Full Length Research Paper

Analgesic and neuromodulatory effects of sea anemone Stichodactyla mertensii (Brandt, 1835) methanolic extract from southeast coast of India

Sadhasivam Sudharsan¹, Palaniappan Seedevi¹, Umapathy Kanagarajan², Rishikesh S. Dalvi²,³, Subodh Guptha², Nalini Poojary², Vairamani Shanmugam¹, Alagiri Srinivasan⁴ and Annaian Shanmugam¹*

¹Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608 502, Tamil Nadu, India.
²Central Institute of Fisheries Education, Off Yari Road, Versova, Mumbai-400061, Maharashtra, India.
³Maharshi Dayanand College, Dr. S.S. Rao Road, Mangaldas Verma Chowk, Parel, Mumbai-400012, Maharashtra, India.
⁴Department of Biophysics, All India Institute of Medical Sciences, New Delhi-110 029, India.

Accepted 8 July, 2013

The biological activity of crude methanolic extract (CME) of sea anemone Stichodactyla mertensii was screened. The CME was fractionated using diethylaminoethyl (DEAE – cellulose) and screened for hemolytic activity, mice bioassay, analgesic activity and neuromodulatory activity. The presence of protein was estimated to be 0.292 mg/ml in crude, followed by 0.153, 0.140 and 0.092 mg/ml in Fractions 1, 2 and 3, respectively. The crude extract and 3 fractions showed the hemolytic activity of 109.58, 52.28, 57.14, 43.47 HT/mg on chicken blood, while in human blood it was recorded as 27.39 and 26.14 HT/mg in crude and F1 fraction and 26.14, 28.57 HT/mg in F1, F2 fractions of ‘AB’ and ‘O’ blood groups. Whereas the F2 and F3 fractions in ‘AB’ group and F3 fraction alone in ‘O’ group did not show any activity. The lethal dose 50% (LD₅₀) in mice was found to be 108.24 mg/kg in the crude extract. The crude extract and the active fractions reported moderate analgesic activity at 93.75 mg/kg concentration. The impact of sea anemone crude extract was found to increase neuromodulatory activity in Na⁺ K⁺ ATP-ase at 86 to 91%, Mg²⁺ ATP-ase at 266 to 438% and AChE at 67 to 24% activities, though the study has provided some justification of sea anemone extract which showed moderate hemolytic activity and prompt analgesic activity, as well as acting variably on the presynaptic sodium pump and promoting the cholinesterase activity.

Key words: Stichodactyla mertensii, hemolytic titration assay, analgesic drug, mice bioassay.

INTRODUCTION

Sea anemones (Actinaria, Cnidaria) are armed with venom-secreting nematocytes, which help in capturing of prey and protection from predators. Venom from most sea anemones is harmless to humans or may induce mild dermatitis, but few species of sea anemones possess highly toxic venoms which may cause some serious
effects to humans. *Phyllodis cussemoni* is one of the most dangerous (Kwietniewski, 1897). The biological role of toxins delivered by nematocysts include the capture and killing of prey, digestion, repelling of predators and interspecies spatial competition (Macek, 1992). Most of the coelenterate venomous animals, sea anemones, contain a variety of interesting biologically active compounds and some potent toxins (Vincent et al., 1980). Previously, different types of protein toxins have been isolated from several species of sea anemones which are neurotoxins of 46 to 49 amino acid residues cross-linked by three intra molecular disulfide bonds having specific affinity for sodium channels associated with synaptic nerve terminals and hemolysins, basic and cysteine-less polypeptides (Schweitz et al., 1981; Thomson et al., 1987). In addition to previous studies, cardio stimulatory activity has also been reported for some toxins from sea anemones (Norton et al., 1990; Simpson et al., 1990).

