IMMUNOMODULATORY POTENTIAL OF SALIVARY GLAND EXTRACTS OF OCTOPUS

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ABSTRACT

Marine environment is emerging as a source for diverse and novel chemical structures such as bioglycan, cyclic tripeptides, polyhydroxylated lactone, polysaccharides, terpenes, etc. with promising biological activities. Secondary metabolites from marine organisms have been found to possess several activities including anti-microbial, anti-proliferative, analgesic and immunostimulation. The present study was designed to study the immunomodulatory effects of the posterior salivary gland extracts of two species of octopus – Cistopus indicus and Octopus fusiformis. The glands were extracted in three solvents, partially purified and the samples assayed for hemagglutination, delayed type hypersensitivity response and phagocytosis. The observations made in the present study suggest that the extracts of the salivary glands of these two octopus species show variations in their activities. The acetic acid, methanol extracts and the fractions FA4, FA5, FA6, FA7, FA10 of the salivary glands of C. indicus showed significant immunomodulatory effect.

Keywords: immunomodulation, octopus, salivary gland.

INTRODUCTION

Several natural products, especially marine bioactive compounds, have shown promising potential of playing an important role in medicine and therapeutics. In recent years, many bioactive compounds such as amines, peptides, steroids, terpenoids, isoprenoids, and sesquiterpenes have been extracted from various marine organisms [1-3]. Soft-bodied
Narayan et al. World Journal of Pharmacy and Pharmaceutical Sciences

Cephalopods, some of the world’s most adept predators, have well-developed salivary glands that inject venom into prey. Biologically active principles such as eledoisin, maculotoxin, tyramine and octopamine have been reported in the posterior salivary glands of octopuses [4-6]. The immune system is known to be involved in the etiology as well as patho-physiological mechanisms of many diseases. Around 35 naturally available marine sources contribute to natural immunomodulators from marine origin [7]. Immunomodulators are agents capable of modifying immune response and provide therapeutic effects by stimulating or suppressing the immune response. The current study was executed to evaluate the immunomodulatory activity of the salivary gland extracts of two octopuses Cistopus indicus and Octopus fusiformis.

MATERIALS AND METHODS

Specimen collection and sample extraction

*Cistopus indicus* and *Octopus fusiformis*, collected from Marve Beach, Mumbai, were brought live to the laboratory and immediately dissected to isolate the posterior salivary glands. The glands were separately extracted in acetic acid [8], methanol [9] and methanol: chloroform 1:2 [10] and will be hereafter referred to as Crude A, Crude M and Crude MC respectively. The extracts were subjected to partial purification using DEAE Cellulose column chromatography and the fractions collected [11-13]. One unadsorbed fraction (FAU, FMU, FMCU) and ten adsorbed fractions (FA1-10 for Crude A, FM1-10 for Crude M and FMC1-10 for Crude MC) were eluted. The samples were estimated for protein content [14] and subjected to the following assays which were carried out in six sets for confirmation of results.

Hemagglutination

Hemagglutination activity of the samples was assayed using human erythrocyte suspension [15]. 3% erythrocyte suspension in 0.9% saline of A, B, O and AB blood groups were used for the assay. 50 µl of the extract, added to 50 µl of 3% erythrocyte suspension of ABO blood types on a blood typing plate. Agglutination was observed during a time period of 30 minutes and the results recorded.

Delayed type hypersensitivity response

Delayed type hypersensitivity response of the samples was assayed by edema forming activity [16]. Groups of five mice were injected subcutaneously in the right foot pad with 50 µl
of the samples containing 0.25 mg/ml of the extracts in phosphate buffer. The left foot pad was injected with 50 µl of phosphate buffer (pH 7.2) as control. After two hours, the size of the paws was measured using vernier calipers. Edema ratio (ER) expressed as the percentage increase in the size of the right foot compared to that of the left one. Minimum edematous dose was defined as the dose causing 105% ER.

**In vitro phagocytosis**

The immunomodulatory activity was analyzed through *in vitro* phagocytosis of *Candida albicans* by polymorphonuclear cells [17]. *C.albicans* cell pellets were resuspended in sterile Hanks balanced salt solution with human serum in the proportion 16:4. Few drops of human blood were collected on slides by finger prick. The clots were gently removed and the slides flooded with different concentrations of the samples and incubated at 37°C for 15 minutes. The suspension of *C.albicans* is added, incubated, fixed and stained with Giemsa. Mean number of phagocytosed cells on the slides were determined microscopically for 100 granulocytes. This number was taken as the Phagocytic Index (PI) and compared with that of the control to calculate % modulation.

**Data Analysis**

Experiments were carried out in six sets; data are expressed as mean ± SEM and evaluated by one way ANOVA. * indicates statistical significance at 0.05 level.

**RESULTS AND DISCUSSION**

None of the samples showed agglutination of the human erythrocytes of A, B, O and AB blood groups. Crude A and M of *C. indicus* and Crude A of *O. fusiformis* caused edema formation in mice which lasted for 24 hours. The fractions FA4, FA5, FA6, FA10 and FM5 of *C. indicus* elicited edema formation. The highest edematous ratio was obtained with the fraction FA5 (Table 1). Crude A of *C. indicus* showed enhancement in phagocytosis at higher concentrations of 250 µg/ml and 500 µg/ml (Fig.1). Anti-phagocytic activity was exhibited by Crude MC and Crude M of *C. indicus* of *O. fusiformis* respectively, both at a concentration of 250 µg/ml. Crude A of *O. fusiformis* shows a concentration dependant increase in the phagocytic activity whereas Crude MC had no effect on phagocytosis (Fig.2). The highest immunostimulation of 40% effected by 500 µg/ml concentration of Crude M of *C. indicus*. Among the fractions, the fractions FA6, FA7 and FA5 of *C. indicus* exhibited significant immunostimulatory activity. The fractions of FMC1-10 of *C. indicus* and all the
fractions of *O. fusiformis* showed no significant immunomodulatory effect.

**Table 1: Edema formation by the extracts of *Cistopus indicus* and *Octopus fusiformis***

<table>
<thead>
<tr>
<th>Octopus species</th>
<th>Type of extract</th>
<th>Protein Content (mg/ml)</th>
<th>Size of left paw (Control) in cm</th>
<th>Size of right paw (Test) in cm</th>
<th>Edema (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cistopus indicus</em></td>
<td>Crude A</td>
<td>9.64±0.02</td>
<td>0.3</td>
<td>0.5</td>
<td>167*</td>
</tr>
<tr>
<td></td>
<td>Crude M</td>
<td>9.72±0.04</td>
<td>0.3</td>
<td>0.5</td>
<td>167*</td>
</tr>
<tr>
<td></td>
<td>Crude MC</td>
<td>0.87±0.06</td>
<td>0.3</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>FA4</td>
<td>0.09±0.03</td>
<td>0.4</td>
<td>0.6</td>
<td>150*</td>
</tr>
<tr>
<td></td>
<td>FA5</td>
<td>0.12±0.05</td>
<td>0.3</td>
<td>0.5</td>
<td>167*</td>
</tr>
<tr>
<td></td>
<td>FA6</td>
<td>0.08±0.05</td>
<td>0.4</td>
<td>0.5</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>FA10</td>
<td>0.04±0.04</td>
<td>0.4</td>
<td>0.6</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>FM5</td>
<td>0.10±0.05</td>
<td>0.4</td>
<td>0.5</td>
<td>125</td>
</tr>
<tr>
<td><em>Octopus fusiformis</em></td>
<td>Crude A</td>
<td>7.49±0.05</td>
<td>0.4</td>
<td>0.5</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>Crude M</td>
<td>8.54±0.08</td>
<td>0.3</td>
<td>0.3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Crude MC</td>
<td>0.55±0.07</td>
<td>0.4</td>
<td>0.4</td>
<td>100</td>
</tr>
</tbody>
</table>

![Fig.1: Phagocytic Index of the salivary gland extracts of *Cistopus indicus*](image1.png)

![Fig.2: Phagocytic Index of the salivary gland extracts of *Octopus fusiformis*](image2.png)
The immune system is a major target for development of treatment strategies, in particular to improve the management of infections, tumors and autoimmune diseases. Approaches include immunomodulation with cytokines or their antagonists [18]. Hemagglutination is one of the preliminary tests to be carried out for evaluating immunomodulatory activity involving antigen-antibody agglutination. The lack of hemagglutinating activity of the samples, when tested with human red blood cells, suggest the absence of component(s) that recognize the different antigens on the human red blood cells.

