Cytotoxic and Analgesic Potentials of Papaver Pavoninum Fisch & Mey.

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Abstract

Ethanol extract of whole plant of Papaver pavoninum was used to investigate its cytotoxic and analgesic potentials. Brine Shrimp Cytotoxic bioassay showed that 100 and 1000 μg/ml doses produced highly significant cytotoxicities causing 83.3±1.924% and 96.7±1.924% lethality respectively, with LD50 value of 2.54μg/ml. The analgesic bioassay, using acetic acid induced writhing behavior in mice showed that all the three doses of the extract (50, 100 and 150 mg/kg) were highly effective and even more effective than the standard analgesic drug (Diclofenic Sodium), which reduced the number of writhes by 13.54%, while the three doses of the plant extract reduced the writhing by 36.91%, 57.01% and 68.39% respectively.

Key words: Papaver pavoninum, Ethanolic extract, Cytotoxicity, Analgesic activity.

Introduction

Plants have a significant role in curing various diseases and complexities affecting human health through their inherent active constituents (Nair et al., 2004; Okigbo et al., 2008). Plant derived medicines have been in use since ancient times for self-medication due to their therapeutic properties (Arkele et al., 1992). An estimated 80% of the world population depends on crude folk medicines (Sing & Hundri, 2000). Strong faith in traditional herbal medicines, their easy availability and reasonable prices make them preferable choice over modern pharmaceutics (Shabbir et al., 2004).

An increase has been noted in the use of medicinal plants, both in traditional and industrial areas and these are being exploited for biological screening of natural bioactive products (Ozturk & Ozturk, 2008). This trend has increased interest of modern plant scientists towards natural active constituents of plant origin for manufacture of useful drugs (Yakuba et al., 2007). The reason behind exponential growth and commercialization in the field of herbal medicines in both developing and developed countries is mainly due to their natural origin, low cost, easy availability and lesser side effects (Mulla & Swamy, 2010).

Cytotoxic activity: Screening of active substances from plant sources has resulted in discovery of new and efficient drugs against various diseases, including cancer (Amara et al., 2008). Brine shrimp bioassay is a quick and cheap bioassay by which physiologically active natural products with potential cytotoxic activity can be screened out (Kivack et al., 2001, Carballo et al., 2002). This bioassay serves as a useful tool for assessment of cytotoxicity by heavy metals, cyanobacterial toxins and plant extracts (Moshafi et al., 2009). Cytotoxic screening provides preliminary grounds for selection of plant extracts having anticancer potentials (Al-Fatimi et al., 2007).

Analgesic activity: Analgesics are pain relieving substances, which reduce pain without losing consciousness. Peripheral and central nervous systems are affected in various ways by such drugs. There are various sources of analgesic drugs, of which some are synthetic drugs for example Disprin, Paracetamol and Diclofenic etc. Medicinal plants also provide a rich source of analgesics (Kumar et al., 2010). Many researchers have worked out to identify the analgesic properties of various plant sources like, Jakson et al., 2011 (Carpolobia lutea), Bukhari et al., 2010 (Acacia modesta), Nisar et al., 2008 (Taxus wallichiana), Perianayagam et al., 2004 (Emblica officinalis).

Some members of family Papaveraceae have also been reported to have analgesic actions like, Glauicium grandiflorum, Glauicium paucilobum (Morteza-Semnani et al., 2002, 2006). Opioids are drugs derived from opium (Papaver somnifarum), and used in various medications as analgesic agents, for relieving severe or chronic pain (Kumar et al., 2010). According to Elpel 2000, California poppy (Eschscholzia) serves as an analgesic tea and Prickly Poppy (Argemone) is used as analgesic wash for sunburn. As at present there is no such report about the analgesic potential of Papaver pavoninum. A bioassay, using experimental animals was carried out to investigate the analgesic potential of this plant.

Materials and Methods

Cytotoxicity: To check the cytotoxic potential of Papaver pavoninum, Brine shrimp bioassay was carried out, adopting the procedures of Atta-ur-Rehman, (2001).

Requirements: Hatching tray (a rectangular dish (22x32 cm), a perforated partition, Brine shrimp (Artemia salina) eggs, Lamp to attract brine-shrimp larvae, Sea Salt (38 g/L of D/W, pH 7.4), methanol, Micro pipette (5, 50,500μl), vials tray and 30 vials.

The hatching tray was half-filled with filtered sea salt solution and a perforated partition was placed in the tray to make two unequal portions. To the smaller portion 50,500μl), vials tray and 30 vials.

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plant was dissolved in 2ml solvent, to serve as stock solution, from which 5, 50 and 500 µl were transferred to vials (3vials/concentration). The solvent was allowed to evaporate overnight. Later 5 ml of seawater was added to each vial. The resultant concentrations obtained were 10, 100 and 1000 µg/ml respectively. Control vials (3 in number) were added only with sea water solution. After 48 hours of hatching and maturation as napulii, 10 larvae were transferred to each vial using a Pasteur pipette. All the vials were incubated at 25-27°C for 24 hours under illumination. After 24 hours, number of shrimps killed in each treatment was counted and the data was analyzed with Finney computer program (Finney, 1981) to determine LD₅₀ values with 95% confidence interval.

