. Neem is thought to have antiseptic, anti-

helminthic, insecticidal, antiseptic, anti-diabetic and anti-hypertensive characteristics. Various inflammatory illnesses are treated using traditional plant medicines worldwide (Kumar et al., 2015).

**Material and Methods**

**Sample collection and chloroform extract of A. indica leaves**
The fully matured leaves of A. indica were collected from different botanical areas of Lahore. The staff members of The University of Punjab, Lahore recognized them. Leaves were cleaned with cotton and then dried at room temperature. The cold maceration method was used for leaf extraction (Dhakal et al., 2016).

**Experimental rats**
Albino rats of either sex female or male (160-200g) were used for anti-inflammatory antipyretic and analgesic activity. Rats were kept in the animal house of The University of Lahore in polypropylene cages. Before experimental work, rats were kept in a fasting condition. After that, they were given distilled water and balanced feed.

**Drugs used**
Normal saline 10ml/kg, Acetic acid induce pain 400mg/kg, Paracetamol 100mg/kg, Yeast 400mg/kg. Diclofenac 100mg/kg, Carrageenan 100mg/kg, Leaves extract (50,100,200,400mg/kg).

**Anti-inflammatory activity model**
For this activity model, 36 rats were alienated into 3 groups.

**Carrageenan induce oedema**
Initially, all groups of rats were treated with carrageenan in which 0.1 ml of 1% carrageenan was induced in the left hind paw. The volume of the paws was measured with a Vernier calliper before and after the injection. Anti-inflammatory activity was calculated by the given formula:

\[
\% \text{ inhibition} = \frac{\text{Control mean} - \text{treated mean}}{\text{Control mean}} \times 100
\]

The rats in the control group were given an injection of normal saline 10ml/kg in the sub-planter region of the hind paw. The rats in the standard group were given a dose of diclofenac 100mg/kg in the sub-planter region of the hind paw. After carrageenan induction, the paw size was increased in the experimental group. After six hours, the chloroform extract of A. indica was injected into group III at doses of 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg correspondingly.

**Yeast induce fever**
All the groups were injected with yeast below the nape of the neck to induce fever. After injecting yeast, the fever developed after 21 hours and the highest temperature was 101.54 Farenheit. In group I, rats were injected with normal saline (10ml/kg) below the nape of the neck. In group II, the rats were treated with a dose of paracetamol 10ml/kg. While in group III the rats were treated with different concentrations of chloroform extract of A. indica at dose of 50, 100, 200 and 400mg/kg. After 1, 2, 3 and 4 hours the body temperature (rectum) of rats was measured with a digital thermometer.

For calculating the anti-pyretic activity, the formula is given below:

\[
\text{Inhibition} = \frac{B - \text{CB}}{B - A} \times 100
\]

B = temperature after fever induction
A = original body temperature
CB = temperature after 1, 2 and 3 and 4 hours

**Writhing induced by Acetic acid**
With the injection of acetic acid, the writhing process began in rats. It was injected to determine the potential of chloroform extract of A. indica pain process. But before 1 hour of the experiment, rats were injected with normal saline (10ml/kg) intraperitoneally in group I. In group II, the rats were treated with a dose of diclofenac 10ml/kg. While in group III rats were treated with different concentrations of chloroform extract of A. indica at doses of 50, 100, 200 and 400mg/kg. For counting the writhes, the stop watch was used. The rats were placed into different cages during the activity.

Analgesic activity was calculated by the given formula:

\[
\text{Analgesic activity} = \frac{N_c - N_t}{N_c} \times 100
\]

Where,
Nt = treated group writhes
Nc = control group writhes

**Statistical analysis**
Collected data were analyzed through on-way ANOVA technique followed by Duncan’s multiple range test using SAS software (version 9.1), the significance level was considered as p ≤ 0.05.

**Results**

**Edema induced by carrageenan**
In this activity, group I remained untreated after carrageenan injection, hence, there was 0% inhibition activity. While, group III at different doses showed 40%, 47%, 49% and 53% inhibition of edema accordingly. Group II and Group III of A. indica showed significant (p<0.05) % inhibition in carrageenan-induced paw edema of rats after 1-5 hours as compared to group I (Table 4.1).

