Evaluation the analgesic and anti-inflammatory activity of aqueous extracts of Iraqi propolis in male Albino mice

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Abstract

The present study was designed to evaluate the analgesic and anti-inflammatory activity of the aqueous extracts of Iraqi propolis (AEIP) in male mice, AEIP at the doses of 100, 200 and 300mg/kg/orally. For the evaluation of analgesic activity hot plate and acetic acid were used, while for anti-inflammatory we using carrageenan to induce paw edema. The results exhibited significant p<0.001 and dose dependent activity compared with the control by increasing in reaction time to thermal stimuli and reduction in abdominal writhing induced by acetic acid, furthermore AEIP was significantly p<0.001 reduced paw edema induced by sub planter injection of carrageenan. In conclusion oral administration of AEIP showed that it has antinociceptive and anti-inflammatory activities.

Introduction

Inflammation is the response of cells and body tissues to injury through different factors such as infections, chemicals, thermal and mechanical injuries (1). Various endogenous mediators like histamine, serotonin, bradykinin, prostaglandins etc are most abundant in inflammatory cell and among them prostaglandins are ubiquitous substances that indicate and modulate cell and tissue responses involved in inflammation. These mediators even in small quantities can elicit pain response. Pain results in dropped muscular activities. Most of the anti-inflammatory drugs now available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are hyperalgesic, potent vasodialators and also contribute to erythema, edema and pain. Hence, for treating inflammatory diseases analgesic and anti-inflammatory agents are required (2). Non steroidal anti-inflammatory drugs (NSAIDs) are the most clinically important medicines used for the treatment of inflammation related diseases like arthritis and cardiovascular disease (3). Having various and severe adverse effects like gastric lesions for NSAIDs, adverse cardiovascular thrombotic effects for selective cyclooxygenase-2 (COX-2) inhibitors (4). Therefore, new anti-inflammatory and analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects.
The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs (5). Propolis is a resinous material collected by bees from exudates and bud of the plants and mixed with wax and bee enzymes. The word propolis (from the Greek pro = in defense or for, and polis = city) reflects its importance to bees, since they use it to smooth out internal walls, as well as to protect the colony from diseases and to cover carcasses of intruders who died inside the hive, avoiding their decomposition (6). More than 300 components have been found in propolis, mainly composed of phenolic compounds (e.g., flavonoids, aromatic compounds), terpenes and essential oil [6-8]. Propolis has been proven to have various bioactivities that are anti-pathogenic, immunoregulatory, antioxidative, anti-tumor, hepatoprotective and anti-inflammatory [8-10]. Despite of these reports few attempt has been ever made in screening the effectiveness of propolis from Iraqi origin. Therefore, The aim of this study was to investigate the analgesic and anti-inflammatory properties of aqueous extracts Iraqi propolis(AEIP).

MATERIAL AND METHODS

Origin of propolis sample
Propolis samples were collected from hives of honey bees of Karbala, Iraq during spring and summer seasons of 2012. Propolis samples were cleaned, free of wax, paint, wood, cut into small pieces, and placed in clean container.

Preparation of aqueous extract of propolis
Aqueous extract of propolis was obtained as described by Nagai et al (11) ,with slight modification. In brief, 100.0 g of propolis was suspended and extracted with 10 volumes of distilled water with shaking at 80 °C for 1 day. The extracts were vacuum filtered using Buchner assembly and filtrates were pooled. The residue was re-extracted under the same conditions. Finally all the extracts were pooled together and solvent was evaporated using rotary evaporator. The dry mass obtained was collected and 100 ml of 10 mg/ml stock solution was prepared. This solution was utilized to prepare different concentration of extract for further assessments.

Experimental animals
Ninety albino Swiss mice (20-25 g) were used in this study. They were obtained from the Iraqi National Center for Drugs Safety and Evaluation. The animals were housed in plastic cages (groups of six mice/cage) in a room with controlled temperature (22±2 °C) under a 12 h light/dark cycle with access to standard certified rodent diet and water ad libitum.

Analgesic activity

Hot plate method: The animals were divided into five groups with six mice in each group. Group I animals received Normal saline and save as control group, animals of Group II,III,IV treated with 100, 200,300mg/kg/orally of AEIP respectively, while the V group received Diclofenac at the dose of 0.71mg/ kg, orally . The animals were placed on Eddy’s hot plate kept at a temperature of (55±0.5)°C. A cut off period of 15 second, was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to 0, 30, 60 and 120 min after oral administration of the samples (12).

