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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 tetradoxin (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 Ist, IInd, IIIrd, IVth, Vth respectively. The Gross Behavioural Changes (GBC) viz. mode of respiration (laboured/abdominal or normal), pole climbing and gripping, eye ball for ptosis development (squent /sedation or normal) and righting reflex action (whether the animal was capable of restoring to normal position when it was kept on its back), had been observed continuously for first 30 min and then for every one hour upto 3 hrs. All these observations were made by following the procedure given by Satoskuv *et al* (1997) and the observations were presented in Table 1. Based on the behavioural changes influenced by the extract, the minimum effective dose that can be taken for pathological study, was ascertained.

After ascertaining the dose range to be used in pathological study, 30 Swiss mice (male) with body weight ranging from 20-25gm were selected and assigned randomly for test and control purpose. They were set into 6 groups; of which one was the control and others were administered with 0.03, 0.1, 02, 0.2 and 0.4ml of extract respectively. The injection was given intraperitoneally and the animals were observed for 24 hours and death in each group was recorded.

As soon as the experiment was over the dead animals were separated and the vital organs of the animals viz. heart, lungs and brain were carefully removed and preserved in Bouin's solution for pathological studies. Sections were prepared and stained with haematoxylene and Eosin as described by Sumner and Sumner (1969).

Each slide was observed under trinocular microscope fitted with SLR camera. The site specific and non-site specific changes in the tissues were examined carefully and photographed.

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.).

Dose (ml)	Respiration	CNS	Pole climbing pattern		Eye ball	Righting reflex
			90º angle	Inclined 45º angle		
GBC after 30 min of extract administration						
0.02	-	-	-	+	+	-
0.04	-	-	-	-	-	-
0.08	-	-	-	-	-	-
0.2	-	-	-	-	-	-
GBC after 1hr of extract administration						
0.02	-	+	+	+	+	+
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 droping. 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/squent = (-) /Sedation (=) normal (+).
6. **Righting reflex:** Whether the animal is able to come back to normal when it is placed on its back. Normal(+); Slowly(-)

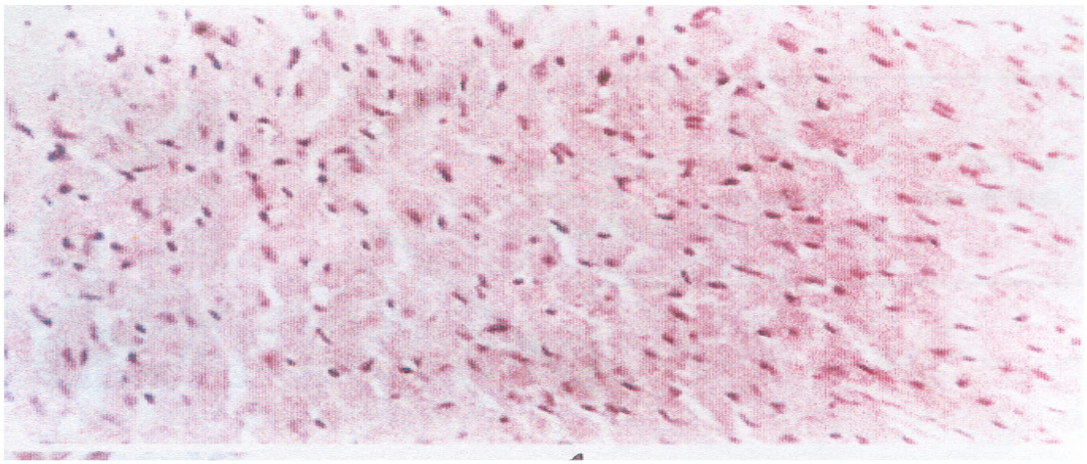

Fig. 1: Sectional view of heart (20X) of control animal showing myocardium without odema and clear cardiac tissue.

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 Gliman (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