In this activity, group III of A. indica leaf and the standard drug paracetamol showed significant Table

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**Table 4.2:** %inhibition of yeast induced pyrexia of rats by group I, II and III (chloroform extract of *A. indica*)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial Temp</th>
<th>Temp at 21 hour</th>
<th>1 hour</th>
<th>2 hour</th>
<th>3 hour</th>
<th>4 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>97.92±0.38</td>
<td>101.93±0.24</td>
<td>101.55±0.13</td>
<td>101.40±0.10</td>
<td>101.20±0.09</td>
<td>100.7±0.08</td>
</tr>
<tr>
<td>Standard</td>
<td>97.18±0.22</td>
<td>101.73±0.10</td>
<td>100.70±0.37</td>
<td>99.90±0.54</td>
<td>98.57±0.43</td>
<td>97.18±0.24</td>
</tr>
<tr>
<td>50mg/kg</td>
<td>97.17±0.19</td>
<td>101.98±0.21</td>
<td>102.85±1.40</td>
<td>101.25±0.13</td>
<td>100.82±0.14</td>
<td>100.48±0.17</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>97.17±0.30</td>
<td>101.50±0.26</td>
<td>101.30±0.19</td>
<td>101.08±0.20</td>
<td>100.92±0.20</td>
<td>100.67±0.15</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>97.50±0.35</td>
<td>101.95±0.15</td>
<td>101.62±0.14</td>
<td>101.08±0.33</td>
<td>100.35±0.42</td>
<td>99.80±0.33</td>
</tr>
<tr>
<td>400mg/kg</td>
<td>97.18±0.24</td>
<td>101.93±0.27</td>
<td>100.85±0.2</td>
<td>100.13±0.21</td>
<td>98.77±0.21</td>
<td>97.68±0.38</td>
</tr>
<tr>
<td>p-value</td>
<td>0.3784</td>
<td>0.5868</td>
<td>0.1886</td>
<td>0.0031</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Superscripts on different means within column differ significantly at p ≤ 0.05.

**Pyrexia test**

In this activity, group III of *A. indica* leaf and the standard drug paracetamol showed significant (p<0.05) % inhibition in yeast-induced pyrexia of rats after 1-4 hours as compared to group I (Table 4.2).

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**Table 4.3:** %inhibition of Acetic acid induced writhing of rats by group I, II and III (chloroform extract of *A.indica*)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Analgesic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>50mg/kg</td>
<td>20.67±0.715%</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>16.50±0.5624%</td>
</tr>
<tr>
<td>200mg</td>
<td>13.17±0.7540%</td>
</tr>
<tr>
<td>400mg</td>
<td>9.50±0.5656%</td>
</tr>
<tr>
<td>Control</td>
<td>21.83±0.790%</td>
</tr>
<tr>
<td>Standard</td>
<td>7.33±0.8866%</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Superscripts on different means within column differ significantly at p ≤ 0.05.

**Discussion**

A. indica leaf chloroform extract is used to assess the analgesic, antiinflammatory, and anti-pyretic effects. Further research is needed to determine the exact mechanism of action.

properties. In terms of analgesic, anti-inflammatory, and antipyretic drug usage, the world today has few options; they include mostly narcotics (morphine) and non-narcotics such as salicylates and corticosteroids. Even though most of us are aware of the negative side effects and high cost of these medications, we nevertheless hopelessly rely on them without any better options (Bhattacharya et al., 2014).

The body uses the inflammation as a physiological defence mechanism to protect itself against allergens, harmful substances, blisters, infections and other unpleasant stimuli (Ghauri et al., 2021). Carrageenan is frequently used to simulate inflammation in lab to identify substances with anti-inflammatory properties. Yeast causes pyrexia in rats, and acetic acids used to assess the writhing test (Alyas et al., 2022). Serotonin and histamine are thought to be released in the first phase of carrageenan-induced edema whereas, prostaglandins, protease and lysosomes are thought to be released in the second phase (Bokanisereme and Okechukwu, 2013). The active ingredients in A. indica are thought to exert their anti-inflammatory effects, preventing the production, discharge or activity of inflammatory mediators such as serotonin, prostaglandins and histamine (Mahabub-Uz-Zaman et al., 2009).