Acetic Acid-Induced Writhing Response:
Mice were divided into five groups each containing 6 mice. The control group received Normal saline (10ml/kg, orally) The test groups were treated with 100,200,300 mg/kg/orally of AEIP while the fifth group received Diclofenac at the dose of 0.71mg/ kg, orally. After 30 minutes of drug administration, the mice were treated with 0.6% acetic acid at 10ml/kg body weight, i.p. (13). Five minutes after acetic acid injection, mice were placed in individual cage and the number of abdominal contractions was counted for each mouse for a period of 10 minutes after 5 minutes latency. A significant reduction of writhes in tested animals compared to those in the control group.
was considered as an antinociceptive response and the percentage inhibition of writhing was calculated as following:

\[
\% \text{ of inhibition} = \frac{(W_c - W_t)}{W_c} \times 100.
\]

**Anti inflammatory activity**

Carr-Induced Mice Paw Edema

Carr-induced hind paw edema model was used for determination of anti-inflammatory activity (14). Male mice (18–25 g) were randomly assigned to five groups (n = 6) including Carr group received 1% Carr (Fifty microliters). AEIP at doses of 100,200,300mg/kg were orally administered 2 h before the injection with 1% Carr in the plantar side of right hind paws of the mice. And Indomethacin (10 mg/kg) was intraperitoneally administered 90 min before the injection with 1% Carr in the plantar side of right hind paws of the mice. Paw volume was measured immediately after Carr injection at 1, 2 and 3 intervals using a plethysmometer (model 7159, Ugo Basile, Varese, Italy). The degree of swelling induced was evaluated by a minus b, where a was the volume of the right hind paw after Carr treatment and b was the volume of the right hind paw before Carr treatment. Indomethacin was used as a positive control.

**STATISTICAL ANALYSIS**

Results were expressed as Mean ± S.D. Statistical significance was calculated by using One Way Analysis of Variance (ANOVA) by SPSS software version 12.0. P<0.05 was considered as significant. Values bearing different letters as superscripts showed significant differences (p < 0.05).

**Results and Discussion**:

Mice treated with AEIP or 0.71mg/kg diclofenac(group II,III,IV,V) showed significant increase in reactive time to thermal stimuli (P<0.0001) in comparison to control animals (group I) both 30, 60 and 120 minutes following treatment tested by using hot plate method, also there is no significant differences between group IV and V (table1). While table 2 shows the responses of mice to acetic acid induced writhing. Treatment with different doses of AEIP or diclofenac reveal significantly (p < 0.0001) reduced the number of writhes compare to control group, furthermore there is significant differences between group IV and V. The results obtained with AEIP or indomethacin on carrageenan induced hind paw edema are shown in Table 3. The extracts significantly (p < 0.0001) inhibited the inflammatory edema. The effect of inhibition by all the AEIP was compared to that of control and indomethacin group. The inhibition was more in group III and IV.

Table 1- Reactive time to the thermal stimuli (second) in male mice treated orally with AEIP and diclofenac.

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 Min.</th>
<th>30 Min.</th>
<th>60 Min.</th>
<th>120 Min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>11.76 ± 0.47</td>
<td>12.36 ± 0.31</td>
<td>12.42 ± 0.34</td>
<td>12.54 ± 0.89</td>
</tr>
<tr>
<td>AEIP (100mg/Kg )II</td>
<td>11.56 ± 0.55</td>
<td>15.02 ± 2.37</td>
<td>17.06 ± 1.59</td>
<td>21.36 ± 2.18</td>
</tr>
<tr>
<td>AEIP (200mg/Kg )III</td>
<td>11.92 ± 0.48</td>
<td>18.17 ± 1.57</td>
<td>22.66 ± 1.45</td>
<td>23.78 ± 2.16</td>
</tr>
<tr>
<td>AEIP (300mg/Kg )IV</td>
<td>11.64 ± 0.56</td>
<td>24.02 ± 1.43</td>
<td>26.36 ± 2.06</td>
<td>28.64 ± 2.34</td>
</tr>
<tr>
<td>Diclofenac (0.71 mg/Kg )V</td>
<td>11.42 ± 0.46</td>
<td>23.92 ± 1.81</td>
<td>27.38 ± 1.59</td>
<td>27.64 ± 2.68</td>
</tr>
<tr>
<td>P</td>
<td>NS 0.63331</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