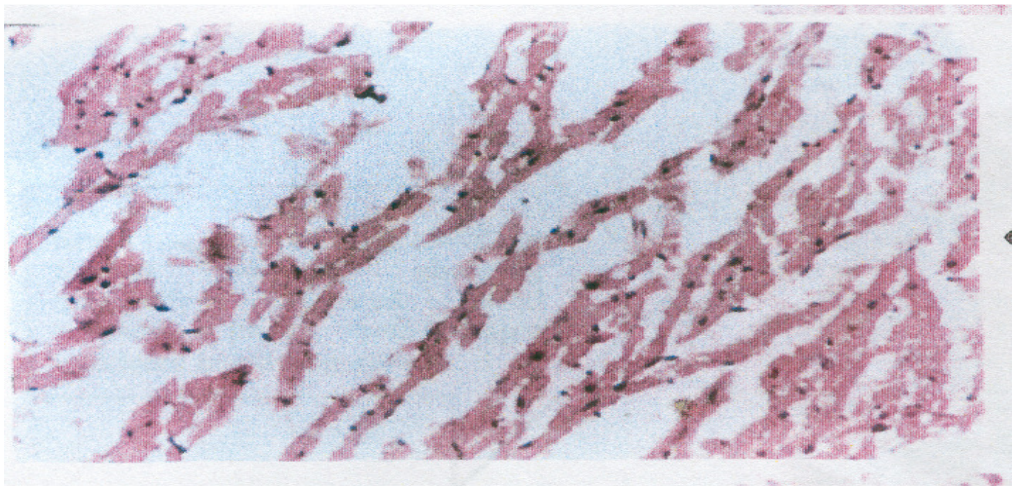

Fig. 2: Sectional view of heart (20X) of treated animal showing marked oedema separating cardiac fibers and degeneration of cardiac tissue

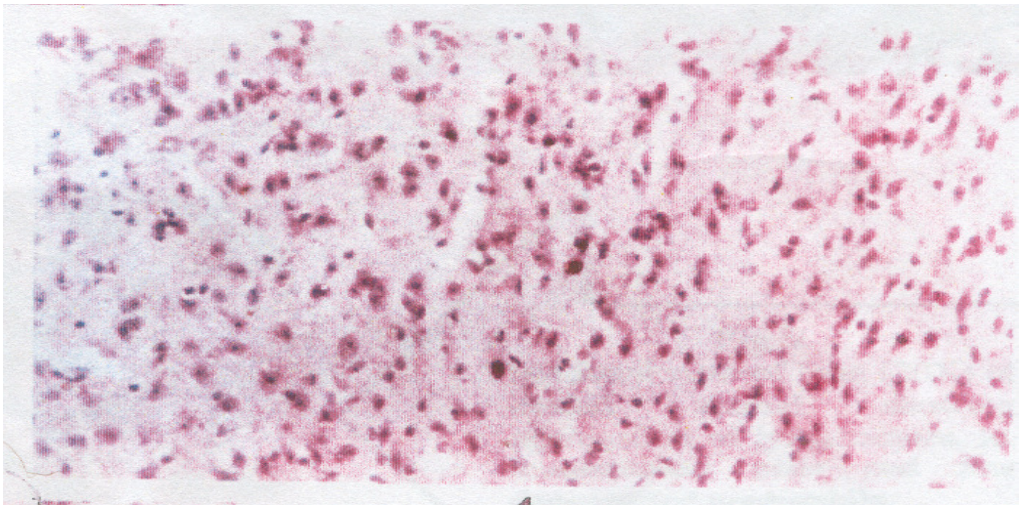

Fig. 3: Sectional view of brain (20X) of control animal showing no oedema.

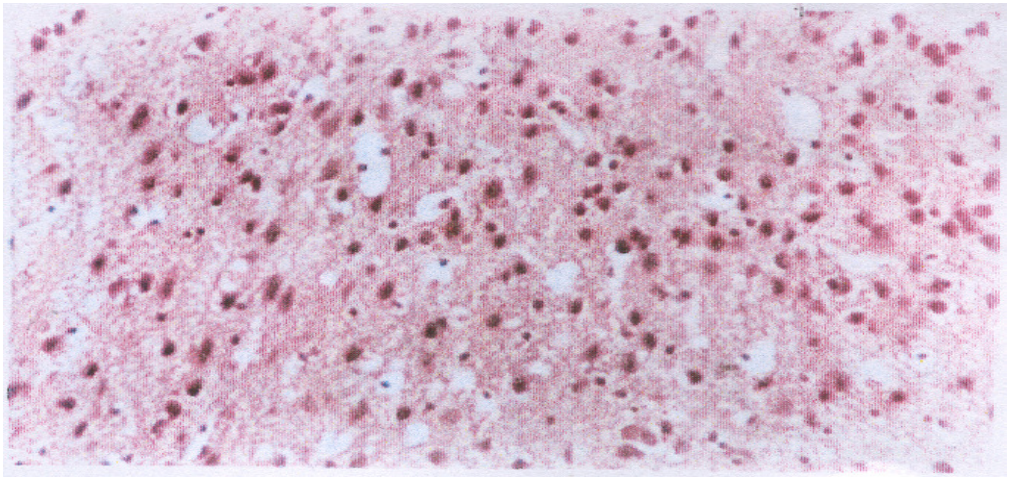

Fig. 4: Sectional view of brain (20X) of treated animal showing marked oedema without any site specific change.

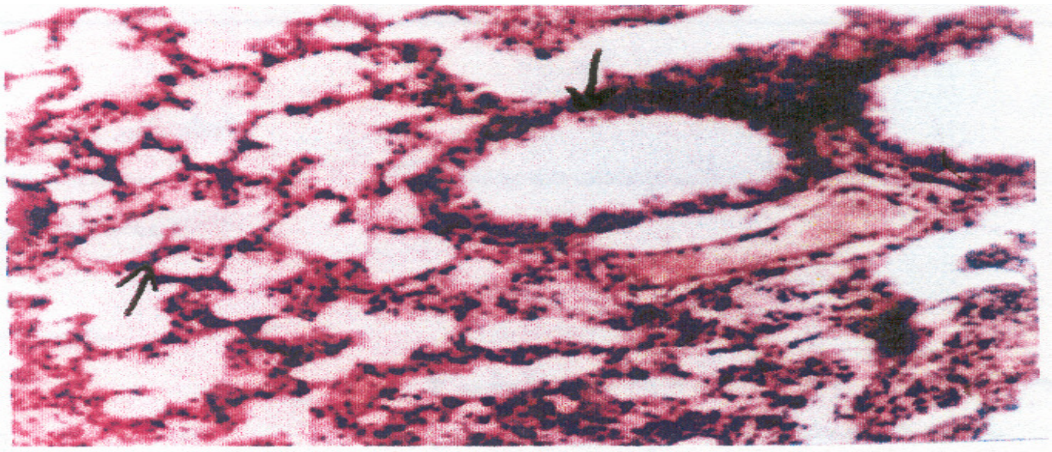

Fig. 5: Sectional view of lung (10X) of control animal showing bronchiole and alveoli.

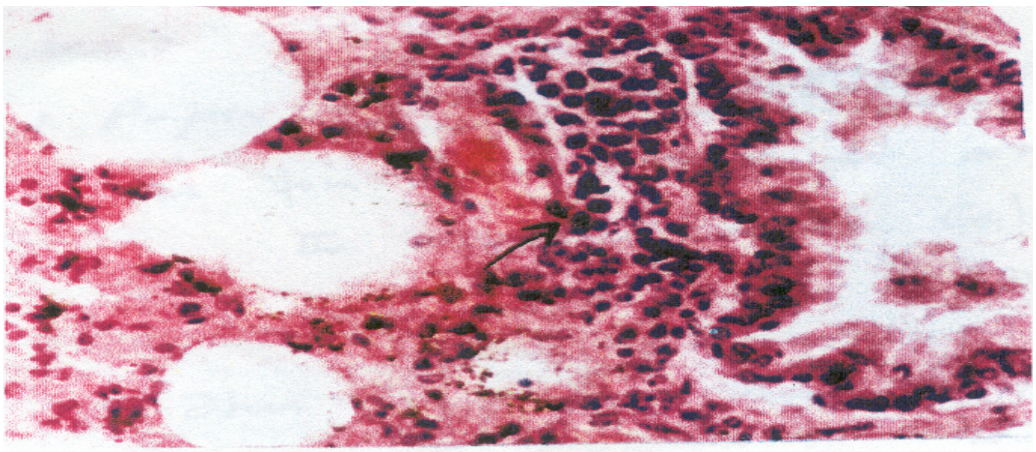

Fig. 6: Sectional view of lung (20 X) of treated animal showing markedly congested blood vessels and hemorrhagic exudates involving alveoli.

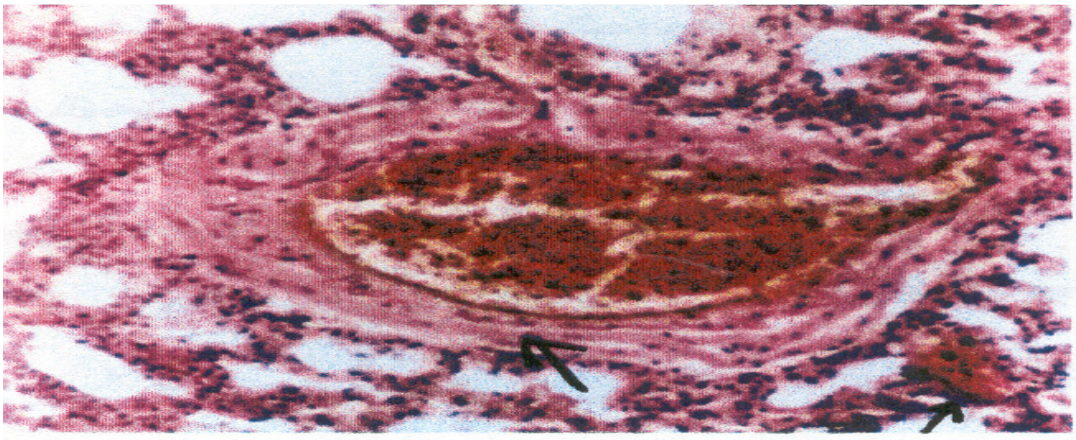

Fig. 7: Sectional view of lung (20X) of treated animal showing aggregation of lymphocytes. Le. Mononuclear cells and few degenerated neutrophils.

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 effecting 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.

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