According to our findings, anti-inflammatory efficacy was dose-dependent. Group III showed less notable inhibition at the 50 mg/kg and 100 mg/kg doses, respectively. At a dose of 400 mg/kg, it demonstrated the most notable edema inhibition as compared to group II. At 500 mg/kg b.w, ethanol extract of the A. indica showed a significant dose-dependent decrease. However, at eight hours into the research at a dose of 500 mg/kg b.w, the maximum edema inhibition was reported to be 22.22 percent (Emran et al., 2015). Numerous biological activities, including anti-allergic and anti-inflammatory actions, have been linked to flavonoids. They accomplish this by inhibiting the activity of the enzymes cyclooxygenase and lipoxygenase, lipid peroxidation, capillary permeability and platelet aggregation (Avior et al., 2013).

Pathogenic fever, which is caused by injecting yeast suspension into albino rats' nape of the neck to induce pyrexia, is linked to an increase in prostaglandin synthesis. Antipyretic drugs can lower elevated body temperature in specific clinical circumstances. The plant extract inhibits the enzyme cyclooxygenase, which reduces the release of prostaglandins and gives rise to the anti-inflammatory characteristics of the substance (Safari et al., 2016). Similar to how paracetamol affects cyclo-oxygenase enzyme activity, antipyretic effectiveness may be mediated by suppressing prostaglandin synthesis. Neem leaf extract may lessen pyrexia by lowering hypothalamic PGE2 levels, blocking the processes that link peripheral inflammation to the formation of PGE2 or both (Santra et al., 2014).

In this study, the experimental group receiving A. indica leaf chloroform extract experienced a marked reduction in fever. Flavonoids are recognized to target prostaglandins, which are intricate in pyrexia. Therefore, flavonoids in the chloroform leaf extract of A. indica may help explain its anti-pyretic properties. By blocking the synthesis of prostaglandins, paracetamol also lessens fever brought on by yeast (Saini and Singha, 2012). Neem leaves extract has flavonoids, tannins, alkaloids, nimbin, nimbinin, nimboleide and nimbidic acid which may be the cause of its anti-inflammatory, analgesic and anti-pyretic properties (Timothy et al., 2011).

The well-proposed acetic acid-induced writhing is a useful tool for assessing the analgesic potential of drugs. In the acetic acid-induced writhing paradigm, the discharge of free arachidonic acid from tissue phospholipids via COX results in a localized inflammatory response, and prostaglandins production, notably PGE2 and PGE2 and an increase in the level of lipoxygenase products in peritoneal fluid (Afsar et al., 2015). These prostaglandins and lipoxygenase products increase capillary permeability, releasing endogenous chemicals that excite pain nerve endings, resulting in swelling and anguish. NSAIDs inhibit the COX enzyme in peripheral tissues and impact the major afferent nociceptors' transduction mechanisms (Shukla and Mehta, 2015).

The abdominal muscle contracts, the hind limbs extend, and the affected body part lengthens. This characteristic of pain activity caused by intraperitoneal injection of acetic acid is assumed to be mediated via the local peritoneal receptor. The number of writhes decreased, indicating an analgesic effect. Abdominal constriction brought on by acetic acid is a susceptible method for analyzing peripherally acting analgesics. When acetic acid is subjected to a painful stimulus, endogenous chemicals such bradykinins, serotonin, progesterone, histamine and substance P are produced (Subedi et al., 2016).

Reference medication diclofenac, reduced pain by preventing the synthesis of pain mediators in peripheral tissues (Alyas et al., 2020). In the current investigation, the 400 mg/kg dose of chloroform leaf extract from A. indica significantly reduced pain in the experimental group. The experimental group showed significant (p>0.05) analgesic potential in a dose-dependent manner. Alkaloids are necessary for the analgesic effect (Emran et al., 2015).

Conclusion

From the overall results, we deduced that the occurrence of phytoconstituents for instance flavonoids, alkaloids, tannin, sponins, terpenoids, glycosides, phenolics, and steroids in the chloroform extract of A. indica contributed to its considerable

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analgesic, anti-pyretic and anti-inflammatory effects. Our research supports the neem plant's traditional usage as medicine. Future anticipated phytochemicals will aid in identifying clinically useful molecules for the occurrence of their primary and secondary metabolites. Flavonoids may be account for the effects mentioned above.

**Conflict of interest**
The authors declared absence of conflict of interest.

**References**


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