- Means with different letters refers to significant differences between groups vertically.
- P ≤0.0001
Table 2- The effect of AEIP and diclofenac on acetic acid induced writhes in male mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of writhing per 15 Min.</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>38.8 ± 1.60</td>
<td>A</td>
</tr>
<tr>
<td>AEIP (100mg/Kg)II</td>
<td>17.6 ± 1.42</td>
<td>54.64</td>
</tr>
<tr>
<td>AEIP (200mg/Kg)III</td>
<td>12.2 ± 1.43</td>
<td>68.56</td>
</tr>
<tr>
<td>AEIP (300mg/Kg)IV</td>
<td>9.4 ± 0.92</td>
<td>75.77</td>
</tr>
<tr>
<td>Diclofenac (0.71 mg/Kg) V</td>
<td>14.8 ± 1.10</td>
<td>61.86</td>
</tr>
</tbody>
</table>

Means with different letters refers to significant differences between groups vertically.

- P ≤0.0001

Table: 3- Effect of AEIP and Indomethacin on carrageenan-induced paw edema in male mice.

<table>
<thead>
<tr>
<th>Groups TRT</th>
<th>1 hr.</th>
<th>2 hr.</th>
<th>3 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>31.2 ± 0.3</td>
<td>60.6 ± 0.1</td>
<td>55.3 ± 0.3</td>
</tr>
<tr>
<td>AEIP (100mg/Kg)II</td>
<td>26.3 ± 0.2</td>
<td>32.5 ± 0.3</td>
<td>28.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>(15.70 %)</td>
<td>(46.37 %)</td>
<td>(47.92 %)</td>
</tr>
<tr>
<td>AEIP (200mg/Kg)III</td>
<td>15.1 ± 0.5</td>
<td>14.8 ± 0.4</td>
<td>11.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>(51.60 %)</td>
<td>(75.58 %)</td>
<td>(79.57 %)</td>
</tr>
<tr>
<td>AEIP (300mg/Kg)IV</td>
<td>13.2 ± 0.3</td>
<td>15.6 ± 0.4</td>
<td>10.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>(57.69 %)</td>
<td>(74.25 %)</td>
<td>(81.19 %)</td>
</tr>
<tr>
<td>Indomethacin (10 mg/Kg) V</td>
<td>23.2 ± 0.4</td>
<td>38.6 ± 0.2</td>
<td>24.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>(25.64 %)</td>
<td>(36.30 %)</td>
<td>(56.42 %)</td>
</tr>
</tbody>
</table>

Means with different letters refers to significant differences between groups vertically.

- P ≤0.0001

The brain and spinal cord play a major role in central pain mechanisms. The dorsal horn of the spinal cord is endowed with several neurotransmitters and receptors including: substance P, somatostatin, neuropeptide Y, inhibitory amino acid, nitric oxide, endogenous opioids and the monoamines which are the major targets for pain and inflammation (15). The hotplate method was considered to be selective to examine compounds acting through opioid receptor; all the treated groups increased pain threshold which mean basal latency which indicates that it may act via centrally mediated analgesic mechanism. Narcotic analgesics inhibit both peripheral and central mechanism of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain (16). The AEIP inhibits pain with both mechanisms, suggesting that the extract may act as a narcotic analgesic. On the other hand, acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from the tissue phospholipid (17). The acetic acid induced writhing response is a
sensitive procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (18), acid sensing ion channels (19) and the prostaglandin pathways (20). Flavonoids may increase the amount of endogenous serotonin or may interact with 5-HT2A and 5-HT3 receptors which may be involved in the mechanism of central analgesic activity (21). Moreover, AEIP showed highest analgesic activity in all the experimental model which may be due to its high flavonoid content as well as free radical scavenging activity, as these free radicals are involved during pain stimulation and antioxidants showed reduction in such pain (22). Carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages (23). Since the AEIP significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme. Flavonoids and saponins are well known for their ability to inhibit pain perception as well as anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (24). The ability of flavonoids to inhibit eicosanoid biosynthesis has been documented. Eicosanoids, such as prostaglandins are involved in various immunological responses and are the end products of the cyclooxygenase and lipoxygenase pathways (25). Further flavonoids are might able to inhibit neutrophils degranulation and thereby decrease the release of arachidonic acid (26). Thus the presence of flavonoids in the AEIP may be responsible for the anti-inflammatory and analgesic activity in Swiss albino mice. This study may be concluded that the AEIP possesses analgesic and anti-inflammatory properties, which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanisms. The extract will, therefore, be of potential benefit in the management of pain and inflammatory disorders.

Reference:


