Chapter 7

BIOMEDICAL APPLICATIONS OF S. ARGUS VENOM
7.1. INTRODUCTION

The increased demand for effective, newer and better drugs has made man turn towards sea as a perennial source largely because marine natural products continue to be viewed as one of the few de novo sources of drug discovery yielding unorthodox and often unexpected chemical structure that offer novel points of departure for molecular modification leading to clinically available drugs.

The scientific community is focusing its efforts on the isolation and characterization of biologically active compounds derived from marine organisms with various pharmacological activities. Marine toxinologists in the past decade were involved in the search for potential pharmaceuticals from marine resources. Although it is virtually difficult to single out a particular bioactive molecule that will find place in medicine, many compounds have shown promise. During the past three decades there had been an increase in awareness of the remarkable potential of marine flora and fauna in areas as diverse as health, food additives, material for orthopedics, thermostable polymerases for polymerase chain reaction and bioactive material.

Toxicity being indicative of potent physiological activity it is quite possible that most of the toxic substance could yield valuable biomedical compounds. Although all of them may not enter the realm of material medica as such, they could at least serve as useful models to the synthesis of valuable medical compounds. The present investigation was aimed at elucidating some of the bioactivities of S. argus venom that would qualify them as potential biopharmaceutical compounds.

Oedematic Activity

Oedema formation is a common feature of the cutaneous inflammatory processes and is dependent on a synergism between mediators that increase vascular permeability and those that increase blood flow. The mediators include
prostaglandins, bradykinin, histamine, ATP and acetylcholine that cause the classical signs of inflammation i.e., swelling, redness, hyperthermia and pain.

**Haemolytic Activity**

Haemolysis is the breakdown of red blood cells and in the final stage of breakdown haemoglobin is released from the red cells. Haemolysis in small amounts is a normal body process. About 0.8 – 1% of all red cells in the body are haemolysed every day. It is usually balanced by red cell production in the marrow of the bones. But sometimes, so many cells breakdown so that marrow production is insufficient and anemia may result. Many biotoxins are known to cause haemolysis of RBC and they do have considerable potential as anticancer agents.

**Blood Clotting**

Animal venoms are usually complex mixtures of bioactive molecules which mainly include proteins and peptides. These toxins interact with physiological targets causing immobilization, death, or digestion of tissues. The vasculature and the blood coagulation system constitute relevant targets owing to their prominent role in homeostasis.

**Platelet Lysis**

Platelets play a key role in normal blood clotting. During the clotting process, platelets clump together to plug small holes in damaged blood vessels. Platelets also activate factor VIII and release phospholipids as part of the blood clotting process.

**Cytolysis**

Cytolytic proteins and peptides play an important role in performing offensive and defensive actions in a number of organisms by lysing cells through enzymic and non-enzymic mechanisms. Many cytolytic toxins lyse cells directly or
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make cells more susceptible to damage by hydrolyzing membrane lipids through enzymic action. On the other hand a wide variety of non-enzymic proteins and peptides that possess cytolytic activity have been isolated. Most of these cytolysins lyse these cells by formation of discrete transmembrane pores. Through these pores osmoticants can move in or out whereas the large molecules such as protein cannot. Thus the cell interior is hyperosmotic, attracting a net influx of water, which results in sustained cell swelling and subsequently cell lysis. Pore forming toxins bind to either lipids or proteins in the cell membrane.

7.2. REVIEW

Fish venoms are known to induce intense and sustained oedematogenic responses space. Envenomations by venomous fish are associated with oedema observed both clinically and experimentally. The acute inflammatory responses in experimental animal after envenomation with the toadfish venoms have been well demonstrated in Thalasophryne nattereri and T.maculosa [Lopez-Ferriera et al., 1998; Lima et al., 2003; Sosa-Rosales et al., 2005]. The stonefish S.horrida and the lethal factor stonustoxin have been studied for oedematic activity [Poh et al., 1991; Khoo et al., 1992]. Studies have been carried out on the Potamotrygon stingrays and scorpion fish, S.plumeiri for oedematic activity [Carrijo et al., 2005 and Magalhaes et al., 2006].

Considerable work has been done on the haemolytic properties of fish venoms [Auerbach et al., 1987]. Most of the piscine venom exhibit haemolytic activity. The venom of the catfish Plotosus canius and Heteropneustes fossilis both produce haemolysis [Datta et al., 1982; Auddy et al., 1994]. Studies on the weeverfish Trachinus draco and dracotoxin the lethal toxin from it have shown to be potent haemolytes [Chhatwal and Dreyer, 1992 a; b]. Haemolytic studies on crude venom of stonefish have been carried out in S. horrida, S. trachynis and S. verrucosa as well as for the lethal factors stonustoxin, verrucotoxin and trachylysin [Kreger, 1991; Poh et al., 1991; Khoo et al., 1992; Garnier et al., 1995;
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Ouanounou et al., 1999]. The toadfishes, Thalassophryne nattereri and T. maculosa have also shown haemolytic activity [Lopes-Ferriera et al., 1998; Sosa-Rosales et al., 2005]. Species specific haemolytic activity has been observed in the six species of fishes S. verrucosa, Pterois lunulata, P. volitans, P. antennata, Dendrochirus zebra and Inimicus japonica [Shiomi et al., 1989]. Stonustoxin, the lethal factor isolated from stonefish venom was studied for platelet lytic activities [Khoo et al., 1995]. Lysis of human platelets by the toadfish T. nattereri was also studied [Lopes-Ferriera et al., 2002].

Several marine peptides have displayed high order of antitumor activity. The approach of testing venoms as antitumour agents dates back to the beginning of the last century, when Calmette et al. [1933] reported on the antitumour activity of snake venom, [Naja species venom] in adenocarcinoma cells. Since then many anticancer compounds have been isolated from toxins of marine sponges e.g. Sesterstatin, Dolastatin 10, Crellastatin etc. and Ecteinascidin 743 from tunicate is under Phase II clinical trial [Mayer 1999]. It was also demonstrated that purified protein from cobra venom was selectively cytotoxic to cancer cells [Baldi et al., 1988; Braganaca 1976]. Particularly, proteinacious venom from several animals like snake, scorpion, spider etc. was reported to have excellent cytotoxicity in cultured cancer cell lines and also reduced tumor growth in mice [Abu-Sinna et al., 2003; Orsolic et al., 2003]. Among all, snake venom has been studied extensively, however, the marked curative properties of the snake venoms are always hindered by their high toxicities, and hence less toxic species, fish venom are proving to be a promising drug for research. The role of active component of the venom, its molecular target and signaling pathways through which it cause apoptosis in cancer cells are still in their early stages of study.

Cell lysis was determined for G. marmoratus, P. volitans and S.trachynis venom by measurement of Propidium Iodide Fluorescence [Church et al., 2003]. Mitogenic and cytotoxic effects of S.verrucosa and Hypodytes rubripinnis on
normal and tumor cell lines have been carried out using flow cytometer [Satoh et al., 2002]. The oriental catfish *Plotosus lineatus* was cytotoxic to cultured Ehrilisch ascites tumor cells, are a common model of tumor growth [Fahim et al., 1996]. The cytolytic effect of *T. nattereri* venom on cultured myoblast, endothelial cells and mononuclear cell lines [J774Al] was studied [Lopes-Ferriera et al., 2001; 2002]. The present study tries to emphasise the biomedical applications of *S. argus* venom.

### 7.3. MATERIAL AND METHODS

#### 7.3.1. Oedematic activity

The ability of *S. argus* venom to induce oedema was studied in mice. 30μl of sterile 0.9 % [W/V] saline solution with venom in different concentrations [12.5, 25 and 50μg/paw] were injected in the sub plantar region of the right hind paw. The left hind paw received an equal volume of sterile saline alone and served as the control. Prior to injection the venom solutions were filtered through 0.22μm Millipore filters. The volumes of both paws were measured using calipers at 0.5, 2, 4, 6, 24 and 48 hours after venom administration.

Percent oedema was calculated as follows:

\[
\text{Percent oedema} = \frac{\text{Right paw thickness} - \text{Initial thickness}}{\text{Right paw initial thickness}} \times \frac{\text{Left paw volume} - \text{Initial volume}}{\text{Left paw initial volume}} \times 100
\]

Each point represents mean ± SEM.

The effect of the antihistamine, pheniramine maleate on oedema inducing activity was tested by injecting the pheniramine maleate [50mg/kg] 30min before injection of 25 μg of *S. argus* venom. After two hours the volume of the paws were measured using calipers.
7.3.2. Estimation of Nociceptive activity

For nociceptive tests each mouse was kept in an adapted chamber. After 10 minutes adaptation period, the animals were injected with the venom [12.5, 25, 50 μg of protein] into the intraplantar region of the hind foot paw in a fixed volume of saline. The control group was injected only with sterile saline. Each animal was then returned to the observation chamber and the amount of time spent licking or biting each hind paw was recorded for 30 minutes and taken as index of nociception [Hunskaar et al., 1985]. Each point represents mean ± SD of six independent experiments.

