In Vivo Evaluation of the Anti-Inflammatory Activity of Ethanolic Extract of Atropa Belladonna

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Abstract: The aerial parts of the plant Atropa belladonna prevalently known to use in folk remedies as anti-inflammatory. The intention of the current study was primarily to evaluate the in vivo anti-inflammatory potential of the crude ethanolic macerated extract that is obtained from parts of Atropa belladonna (excluding roots and berries). Anti-inflammatory activity is examined in mice by using formalin test. Indomethacin is taken as positive control while Tween 80 is taken as negative control in this study. Atropa belladonna extract appeared weak to nearly moderate in its effect for acute inflammation while in chronic inflammation it has shown significant activity.

Keywords: Atropa belladonna, anti-inflammatory, ethanolic, indomethacin, formalin test.

1. INTRODUCTION
Atropa belladonna Linn is a renowned medicinal plant belongs to solanaceae family, also famous by a common name of deadly nightshade and usually known for its noteworthy sedative as well as local analgesic activity. It is a perennial plant, local to central, European and Southern regions and also cultivated all across the earth. It contains a number of tropane alkaloids, which have exceptional anticholinergic and spasmylic properties. The chief active compound of this plant is atropine. Atropa belladonna and a few other herbal compounds posed significant effect on the experimental inflammation of rat paw caused as a result of injection of the autologous blood. On the contrary, some clinical studies, conducted under selected circumstances on certain plants like Atropa belladonna, Arnica Montana and Hamamelis virginiana, have revealed that alone or else in combination, these plants might have some anti-edemagenic and anti-inflammatory properties.

In the earlier studies, we have found that treatment of dermal abrasions by plant Atropa belladonna Linn discovered the shortening of process of the acute inflammation along with increased in tensile strength as well as collagen deposition in the healing of cutaneous wounds. We have also found by experiment that wounds treated with the Atropa Belladonna plant have revealed to diminish the process of the acute inflammations and herein noticeable increase in the deposition of collagen and superior tensile strength in cutaneous wounds healing as compared to their untreated control.

Additionally minute potencies of plant extracts (together with Atropa belladonna) used in a product of Germany named as Traumeel which have confirmed stimulatory effects on the synthesis of lymphocyte of the inhibitory cell cytokine TGF-beta in entire blood cultures. During TGF-β production, further pro-inflammatory cells of T-lymphocytes (by, for instance, IL-1 and TNFα) are barred to brace the process of inflammation. This action thus supported by the outcomes in vivo.

2. MATERIALS AND METHODS
2.1. Chemicals
The chemicals that used in this assessment include; formalin 36% (Sigma Company chemicals, USA) and Indomethacin (Sigma chemicals company, Germany). Other necessary materials including Normal saline, Tween 80, sodium carbonate, insulin syringe, digital vernier caliper provided by Hamdard Laboratory of Karachi, Pakistan.
2.2. Herbal Extract Preparation

Fresh belladonna plant and its over the ground parts were collected capriciously from the Multan region of Punjab in Pakistan. Whole plant parts (excluding roots & Flowering tops or else berries) were used to make extract. The taxonomic distinctiveness of this plant was established by the Department of pharmacy, Hamdard University, Karachi, Pakistan. Plant matter then washed through clean water, dried in free air and then improved to fine powder and stored in airtight glass container. Afterwards ethanolic extract of Plant was prepared as explained by (Ahmad and Beg 2001) with slight variation and stored in low temperature for its further use.

2.3. Animals

NMRI mice of either sex of weight 25-35 grams were obtained from the animal house of Dr. Hafiz Muhammad Ilyas institute of Pharmacology and Herbal Sciences, Hamdard University, Karachi, Pakistan. Experimental animals were kept under standard condition of environment with free access to water and food.

2.4. Acute Toxicity Test or the Maximum Non-Fatal Dose (Mnfd)

Different calculated doses of Atropa belladonna ethanolic extract and used vehicle (Tween 80) was administered orally to divided groups of six test animals. The experimental animals were observed for any acute symptom of the toxicity, initially for the duration of 1 to 3 hours. The maximum dose which did not bring on any mortality over a period of one week was considered as the highest Non-fatal dose. The figures of deaths were then counted after this treatment and registered. Any fatality occurred in a week of treatment was then noted and the value of LD50 (Dose induced 50% casualty in the experimental animals) was consequently determined as demonstrated in Table - 1.

