Evaluation of methanol extract of mole crab *Emerita asiatica* for its pathological properties using mouse assay

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Abstract: A The methanol extract of marine mole crab, *Emerita asiatica* was tested for its pharmacological property using mouse assay. The vital organs viz. heart and lungs showed oedema and degeneration of cardiac muscles, markedly congested blood vessels and haemorrhagic exudates involving entire alveolar parenchyma in the lungs. The extract is seemed to influence negatively on the structure and functions of heart and lungs and non site-specific changes in the brain of mice.

Key words: Mole crab, Methanol extract, Heart, Lungs, Histopathology.

Introduction

Histopathological study examining the action of organic substances obtained from plants and animals, on the target organs has been evolved as a potential and reliable prognostic tool indicating the risk of relapse in various defined clinical context thereby facilitating therapeutic decisions. The search for bioactive substances in the marine realm indicated the presence of variety of structurally interesting and physiologically effective organic compounds in the marine animals. With regard to marine crustacean members it is reported that crabs (principal members of the group Decapoda) possess bioactive compounds having biomedical properties even similar to tetrodoxin (Venkateshwaran, 1997). The Decapods also possess many other biodynamic compounds significantly influencing the structure and functions of various vital organs of mammalian system (Dudel et al., 1963; Ruggieri, 1976; Watson et al., 1987; Lakshmanan and Venkateshwaran, 1999; Naveenkrishna and Venkateshwaran, 1999). Present study is aimed to ascertain the pharmacological properties of the methanol extract of an intertidal crustacean member *E. asiatica* using mouse assay.

Materials and Methods

For the present study, live animals (*Emerita asiatica*) weighing between 30-40 g were collected from the Pondicherry sandy coast during summer from the intertidal region of the Pondicherry coast. From the freshly collected crabs, a total of about 100 g of body muscle, was removed and was macerated. The whole mass was divided into two sub samples each weighing 50g. Each sub sample was dehydrated with sufficient dehydrating agent (sodium sulphate) following the method described by Clemmen (1985) and the two sub samples were refluxed in methanol for 6 hours and the extracts were used for pathological study as described by Harris et al (1956).

To ascertain the dose range that can be used for pathology, a pilot study was undertaken adopting the method described by Harris et al. (1956). 5 male Swiss mice weighing 20-25g were selected and kept in separate cages. The extract was injected intra- peritoneally on the ventral side of the abdomen. The doses given were 0.02, 0.04, 0.08, 0.2 and 0.4 ml of the crude extract for 5 mice and were grouped as I

Results and Discussion

The histopathological studies indicated that the methanol extract of the crab structurally affected the tissues of heart and lungs. The microscopic observations revealed
Table – 1: Gross behavioral changes (G.B.C.).

<table>
<thead>
<tr>
<th>Dose (ml)</th>
<th>Respiration</th>
<th>CNS</th>
<th>Pole climbing pattern</th>
<th>Eye ball</th>
<th>Righting reflex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>90º angle</td>
<td>Inclined 45º angle</td>
<td></td>
</tr>
<tr>
<td>0.02</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>0.04</td>
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<tr>
<td>0.08</td>
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<td>+</td>
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<td>0.2</td>
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<td>+</td>
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</table>

GBC after 30 min of extract administration

| 0.02      | -           | +   | +         | +       | +              | +               |

GBC after 1hr of extract administration

| 0.04      | -           | +   | +         | +       | +              | +               |
| 0.08      | -           | +   | -         | +       | +              | +               |
| 0.2       | -           | +   | -         | -       | +              | +               |

GBC after 2 hrs of extract administration

| 0.02      | -           | +   | +         | +       | +              | +               |
| 0.04      | -           | +   | +         | +       | +              | +               |
| 0.08      | -           | +   | +         | +       | +              | +               |
| 0.2       | -           | +   | +         | +       | +              | +               |

GBC after 3 hrs of extract administration

| 0.02      | -           | +   | +         | +       | +              | +               |
| 0.04      | -           | +   | +         | +       | +              | +               |
| 0.08      | -           | +   | +         | +       | +              | +               |
| 0.2       | -           | +   | +         | +       | +              | +               |