Analgesic activity

Requirements: Albino mice (about 22 grams in weight), Diclofenic sodium, Acetic acid, Sterile normal saline (used as control), plant extract prepared in normal saline. The animals were maintained under standard laboratory conditions (25°C and light/dark cycles i.e. 12/12 h and were fed with standard food and water.

Procedure

Acetic acid induced writhing test: Animals were kept on fast for 2 hours before the start of the experiment. Animals were divided into five groups (6 mice/group). Group I was injected with normal saline (10 ml/kg i.p.) to serve as negative control while group II was injected with standard drug (diclofenac sodium, 50 mg/ kg i.p.) as positive control. Group III, IV and V were injected with 50, 100 and 150 mg/kg i.p. of the plant extract. After 30 minutes of administration of these doses, pain was induced by injecting 1% acetic acid into the peritoneal cavity of all mice. The writhing (contraction of abdomen, turning of trunk and extension of hind limbs) that occurred within the next 10 minutes following acetic acid administration were counted and recorded for all the mice and the results were expressed as percentage inhibition (Akuodor et al., 2011).

Results and Discussion

Cytotoxic activity of Papaver pavoninum: Phytochemicals derived from various plants are extensively being employed in formulating useful drugs for various diseases, including cancers, the mimic of the present time (Amara et al., 2008). Toxic effects of various chemicals on Artemia salina (Crustacean) larvae is correlated to the antitumor potential, as brine shrimps respond in quite a similar way as do the mammalian tissues (McLaughling, 1991; Solis, 1993). Thus evaluation of cytotoxic potential through brine shrimp lethality bioassay is significant in searching new anticancer drugs (McLaughling, 1998).

In the present study Brine shrimp lethality bioassay was carried out to evaluate the basic cytotoxic potential of the crude ethanolic extract of Papaver pavoninum (Table 1, Fig. 1). Three concentrations (10µg/ml, 100µg/ml and 1000 µg/ml) were applied. The extract was found to produce outstanding dose dependent cytotoxicity, based on the following criteria:

- 30-40% lethality → Low activity
- 50% lethality → moderate activity
- 60-70% lethality → good activity
- Above 70% lethality → significant activity

The 100 and 1000 µg/ml doses produced highly significant cytotoxicity causing 83.3±1.924% and 96.7±1.924% lethality respectively, while the 10 µg/ml dose caused moderate lethality (56.7±5.091%). LD₅₀ value was 2.54µg/ml, which indicates that the plant may serve as an effective cytotoxic source. Other researchers have also carried out such brine shrimp lethality tests on various plants such as Luffa acutangula and Luffa cylindrica (Bulbul et al., 2011), Polygonatum verticillatum (Saeed et al., 2010), Agave cantula (Ateeq-ur-Rehman et al., 2009), Hibiscus sabdariffa (Tolulop, 2007) . Some researchers have also reported cytotoxic potentials of various members of family Papaveraceae like, Chelidonium majus L. (Ernst & Schmidt, 2005), Argemone maxicana (Dahawi, 2009; Lagnika et al., 2011), Corydalis cava (Nawrot et al., 2010). The present study showed that P. pavoninum has a very promising cytotoxic properties and it can further be worked out to isolate and identify the exact phytochemical responsible for cytotoxicity. Taborska et al., (1988) reported isolation of various alkaloids (N²-methyl-1, 2, 3, 4-tetrahydro-β-caroline alkaloid, allocryptopine, isocorydine, protopine, magnoflorine, corydine, corytuberine and coptisine) from P. pavoninum. Lagnika et al. (2011) suggested alkaloids to be responsible for the cytotoxic potentials of Argemone mexican, which may be similar to the effect of the alkaloids of P. pavoninum.

Terpenes may also be responsible for cytotoxic effect, because sesquiterpines derivatives are known to have cytotoxic potentials (Evans, 1989).

Flavonoids might also be the contributing agents as these are found to have some role in preventing various types of cancers (Wang et al., 2000).

Various classes of phytochemicals such Saponins, Terpines (monoterpenes, diterpenes, triterpenes and Sesquiterpines), Isoquinoline and pyrrolizidine alkaloids, Steroids, Quinones and Cucurbitacin possess anticancer potentials (Evans, 1989).Since most of these phytochemicals were also detected during the present phytochemical screening of the ethanolic extract of P. pavoninum, its cytotoxic/anti-cancer potential is strongly supported by these findings.

<table>
<thead>
<tr>
<th>Extract conc. (µg/ml)</th>
<th>T. no. of Larvae</th>
<th>No. of survivors</th>
<th>No. of dead shrimps</th>
<th>Percent dead</th>
<th>Intercept</th>
<th>R-Square</th>
<th>Slope</th>
<th>LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>30</td>
<td>13</td>
<td>17</td>
<td>56.7 ± 5.091</td>
<td>2.385</td>
<td>0.942</td>
<td>0.09</td>
<td>2.54</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>05</td>
<td>25</td>
<td>83.3 ± 1.924</td>
<td>2.385</td>
<td>0.942</td>
<td>0.09</td>
<td>2.54</td>
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<tr>
<td>1000</td>
<td>30</td>
<td>01</td>
<td>29</td>
<td>96.7 ± 1.924</td>
<td>2.385</td>
<td>0.942</td>
<td>0.09</td>
<td>2.54</td>
</tr>
</tbody>
</table>
Table 2. Analgesic effect of *Papaver pavoninum* ethanol extract in acetic acid induced writhing test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal No.</th>
<th>No. of writhes</th>
<th>Mean no. of writhes</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid (negative</td>
<td>1.</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control)</td>
<td>2.</td>
<td>84</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>3.</td>
<td>81</td>
<td>76.33</td>
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<tr>
<td></td>
<td>4.</td>
<td>69</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5.</td>
<td>90</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6.</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac (positive</td>
<td>1.</td>
<td>104</td>
<td></td>
<td></td>
</tr>
<tr>
<td>control)</td>
<td>2.</td>
<td>79</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>3.</td>
<td>55</td>
<td>66</td>
<td>13.54</td>
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<tr>
<td></td>
<td>4.</td>
<td>53</td>
<td>48.16**</td>
<td>36.91</td>
</tr>
<tr>
<td></td>
<td>5.</td>
<td>47</td>
<td></td>
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<tr>
<td></td>
<td>6.</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>3.</td>
<td>35</td>
<td>32.82***</td>
<td>57.01</td>
</tr>
<tr>
<td></td>
<td>4.</td>
<td>18</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5.</td>
<td>54</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>6.</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150 mg/kg</td>
<td>1.</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.</td>
<td>20</td>
<td>24.17***</td>
<td>68.39</td>
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<tr>
<td></td>
<td>4.</td>
<td>36</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>5.</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.</td>
<td>39</td>
<td></td>
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</tbody>
</table>

Analgesic effect of diclofenac (50 mg/Kg b.wt.) and 3 different doses of *Papaver pavoninum* administered i.p in abdominal writhing assay in mice. Each column represents Mean ± sem (n = 6). **p< 0.01, ***p< 0.01 ANOVA with Dunnett’s post-hoc analysis showing significant difference between control and treatment groups.
Analgesic effect of *Papaver pavoninum*: Various plants have served as outstanding source of natural pain relieving drugs acting in various ways upon the nervous system to eliminate sensation of pain (Kumar *et al*., 2010). Several species of family Papaveraceae have also been reported for their analgesic potentials e.g. *Argemone mexicana* Linn. (Sharma *et al*., 2010); *Papaver somniferum* (Kumar *et al*., 2010); *Glaucoma grandiflorum* and *Glaucoma paucilobum* (Morteza-Semnani *et al*., 2002, 2006); *Eschscholzia* (Elpel, 2000). These findings provoked the interest to screen out the present research plant i.e. *P. pavoninum* for its possible analgesic potentials.

Analgesia of *P. pavoninum* ethanol extract was investigated via acetic acid induced writhing behaviour test in mice. The extract significantly reduced pain in mice at all the three doses (50, 100 and 150 mg/kg body weight), as indicated by reduction in number of writhes. The results were analyzed through ANOVA with Dunnett’s post-hoc (P 0.01 Level of significance) and are summarized in Table 2, Figs. 2 & 3. The results showed that reduction in pain was dose dependent, hence the 150 mg/kg dose proved to be most effective. All the three doses were found to be more effective in reducing sensation of pain than diclofenac (the standard analgesic drug, used as positive control), as indicated by reduction in no. of writhing response in mice. Diclofenac reduced the no. of writhes by 13.54% while the 50, 100 and 150 mg/kg doses of the extract produced 36.91%, 57.01% and 68.39% reductions in acetic acid induced writhing response (Fig. 2). The results clearly proved the marked effectiveness of *P. pavoninum* as an analgesic drug.

Opioids are compounds derived from opium, which is obtained from *Papaver somniferum* capsules and used in various drug formulations. These opioids bind to opioid-receptors in the central nervous system to relieve severe and chronic pains (Kumar *et al*., 2010). Such compounds may also be present in *P. pavoninum* and have produced the analgesic effect in the present assay. Sharma *et al.* (2010) reported significant analgesic activity of *Argemone mexicana* (family Papaveraceae), and suggested that this analgesia might be due the presence of flavonoids and alkaloids (herberine, sarguinarine, protopine, chelerytherine, optisine etc.). Since *P. pavoninum* also belongs to same family and might has similar chemical constituents, with similar analgesic potential. Similarly morphine, codeine, etc. which are known for their analgesic, anodyne and somniferous effects (Tyler *et al*., 1988), are obtained from poppy plant, which also belongs to the same genus. These evidences strengthen the validity of the present findings.

References


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