Further studies dealing with the depiction and the mechanism of action of venoms and their constituents produced by sea anemones are imperative to explicate the function of central nervous system and thus become noteworthy tools for the study of synaptic transmission (Kozlov et al., 2009; Peterson, 1977). Thus in the present study, the crude and fractionated extracts comprises an initial effort to evaluate the bioactivity of secondary metabolites from sea anemone *Stichodactyla mertensii* collected from Devipattinam coast of India.

**MATERIALS AND METHODS**

**Extraction of methanolic extract**

*S. mertensii* was collected from the depth of 6 m along the Devipattinam (Lat. 09° 28’ N; Long. 78° 53’ E) Southeast coast of India. The sea anemones were first thoroughly washed with seawater, followed by tap water, and later with distilled water to remove the epiphytes and other materials attached on their body surface. Tentacles were separated from the anemones and ground in methanol using tissue homogenizer (Remi RQ-127, India), following the method of Kozlov et al. (2009).

**Partial purification of the crude extracts (column chromatography)**

A properly cleaned glass column (55 × 2.75 cm) was mounted vertically in a stand and filled with DEAE cellulose, 15 cm from the bottom of the column. The column was pre - equilibrated with phosphate buffer before loading the sample. The crude extract was dissolved in Phosphate Buffer Saline (PBS pH 7.4) at a concentration of 5 mg/ml and was eluted with a linear gradient of 0.1 M to 1 M NaCl in phosphate buffer saline.

**Estimation of protein**

Protein contents of the crude extract and the fractions eluted from the DEAE column were estimated following the method of Peterson (1977) using Bismuth sulfite agar (BSA) as the standard.

**Hemolytic activity**

The hemolytic activity was estimated following the method of Pani Prasad and Venkateshvaran (1977). The hemolytic assays were performed in 96-well "V" bottom microtiter plates. The crude extract and the fractions were prepared (5 mg/ml) in 100 μl of normal saline, and 100 μl of 1% erythrocyte suspension was added to all wells. The positive and negative controls were maintained using distilled water and normal saline, respectively. The plates were gently shaken and allowed to stand for 3 h at room temperature (26°C). Occurrence of uniform red-coloured suspension in the wells was considered as positive hemolysis whereas formation of a button at the bottom of the wells was considered as absence of hemolysis. The hemolytic titer was defined as the reciprocal of the highest dilution giving positive hemolysis (HT/ml). Specific activity was expressed as hemolytic units per mg of soluble protein (HT/mg).

**Mice bioassay**

An acute toxicity test of the extract and fractions was carried out as per the Association of Official Analytical Chemists (AOAC, 1990). Clinically healthy male albino mice (weigh 20 ± 2 g) were maintained at the animal holding facility of CIFE, Mumbai and were housed under standard conditions of temperature (25 ± 2°C), relative humidity (60%), 12/12 light/dark cycle, and fed with standard pellet diet and tap water following the guidelines of the Institute’s Ethical Committee. Animals (Regulation number FGB/CPCSEA/2001-02) were fasted prior to dosing and the test substances were administered in a single dose intraperitonially (i.p.). The dried crude methanolic extract and fractions (F1 to F3) was dissolved in PBS (5 mg/ml) and was injected intraperitonially to mice in dosages ranging from 0.25 ml (1.25 mg), 0.50 ml (2.5 mg), 0.75 ml (3.75 mg) and 1.0 ml (5.0 mg). All the experiments were performed in triplicates. Control mice were injected with a corresponding volume of PBS. Behavioral changes during the experimental period and the time required for death of each animal were recorded. The LD₅₀ was estimated following the method of Lorke (1983).

**Analgesic activity**

Analgesic activity was assessed by tail flick method following the method of Gray et al. (1970) using Tail Flick Analgesia Meter (Harvard, USA) with a variable 150 W, 25 V lamp as the heat source. Male albino mice (20 ± 2 g) were housed under standard conditions as described above. Prior to the experiment, the animals were restrained in a plastic tube, for 10 min twice a day for three days, for adaptation. The crude methanolic extract and fractions (F1, F2 and F3) were dissolved in PBS (1.25 mg/ml) and 0.25 ml of this solution was injected intraperitonially to mice, and tested at 30, 60 and 90 min after injection. Mice without administration of any extract were used as controls while those injected with a corresponding dose of paracetamol served as reference standards. All the experiments were performed in triplicates. The application site of the heat on the tail was maintained about 5 cm from the tip of the tail of each animal. The tail flick response was noted and cut-off time of 15 s was maintained. Analgesic activity was expressed as a ratio between the difference in reaction time of mice treated with the samples and control.
Neuromodulatory activity

In vitro effect of the sea anemone crude methanolic extract on the ATPase activity of mice brain was given below.

P₂ fraction preparation

Clinically healthy male albino mice (weight 20 ± 2 g) were housed as mentioned above. P₂ fraction (mitochondrial nerve endings) from the brain of mice was prepared following the method of Green et al. (1957). Brain was homogenized in chilled sucrose solution (0.32 M) and centrifuged (Remi refrigerated centrifuge, India) at 2,500 rpm for 15 min at 4°C to remove cell debris, nuclei and plasma membrane fragments. The supernatant was collected in separate tubes and centrifuged at 15,000 rpm for 20 min at 4°C. Later, the supernatant was discarded and the pellet was dissolved in sucrose solution and centrifuged at 15,000 rpm for 20 minutes at 4°C. This step was repeated twice to wash the pellet and resuspended in same sucrose solution, and kept in glass vials at -20°C until use as the enzyme source. Protein was estimated following the method of (Peterson, 1977) using BSA as the standard.

\[
\text{AChE} = \frac{\text{Absorbance of the sample} \times \text{Amount of Ach present in the standard} \times 60}{\text{Absorbance of the standard} \times \text{Amount of protein in the sample} \times \text{incubation time}}
\]

Absorbance of the sample × Amount of phosphate present in the standard × 60

ATP = \[
\frac{\text{Absorbance of the sample} \times \text{Amount of protein in the sample} \times \text{incubation time}}{\text{Absorbance of the standard}}
\]

In vitro effect of the sea anemone crude methanolic extract on the AChE activity of mice brain

The mice brain AchE enzyme activity was assessed according to Ellman et al. (1961). The brain isolated from the male albino mice weighing 20 ± 2 g was homogenized with 0.25 M (8.55 g in 100 ml) ice cold sucrose solution and 2% (w/v) tissue homogenate was prepared in the same sucrose solution and stored in the freezer as the enzyme source. Three millilitres of phosphate buffer (pH 8.0) was placed in each tube, to which 0.1 ml of enzyme source (2% w/v homogenate) was added and stirred. Then 100 μl of 0.01 M DTNB (0.5-5'-dithiobis-2-nitrobenzoic acid) was added and the initial color was measured spectrophotometrically at 412 nm. The test solutions of crude extract (100 μl) at each of four concentrations (250, 500, 750 and 1000 μg) were added. Control experiment was run simultaneously with 100 μl of triple distilled water instead of crude extract. To start the reaction, 20 μl of acetylthiocholine iodide (ATCI) (0.075 M) was added to each tube as substrate and then the reaction was allowed to incubate for 15 min at room temperature. The colour development was measured at 800 nm in a spectrophotometer after 15 min. The control experiment was run simultaneously with 100 μl of triple distilled water instead of toxins.

Statistical analysis

The values were expressed as the mean value ± standard error, and the data were subjected to statistical analysis (ONE-WAY ANNOVA).

Histopathology

For the histopathology examination, all the organs (brain, heart, liver, kidney and lungs) were dissected out from one mice in each group of experimental animal. The organs was fixed in 10% formalin and after proper dehydration, the tissue were embedded in paraffin wax. The sections of 6 μ thickness were cut using a rotary microtome and stained with hematoxylin and eosin (Pantin, 1962). The sections were observed under Motic trinocular stereozoom digital microscope (DMWB1 series) and photographs were taken.

RESULTS

About 2.3 g of crude methanolic extract was obtained from a starting material of 265 g of whole body tentacle. Protein content in the crude methanolic extract was estimated to be 0.292 mg/ml. The crude extract was purified with DEAE cellulose and fractions of 5 ml (F1 to F10) were collected. Among them, fractions with higher protein content were considered for further screening. Hence, the partially purified fractions F1 (0.153 mg/ml), F2 (0.140 mg/ml) and F3 (0.092 mg/ml) were dialyzed
against distilled water, freeze dried and used for further study.

**Hemolytic assay**

The crude methanolic extract, F1, F2 and F3 fractions showed a specific hemolytic value of 109.58, 52.28, 57.14 and 43.47 HT/mg, respectively, on chicken erythrocytes (Table 1). Whereas crude and F1 fraction reported 27.39 and 26.14 HT/mg (in ‘AB’ blood group) and crude, F1 and F2 fractions recorded 27.39, 26.14 and 28.57 HT/mg (in ‘O’ blood group) hemolytic activity in human blood. In the case of ‘AB’ blood group the F2 and F3 fractions and in ‘O’ blood group only the F3 fraction did not exhibit hemolytic activity (Table 2). The crude extract showed higher hemolytic titer on chicken than on the human erythrocytes.

**Mice bioassay**

The mice bioassay was done by crude and fractionated samples from *S. mertensii* by various concentrations 0.25 ml (1.25 mg), 0.50 ml (2.5 mg), 0.75 ml (3.75 mg) and 1.0 ml (5.0 mg) respectively and their response is given in Table 3.

**Analgesic activity in tail flick method**

The analgesic activity of crude and fractions showed moderate analgesic ratio when compared to standard (Table 4). The analgesic ratio of the crude extract was 0.49, whereas of the F1, F2 and F3 fractions was 0.17, 0.14 and 0.10 respectively.

**Neuromodulatory activity**

Effect of Na+-K+ ATP-ase, Mg++ ATP-ase and AChE activity were evaluated by using different concentrations (250, 500, 750 and 1000 μg/ml) of crude extract to observe the neuromodulatory activity (Figure 1). Na+-K+ ATP-ase activity was inhibited by methanolic (86 to 91%) extract, but when the concentration was increased from 250 to 1000 μg/ml it showed the positive modulation. Whereas its crude extract showed 266% of Mg++ ATP-ase activity at 250 μg/ml and 438% at 1000 μg/ml concentration (Figure 2). The crude extract exhibited the positive modulation in AChE activity up to 67% at 250 μg/ml and 24% at 1000 μg/ml concentrations (Figure 3).

**Histopathology**

Observed histopathological changes in the test mice suggest that all the main organs: brain, heart, liver, kidney and Lungs were affected by sea anemone toxins (crude extract and fractionated) Figure 4 to 8.

**DISCUSSION**

Natural products play a vital source of novel therapeutic agents for various conditions, including infectious diseases (Selim et al., 2013). Hence in the present study, the crude methanolic extract (CME) of sea anemone *S. mertensii* was investigated for their pharmacological potential to develop it as a therapeutic agent. The crude extract of 2.3 g from 265 g of sea anemone (tentacle tissue) was obtained. Earlier, Ravindran et al. (2010) reported that 500 g fresh weight of *Heteractis magnifica* yielded 9.73 and 7.84 g in *Stichodactyla haddoni* followed by 5.37 g in *P. sinensis* of crude extracts. The total protein was estimated to be 0.219 mg/ml in *S. mertensii* crude extract which is lower than that of *H. magnifica* (605 μg/ml) and *Paracondactylis sinensis* (860 μg/ml) (Ravindran et al., 2010) in the low level of protein content may be depending on the habitat (in terms of abiotic parameters) from where it has been collected. Karthikayalu et al. (2010) reported the hemolytic property in sea anemone *H. magnifica* crude extract at concentrations as low as 120 μg/ml, whereas in the present study the *S. mertensii* showed moderate hemolytic activity in both chicken and human erythrocytes at 5 mg/ml concentration.

Vakorina et al. (2005) previously reported that actinoporin compound isolated from sea anemone *Radianthus macrodactylus* showed the hemolytic activity, which significantly correlates with our present study and indicates the presence of considerable hemolytic unknown toxic compound in the *S. mertensii* tentacle extract. The lethal dose (LD₅₀) in mice was found to be 108.24 mg/kg in the crude extract and changes in their behaviour was observed based on the dosage (Table 3). Ravindran et al. (2010) observed toxicity in mice for three species of sea anemone. *H. magnifica*, *S. haddoni* and *P. sinensis* at different concentrations (48, 30 and 15 μg/ml) tested wherein death rate occurred in 68.3, 135, 86.33 seconds, respectively. The aqueous ethanol extract from root bark of the plant *Ximenia americana* L. (Olacaceae) showed LD₅₀ of 345 mg/kg of body weight of mice besides inhibiting the oedema, pain, cell migration and increasing vascular permeability (Olabissi et al., 2011).

Similarly, CO₂ extract from *Ilex paraguariensis* showed acute toxicity in mice at 500 mg/kg. Further significant changes were also observed in the histology of liver and kidney cells when compared to the normal cells (Pasquali et al., 2011).

Sea anemones are known to contain mainly polypeptides and proteins, which account for most of their
Table 1. Hemolytic activity in Chicken blood against *Stichodactyla mertensii*

<table>
<thead>
<tr>
<th>S/No</th>
<th>Type of extract</th>
<th>Protein (mg)</th>
<th>Source of blood</th>
<th>Total hemolysis (up to dilutions)</th>
<th>Hemolytic titer</th>
<th>Specific hemolytic activity (HT/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol (crude)</td>
<td>0.292±0.001</td>
<td>Chicken blood</td>
<td>5</td>
<td>32</td>
<td>109.58±0.67</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>0.153±0.001</td>
<td></td>
<td></td>
<td>3</td>
<td>52.28±0.96</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>0.140±0.002</td>
<td></td>
<td></td>
<td>3</td>
<td>57.14±0.75</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>0.092±0.001</td>
<td></td>
<td></td>
<td>2</td>
<td>43.47±0.87</td>
</tr>
</tbody>
</table>

Table 2. Hemolytic activity in Human blood against *Stichodactyla mertensii*.

<table>
<thead>
<tr>
<th>S/No</th>
<th>Type of extract</th>
<th>Protein (mg)</th>
<th>Source of blood “AB” and “O” group</th>
<th>Total hemolysis (up to dilutions)</th>
<th>Hemolytic titer</th>
<th>Specific hemolytic activity (HT/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methanol (crude)</td>
<td>0.292±0.001</td>
<td>AB</td>
<td>4</td>
<td>4</td>
<td>27.39±0.87</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>0.153±0.001</td>
<td>O</td>
<td></td>
<td>2</td>
<td>26.14±0.42</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>0.140±0.002</td>
<td>AB</td>
<td>ND</td>
<td>2</td>
<td>28.5±0.59</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>0.092±0.001</td>
<td>O</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
</tr>
</tbody>
</table>

*Non detectable.

Table 3. Mice bioassay for crude and their fractions.

<table>
<thead>
<tr>
<th>S/No</th>
<th>Extract/ fractions</th>
<th>Injected volume (ml)</th>
<th>Death time (min)</th>
<th>Symptoms</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1.00 (saline)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Crude</td>
<td>0.25</td>
<td>NA</td>
<td>Escape reaction, defecation</td>
<td>Non lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50</td>
<td>NA</td>
<td>Palpitation, escape reaction, defecation</td>
<td>Non lethal</td>
</tr>
<tr>
<td>2</td>
<td>Crude</td>
<td>0.75</td>
<td>2.68±0.025</td>
<td>Palpitation, escape reaction, defecation, over active, dragging of hind limb</td>
<td>Lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>2.45±0.015</td>
<td>Palpitation, escape reaction, defecation, sniffing and scratching, dragging of hind limb</td>
<td>Lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>NA</td>
<td>Escape reaction, defecation</td>
<td>Non lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50</td>
<td>NA</td>
<td>Escape reaction, defecation</td>
<td>Non lethal</td>
</tr>
<tr>
<td>3</td>
<td>F1</td>
<td>0.75</td>
<td>1.33±0.020</td>
<td>Palpitation, escape reaction, sniffing and scratching, dragging of hind limb</td>
<td>Lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>1.20±0.025</td>
<td>Palpitation, escape reaction, micturition, dragging of hind limb, excess breathing</td>
<td>Lethal</td>
</tr>
<tr>
<td>4</td>
<td>F2</td>
<td>0.25</td>
<td>NA</td>
<td>Escape reaction, defecation</td>
<td>Non lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50</td>
<td>NA</td>
<td>Palpitation, escape reaction</td>
<td>Non lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td>NA</td>
<td>Palpitation, escape reaction,</td>
<td>Non lethal</td>
</tr>
<tr>
<td>5</td>
<td>F3</td>
<td>0.25</td>
<td>NA</td>
<td>Escape reaction, defecation</td>
<td>Non lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.50</td>
<td>NA</td>
<td>Over active, escape reaction</td>
<td>Non lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td>NA</td>
<td>Over active, escape reaction</td>
<td>Non lethal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>NA</td>
<td>Over active, palpitation, escape reaction</td>
<td>Non lethal</td>
</tr>
</tbody>
</table>

*Not applicable.*
Table 4. Analgesic activity of *Stichodactyla mertensii*

<table>
<thead>
<tr>
<th>S/No</th>
<th>Weight of mice in (g)</th>
<th>Type of Extract</th>
<th>Amount of sample injected</th>
<th>Time of tail flick (Sec)</th>
<th>Analgesic ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20±2</td>
<td>Crude</td>
<td>0.25 93.75 mg/kg</td>
<td>29.3±1.81</td>
<td>0.49±0.1</td>
</tr>
<tr>
<td>2</td>
<td>20±2</td>
<td>F1</td>
<td>0.25 29.3±1.81</td>
<td>20.1±0.68</td>
<td>0.17±0.1</td>
</tr>
<tr>
<td>3</td>
<td>21±2</td>
<td>F2</td>
<td>0.25 18.2±1.69</td>
<td>0.14±0.1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>20±2</td>
<td>F3</td>
<td>0.25 15.2±0.73</td>
<td>0.10±0.1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>21±2</td>
<td>Standard</td>
<td>0.25 15.2±0.14</td>
<td>1±0.57</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>20±2</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** *In vitro* Evaluation of the effect of the Seaanemone crude methanolic extract on mice Brain ATP-ase enzyme.

**Figure 2.** *In vitro* Evaluation of the effect of the sea anemone crude methanolic extract on mice brain Mg^{++} ATP-ase.
The peptides (APHC2 and APHC3) from Heteractis crispa sea anemone showed analgesic activity in mice at the dosage of 0.1 mg/kg (Kozlov et al., 2009). Likewise, the aqueous extract of jelly fish Crambionella stuhalmanni and Chrysaora Quinquecirrha exhibited the significant analgesic activity at 5 mg/ml (Suganthi et al., 2011) which greatly corresponds with our present results.
Figure 6. Histopathology of liver from mice. (a) Control b and c) Hepatocyte degeneration and vacuolation and infiltration of mononuclear cells d and e) Pyknotic nuclei and centrilobular necrosis disorganization of hepatic laminae.

Figure 7. Histopathology of kidney from mice. (a) Control; b and e) Hemorrhage and tubular degeneration; c) interstitial congestion and tubular necrosis; d) tubular necrosis; f) disruption of the renal corpuscles in some areas and infiltration of inflammatory cells was also observed.
Further, the crude nematocyst extract of sea anemone *Paracondactylis indicus*, *Paracondactylis sinensis*, *Heteractis magnifica* and *Stichodactyla haddoni* showed analgesic activity at 2 mg/ml concentration (Bragadeeswaran et al., 2011).

The ATPase enzyme system is widely accepted as deriving a part of its energy from ATP hydrolysis for active transport of Na\(^+\)-K\(^+\)-ATPase. The Na\(^+\)-K\(^+\)-ATPase (sodium pump) is a pre synaptical membrane protein of higher organisms which hydrolytes cytoplasmic ATP, interacts with neighbouring membrane proteins and organized cytosolic cascades of signalling proteins to send messages to intercellular organelles (Xie et al., 2002). In the present study, the analgesic activity was carried out in *S. mertensii* tentacle methanolic extract. The crude extract and fractionated possessed the analgesic activity at the dosage level of 1.25 mg/ml concentration, and their values are expressed in analgesic ratio. It is interesting to note that the CME protein is capable of stimulating the sodium pump based on the dosage level. In the present study the CME of *S. mertensii* tentacle inhibited the level of Na\(^+\) K\(^+\) ATP.-ase (86 to 91%). However, the CME showed dose dependant effect on the activity of the sodium pump, the enzyme which is responsible for ATP activity is higher at low dosage of CME (250 µg) and it gives diminished activity at higher concentration (1000 µg). Mg\(^++\) ATP-ase activity of methanolic extract of sea anemone was elevated in lower concentration (250 µg) and the activity was inhibited at higher concentration (1000 µg), which is comparable to previous reports in *Gyrostoma helianthus* neurotoxins which delayed the activation of K\(^+\) channel.

**Figure 8.** Histopathology of lungs from mice. (a) Control; b) disruption of alveoli or air spaces are seen; c) excessive congestion near the bronchiol; d) excessive congestion in lungs and massive infiltration of macrophages; e, f and g) RBC’s and macrophage and excessive congestion in lungs.
and showed reversible acetylcholine esterase inhibition in both brain and blood of mice (Kamal et al., 2006).

Wankhede (1996) observed similar neuroinhibitory activity by bile extracts of freshwater crabs on the Na-K ATP-ase enzyme system in mammalian models. Besides them crude toxin from Protonicea diacanthus, Otolithoides biauritus and Muraenesox talanbonides also elevated the Mg’’ ATP ase activity (Malarvannan, 2002). The ATP-ase enzyme system is widely accepted as a structure that employs part of the free energy from ATP hydrolysis for active transport of Na-K. The methanolic extract of sea anemone inhibited AchE enzyme activity in lower concentration and elevated in higher concentration. In the in vitro neuromodulatory activity screening, CME from S. mertensii increased the AchE activity on dose dependent manner. It is characterized as neuromuscular transmission which leads to the behavioural changes on mice patapitation, escape reaction, defecation, over active, dragging of hind limb and micturition. Likewise, paper fish Arothron hispidus exhibited positive modulation in Na’-K’ATP ase, Mg’’ ATP ase and Ach E enzyme activity (Bragadeeswaran et al., 2010).

Conclusion

The present study demonstrated that the marine sea anemone extract have potent pharmacological properties and without any series of toxic effects at low dosage level. These finding strengthen the health care industry and indigenous medicine, and it can be used as remedies for analgesic and neurological disorder.

ACKNOWLEDGEMENT

Thanks to the Director, Centre of Advanced Study in Marine Biology, Annamalai University, (India) and the Director, Central Institute of Fisheries Education, Mumbai (India) for the facilities provided. The first two authors thank (SS& PS) the Ministry of Earth Sciences, New Delhi (India) for financial assistance.

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