Edema is an abnormal accumulation of fluid beneath the skin or in one or more cavities of the body. The amount of interstitial fluid is determined by the balance of fluid homeostasis, and increased secretion of the fluid into the interstitium or impaired removal of this fluid may cause edema. The sub-cutaneous injection of the extracts into the mice paw produce an inflammation characterized by increased tissue water and plasma protein exudation, as in the case of biphasic effects in carrageenan induced edema. The first phase that begins immediately after injection could be due to the release of histamine or serotonin, bradykinin and diminishes in one hour. The second phase begins after an hour and is caused by the release of protease, prostaglandins, oxygen-derived free radicals and the production of inducible cyclooxygenase (COX-2) and local neutrophil infiltration and activation [19-23].

The present study demonstrated that the salivary gland extracts of the octopuses possess significant inflammatory effect as seen in the form of edema in the mice paw. The results suggest that the extracts elicit this response by either promoting the synthesis, release or action of inflammatory mediators like histamine, serotonin or it may itself act as a mediator of inflammation and is in good agreement with the earlier studies [7, 24].

The present study shows that Crude M of C. indicus, at a concentration of 500 µg/ml, with the highest stimulatory activity, when tested in vitro for phagocytosis is indicative of the presence of the active principles in the extract that stimulate phagocytosis. This can be correlated with the high protein content in this extract. When the concentration of the Crude M extract was reduced from 500 µg/ml to 250 µg/ml, the stimulatory effect was also approximately halved. The effect of the Crude A of C. indicus is more than 6 times that of the Crude M of O. fusiformis, and the effect of 500 µg/ml Crude M of O. fusiformis is identical that of 50 µg/ml of Crude M of C. indicus. The present observations clearly indicate differences in the composition of the extracts from the salivary glands of these two species.
which can cause variation in the phagocytic activity due to differences in the composition of the salivary gland extracts of *C. indicus* and *O. fusiformis*.

The Crude MC extract of the salivary glands of *Cistopus indicus*, at a concentration of 500 µg/ml, showed very less immunostimulatory activity when compared to the other extracts at the same concentration. In the case of *Octopus fusiformis*, the Crude MC extract, in different concentrations, showed no effect on phagocytosis. Decreased content of protein in this extract may be the reason for this effect. The observations made in the present study signify that the Crude MC extract of the salivary glands of both the species may either lack the active principles that affect phagocytosis, or may have it in a very low concentration.

Among the fractions, FA6 exhibited maximum stimulatory effect on phagocytosis. The effect of the fraction FA6, at a concentration of 100 µg/ml is much more than that of the Crude A extract at various concentrations. This could be due to the presence of the active component(s) in the FA6 fraction, the effect of which is minimized due to other compounds present in the Crude A extract of the salivary glands of *Cistopus indicus*. None of the fractions of the Crude M extract of the salivary glands of *Cistopus indicus* exhibited such a high stimulation as compared to the crude M extract. It is therefore possible that the activity of the Crude M extract is due to the synergistic effect of the various components present.

**CONCLUSION**

The present study revealed that the salivary gland extracts of the octopus have an immunostimulating effect which showed no strict relation with concentration of the sample. The acetic acid fraction FA6 of the salivary glands of *Cistopus indicus* elicited the maximum stimulatory activity when compared with the other fractions. The active factors responsible for eliciting the inflammatory response can be characterized and used to initiate inflammation study in animal model to understand the effect of other anti-inflammatory compounds. The immunoactive principle(s) from the salivary gland extracts of octopus can find applications in fisheries for enhancing the immunity of commercially important fishes.

**REFERENCES**

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