Phytochemical screening and evaluation of analgesic, anti-inflammatory activities of Peganum harmala Linn., seeds in rodents

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ABSTRACT
The objective of this research work is to carryout the phytochemical screening and evaluate the analgesic, anti-inflammatory activities of Peganum harmala (Linn) seeds. In this study different extracts of Peganum harmala (Linn) seeds were evaluated for analgesic and anti-inflammatory activities using glacial acetic acid induced writhing and carrageenan induced rat paw edema models respectively. For analgesic and anti-inflammatory activities aspirin and diclofenac were used as standard drugs respectively. The ethyl acetate extract showed significant analgesic and anti-inflammatory activities, thus it can be considered as a potential candidate for analgesic and anti-inflammatory activities. The presence of alkaloids, steroids, flavonoids in ethyl acetate extract of Peganum harmala (Linn) seeds could be attributed for the claimed analgesic and anti-inflammatory activities.

INTRODUCTION
Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, thermal and mechanical injuries (Oyedapo et al., 2008). Most of the antiinflammatory drugs presently available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are the mediators of inflammation. Inflammation is accompanied by redness, swelling, pain (analgesia). Inhibition of prostaglandins synthesis is essential to treat inflammation. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required (Anilkumar, 2010). Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions (IMS Health, 2005). But, the long-term administration of NSAIDs may induce gastro-intestinal ulcers, bleeding and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenase enzymes (Robert, 1976; Peskar, 1977; Tapiero et al., 2002). Therefore, there is a need to develop new anti-inflammatory and analgesic drugs with potent activity, less side effects are being searched all over the world as alternatives to NSAIDs and opiates (Dharmasiri et al., 2003; Kumara, 2001). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects.

Plants and plant extracts have been used since the dawn of civilization by mankind. The uses of ethnobotanical preparations for various reasons justified or not, are still continued by various cultures all over the world. Considering structural and biological diversity of terrestrial plants, they offer a unique renewable resource for the discovery of potential new drugs and modern medicine has developed a rational strategy for drug discovery which involves the study of plants and plant materials based on their ethnobotanical usage (Cordell et al., 1991). Natural products are sources of active compounds that may be useful in the development of new and potent drugs.
Peganum harmala L. belongs to the family of Zygophyllaceae (Qazan, 2009). It is a wild growing flowering plant. It is also called African rue, Syrian rue and wild rue. The plant is widely distributed in predesertic regions of south-east Morocco, North Africa and the Middle East (EL-Bahri et al., 1991). Literature survey revealed that Peganum harmala L., shows different pharmacological activities like antioxidant (Dickson et al., 2006), antileishmanial (Di Giorgio et al., 2004), antihemoparasitoidian (Fan et al., 1997), antihistaminic (Gholamreza Asghari et al., 2002), vasorelaxant (Berrougui et al., 2006), antitumor (Lamchouri et al., 1999), wound healing (Derakhshanfar et al., 2010), antiplasmodial (Astulla et al., 2008), MAO inhibition (Herraiz et al., 2010), DNA topoisomerase I inhibition (Sobhani et al., 2002), myeloperoxidase inhibition (Bensalem et al., 2010), antibacterial and antitubercular activity (Pradeep Kumar et al., 2014) etc. However, Peganum harmala (Linn) seeds have not been investigated for analgesic and antiinflammatory activities. Hence, this study was carried out to evaluate the potent bioactive constituents for analgesic and anti-inflammatory activities in Peganum harmala (Linn) seeds.

MATERIALS AND METHODS

The Seeds of Peganum harmala (Linn) were collected from the local areas of Dharwad in Karnataka and were authenticated by Dr. S. S. Hebbar, Department of Botany, Government Pre-university College Dharwad. A voucher specimen (No- SETCPD/Pharmacog/Herb/2013/14) has been deposited in the Herbarium of Department of Pharmacognosy, S.E.T.’s College of Pharmacy, Dharwar, Karnataka.

The Seeds of Peganum harmala (Linn) were shade dried and finely powdered to particle size (#) 40. About 300g of dried powder was subjected to continuous hot soxhlet exhaustive extraction with petroleum ether, chloroform, ethyl acetate and ethanol (95%). Aqueous extract was also obtained by cold maceration of the drug (300 g) with 2% chloroform water. After the extraction, the extracts were filtered and concentrated under reduced pressure using a rota evaporator. The yield of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract was found to be 9.45 g (3.15 % w/w), 8.4 g (2.8 % w/w), 16 g (5.33 % w/w), 22 g (7.33 % w/w) and 20 g (6.66 % w/w) respectively. All the extracts were kept in a dessicator for drying.

Pharmacological activity

The pharmacological activities of synthesized compounds were investigated in albino rats and mice. Rats were used for acute anti-inflammatory and chronic anti-inflammatory activities. Mice were used for analgesic activity. All animal experiments were approved by institutional animal ethical committee. All the animals were stabilized to laboratory conditions before starting the experiments.

Acute toxicity

The acute toxicity test was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines (OECD/OCDC, 2000) to establish the effective dose of all the synthesized and extracted compounds.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the well known Carageenan induced rat paw oedema model (Winter et al., 1962) using groups of six animals each. A freshly prepared aqueous suspension of carageenan (1.0% w/v, 0.1 ml) was injected in the sub planter region of right hind paw of each rat. One group was kept as control and the animals of the other group were pre-treated with the test extract at a dose of 200 mg/ kg body weight of rat, 1 h before the carageenan treatment. The volume was measured before and after carageenan treatment at the 30 min. interval with the help of digital plethysmometer. Standard drug used was diclofenac at a dose of 200 mg/kg body weight of rat.

Analgesic activity

Twenty four hours prior to actual testing a large number of mice (20-25 gm) received intraperitoneally (i.p) 10 ml/kg of 0.6% glacial acetic acid. Animals were observed for writhing movements. Only those showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day the responders received compounds half an hour prior to glacial acetic acid challenge (Koster et al., 1959). Extracts were given at a dose of 200 mg/kg orally. Standard drug used was aspirin at a dose of 30 mg/kg body weight of mice.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, flavonoids in the alcoholic extract and alkaloids, flavonoids and steroids in the ethyl acetate extract. The results are shown in Table-2. Physico chemical parameters for the Peganum harmala Linn., seeds are shown in Table-1.

Hence, the presence of alkaloids, flavonoids and steroids in the ethyl acetate extract could be attributed for observed significant analgesic (Jain et al., 2011; Parveen et al., 2007) and anti-
inflammatory (Sakat et al., 2010; Nagore et al., 2010) activities. However, research work is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principles for the claimed analgesic and anti-inflammatory activities.

Table 1: Physico-chemical parameters of Peganum harmala Linn., seeds.

<table>
<thead>
<tr>
<th>Sl No</th>
<th>Parameter</th>
<th>Determined values in % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alcohol soluble extractives</td>
<td>7.88</td>
</tr>
<tr>
<td>2</td>
<td>Hydro-alcoholic extractives</td>
<td>14.11</td>
</tr>
<tr>
<td>3</td>
<td>Water soluble extractives</td>
<td>18.73</td>
</tr>
<tr>
<td>4</td>
<td>Ether soluble extractives</td>
<td>6.98</td>
</tr>
<tr>
<td>5</td>
<td>Total ash value</td>
<td>7.64</td>
</tr>
<tr>
<td>6</td>
<td>Acid insoluble ash</td>
<td>1.52</td>
</tr>
<tr>
<td>7</td>
<td>Water soluble ash</td>
<td>3.59</td>
</tr>
<tr>
<td>8</td>
<td>Sulfated ash</td>
<td>8.33</td>
</tr>
<tr>
<td>9</td>
<td>Moisture content</td>
<td>2.4</td>
</tr>
</tbody>
</table>

**TABLE 2:** Preliminary phytochemical analysis of various extracts of Peganum harmala Linn., Seeds.

<table>
<thead>
<tr>
<th>Phytoconstituent</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>Alcohol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Steroids</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Phenolic</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

+ve = Present -ve = Absent

Table 3: Anti-inflammatory activity screening of Peganum harmala Linn seeds.

Extract/ Compound | Percentage inhibition of Paw oedema 1hr | 3hr | 5hr |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>20.08</td>
<td>31.43</td>
<td>22.42</td>
</tr>
<tr>
<td>Alcohol Extract</td>
<td>36.10</td>
<td>64.38**</td>
<td>67.95**</td>
</tr>
<tr>
<td>Chloroform Extract</td>
<td>25.40</td>
<td>48.53**</td>
<td>42.63**</td>
</tr>
<tr>
<td>Pet-ether Extract</td>
<td>19.89</td>
<td>32.28</td>
<td>36.44</td>
</tr>
<tr>
<td>Ethyl acetate Extract</td>
<td>38.13</td>
<td>70.34**</td>
<td>69.78**</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>42.12</td>
<td>79.53</td>
<td>76.68</td>
</tr>
</tbody>
</table>

Animals were dosed 200 mg/kg. Data were analysed by ANOVA. Followed by Dunnett’s test. **P<0.001. P < 0.05. Values are mean ± SEM, of six animals in each group.

CONCLUSION

The present study provides an evidence for the analgesic and anti-inflammatory activities of Peganum harmala (Linn) seeds. Aspirin and diclofenac were used as standard drugs for screening the analgesic and antiinflammatory activities respectively. Aspirin used as standard drug for analgesic activity acts by obtunding of peripheral pain receptors and prevention of PG- mediated sensitization of nerve endings (Tripathi, 2013) and the diclofenac used as standard drug for screening the anti-inflammatory activity act by inhibiting the prostaglandin synthesis and specially it is COX-2 selective (Tripathi, 2013). As the analgesic and anti-inflammatory activities results of the ethyl acetate extract studied are close to those of aspirin and diclofenac, the bioactive principles present in the extract may be having the mechanism of action similar to that of the tested standard drugs. However research is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principles for the claimed analgesic and anti-inflammatory activities.

The present study may form the basis for the selection of plant species for further investigation in potent bioactive compounds for analgesic and anti-inflammatory activities.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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