1. **Respiration**: Whether it is normal/ laboured respiration/abdominal respiration. Normal (+); Abdominal respiration (-)
2. **C.N.S.**: Whether CNS stimulated or depressed is observed by the movement of the animal, whether it exhibit spontaneous movement or not. Spontaneous movement (+); Irregular movement or absence of free movement (-)
3. **Pole climbing pattern**: The pole climbing down pattern in a vertical pole is observed for loss of grip or dropping. Normal(+); Loss of grip(-).
4. **Inclined plane**: The moving pattern of an inclined plane was observed; Normal (+); Abnormal/loss of grip (-)
5. **Watch on eye ball for ptosis development**: ie. eye is protrudable/squint = (-) /Sedation (=) normal (+).
6. **Righting reflex**: Whether the animal is able to come back to normal when it is placed on its back. Normal(+); Slowly(-)

Fig. 1: Sectional view of heart (20X) of control animal showing myocardium without oedema and clear cardiac tissue.

Oedema (separation of cardiac fibres) and degeneration of cardiac muscles (Fig.2). Further, markedly congested blood vessels and hemorrhagic exudates involving entire alveolar parenchyma, were also noticed (Fig.6). Moreover, scattered inflammation mainly composed of lymphocytes, was noticed in the case of lung tissues (Fig.7). It is worth to refer the reports of Goodman and Gilman (1995) that neither drugs similar to norephedrine induce respiratory difficulty by contracting the bronchioles and inducing oedema in alveoli. In the present study, the animal might have suffered from respiratory problem before its death exhibiting laboured respiration and other CNS controlled activities (Table 1). Therefore, it is presumed that the
Fig. 2: Sectional view of heart (20X) of treated animal showing marked oedema separating cardiac fibers and degeneration of cardiac tissue.

Fig. 3: Sectional view of brain (20X) of control animal showing no oedema.

Fig. 4: Sectional view of brain (20X) of treated animal showing marked oedema without any site specific change.
Fig. 5: Sectional view of lung (10X) of control animal showing bronchiole and alveoli.

Fig. 6: Sectional view of lung (20X) of treated animal showing markedly congested blood vessels and hemorrhagic exudates involving alveoli.

Fig. 7: Sectional view of lung (20X) of treated animal showing aggregation of lymphocytes. i.e. Mononuclear cells and few degenerated neutrophils.

extract could induce significant physiological effect on lungs and heart impairing the normal functioning of the vital organs; however, the brain tissues were not affected by the extract significantly except for some non site-specific changes i.e. oedema without showing any specific changes (Fig.4). A peer literature survey on the subject matter pertaining to crustaceans
indicated the possibility of compound responsible for such pharmacological property. Chatterjee (1967) indicated that certain cholinergic drugs are capable of affecting cardiovascular system. Evidences on CNS blocking compounds like γ-amino butyric acid in the members of crustaceans, were also reported earlier by Dudel et al. (1963). A detailed investigation on these lines with regard to crustacean members viz., *Carcinoscorpius rotundicauda* and *Carcinus maenas* also confirmed the presence of amines and carbonyl groups (Marderodian, 1968). Further, according to Chatterjee (1967) amines like tetramine and triamine are capable of influencing Central Nervous System and Cardiovascular System. The edible portunid crab, *Charybdis cruciata* and *Tachypleus gigas* were found to contain bioactive compounds capable of affecting kidney and CNS (Naveenkrishna and Venkateshwaran, 1999). Thus, the crustacean members are reported to possess bioactive substances that could influence the structural and functional impairment of the vital organs of the mammalian members. The observations made in the present study on pathological property of the extract of the mole crab *Emerita asiatica* indicate possibility for the presence of biodynamic substances capable of affecting mammalian lungs and heart.

However, further studies on isolation, purification and characterization of the methanol extract, would bring out the exact organic compound / drug responsible for such pharmacological property.

References


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