2.5. Formalin Test

A volume 20-microlitre of 1% formalin saline solution was injected intradermally into the dorsal surface of the hind paw of mouse. Instantly after the injection, mouse then placed for observation in the plexiglass chamber. The volume increase was recorded by digital vernier caliper subsequent to formalin injection. An hour prior to the formalin injection, the marked animals were intragastrically treated with calculated doses of AB (Atropa belladonna) extract (50, 250, 400 mg/kg, i.g.) and Indomethacin (10 mg/kg PO.) and the equal volume of vehicle in which the drug is dissolved (Tween 80) by means of a special type of Gavage needle having ball at its end. The effects of administered drugs at various doses and time intervals were noted and then compared with the control.

3. Experimental Results

In the above experiment the maximum nonfatal doses has determined by the toxicity test as represented below in the Table-1. The considerable anti-edematous effect found at the dose 400 mg/kg i.e. 38.1%. On the other side Indomethacin as standard reference drug also inhibited the inflammatory response in test mice, showing its activity in Table - 2. It is well understood that NSAIDs (nonsteroidal anti-inflammatory drugs) i.e indomethacin inhibits the production of prostaglandin that enhances the sensitivity of pain perception and nociception. Hence, it can be perceived that anti-inflammatory effect of the ethanolic extract of Atropa belladonna may have occurred by the similar way of inhibition of the synthesis of prostaglandin. The formalin model is very useful for explaining mechanism of inflammatory pain and analgesia, thus it was used to illuminate the mechanism of anti-inflammatory analgesic effect of belladonna ethanolic extract. Therefore this study has also revealed that the atropa belladonna extract has possessed noteworthy dose dependent anti-inflammatory property. The observations further indicated that the effect exerted in the late chronic inflammatory phase of formalin test.

Table 1. Acute Toxicity Testing of Crude Ethanolic Extract of Atropa Belladonna

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose(mg/kg) i.g</th>
<th>n</th>
<th>Percent Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB extract</td>
<td>10</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>12</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>12</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>12</td>
<td>100</td>
</tr>
<tr>
<td>Tween 80(10%)</td>
<td>---</td>
<td>20</td>
<td>0</td>
</tr>
</tbody>
</table>
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Table 2. Effect of crude Atropa belladonna ethanolic extract and Indomethacin in Formalin induced Paw edema in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Paw edema (mm)</th>
<th>% edema decreased over time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1 hr</td>
</tr>
<tr>
<td>Tween 80(control)</td>
<td>1 ml/kg</td>
<td>3.0 ± 0.41</td>
<td>---</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10 mg/kg</td>
<td>3.1 ± 0.32</td>
<td>55.7</td>
</tr>
<tr>
<td>AB extract</td>
<td>50 mg/kg</td>
<td>3.1 ± 0.41</td>
<td>10.8</td>
</tr>
<tr>
<td>AB extract</td>
<td>250 mg/kg</td>
<td>3.2 ± 0.31</td>
<td>19.5</td>
</tr>
<tr>
<td>AB extract</td>
<td>400 mg/kg</td>
<td>3.0 ± 0.42</td>
<td>28.8</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The aforementioned analysis has elucidated that the herbal extract of Atropa belladonna has significant dose dependent anti-inflammatory potential specifically in the late or chronic phase in the process of inflammation following the formalin test rather than acute inflammation.

Subsequently, the current investigation has paved the way for advance studies for the verification of the mechanism that is involved in the anti-inflammatory action of atropa belladonna extract. Apparently anti-inflammatory effect of belladonna extract is probably mediated by the inhibition of prostaglandin synthesis. Additionally, the results validate the traditional medicinal use of the plants as anti-inflammatory and spasmolytic for different conditions associated with inflammatory pain such as arthritis.

REFERENCES