7.3.3. Haemolytic activity

Haemolytic activity was assayed on washed human, mice, goat, chicken and rat erythrocytes as described earlier by Garnier et al., [1995]. To samples, 1ml containing various concentrations of venom protein in 150mM NaCl were added, 200μl of re-suspended 2% erythrocytes of each of the above mentioned organisms and kept for 30 minutes at room temperature. The suspensions were centrifuged [5min, 3000 rpm in Hitachi refrigerated centrifuge]. The absorbance of the supernatant was measured at 540nm to detect released haemoglobin. A negative control [erythrocyte suspension in 150mM NaCl] and a positive control [erythrocyte suspension in distilled water] were prepared, to enable calculation of percentage haemolysis. All assays were carried out in triplicates.

7.3.4. Blood Clotting

The assay was performed according to the protocol described in the United States Pharmacopoeia [1985]. Fresh human blood was collected directly into a test tube containing 8% sodium citrate solution in a proportion of 1:19 [v/v]. It was mixed immediately by gentle agitation. The plasma was then separated by
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Centrifugation of the sample. The separated plasma was pooled together and kept under refrigeration for the subsequent clotting assay. About 1.0 ml of plasma was incubated at 37°C in a water bath. About 0.2 ml of calcium chloride [1%] was added into the test tube and mixed. A solid clot observed within 5 min gave the indication that the plasma is suitable for the following test. The test sample S. argus venom was prepared in normal saline at the following concentrations 10, 20, 30, 40 and 50 x 10^2 μg/ml to determine the effect on blood coagulation. The test samples were added to the test tubes containing 1.0 ml of plasma with and/or without addition of 0.2 ml calcium chloride concentration and observed for changes. The experiments were repeated three times to confirm every observation.

7.3.5. Platelet lysis

Fresh citrated human blood obtained by forearm venepuncture was centrifuged for 10 min at room temperature at 160g to obtain the supernatant platelet rich plasma [PRP]. The effects of S. argus venom on platelets were evaluated by incubating increasing concentration of venom with 100μl PRP for 5 min at 37°C. Controls of 0 and 100% cytotoxicity were prepared incubating platelets with saline and 0.1% Triton X-100 respectively. The mixture was centrifuged at 2000 g for 10 min and lactic dehydrogenase activity was measured according to Wroblewski et al., [1955] with slight modifications.

7.3.6. Cytolytic activity

MTT [3-4, 5 – dimethylthiazol –2- yl ] – 2,5 diphenyl tetrazolium bromide assay was used to evaluate the cell viability after treatment with venom. The assay detects living cells and the signal generated is dependent on the degree of activation of the cells [Mosmann, 1983]. HeLa cells were cultivated in RPMI 1640 medium containing 10% FCS at 37°C and 5% carbon dioxide. HeLa cells (5x10^4
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cells per well] were incubated for 24 hours in a 96 well microplate. After changing the medium in each well, to the cells were added sterile PBS or venom diluted in medium in different concentrations [0.1, 0.5, 1, 2.5, 5 and 10μg of protein venom / ml]. After 72 hrs the microplates were centrifuged at 1000 rpm for 10min at 4°C. The medium was removed and 20μl of MTT [5mg/ml] in PBS/ well was added. The microplate was incubated for 3h at 37°C and 5% CO₂. After incubation 100μl of SDS [10%] diluted in PBS were added to each well [18h at 37°C and 5% CO₂]. The results were read on a multiwell scanning spectrophotometer [ELISA Reader] at 570nm. The results were expressed as the percentage of viable cells compared with PBS treated cells. Similar results were obtained in six separate experiments.

7.3.7. Statistical Analysis

The SPSS® statistical software for windows, version 13.0 [SPSS Inc., Chicago, USA] was used in all data analyses. One-way ANOVA was used to determine the levels of difference between all groups. GLM repeated measure was used to determine the significance of change in oedematous activity over time.

7.4. RESULTS

For determination of oedematogenic response induced by S. argus venom, concentration of 12.5, 25 and 50 μg venom/paw were used. The thickness of right different doses of venom produced a dose dependent oedema. The maximal response was observed from 1-3 hours oedema remaining significantly elevated compared with control [Table7.1]. Oedematic activity in mice persisted for more than 24 hours.

In sets of mice administered with highest dose of venom, haemorrhage was observed in the injected paw after 4 hours and tissue necrosis was observed after 24 hours. The time course of oedematous activity for S. argus venom at a concentration of 25μg/paw showed an increased activity during the initial 4 hours, though a decrease in oedema was observed compared to control. The oedema
Table 7.1: Concentration of Venom

<table>
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<tr>
<th>Time (Hours)</th>
<th>12.5µg/paw</th>
<th>25µg/paw</th>
<th>50µg/paw</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>65.00 ± 3.16</td>
<td>89.17 ± 6.65</td>
<td>118.33 ± 5.16</td>
</tr>
<tr>
<td>2</td>
<td>76.67 ± 5.16</td>
<td>103.33 ± 6.06</td>
<td>132.50 ± 4.47</td>
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<tr>
<td>4</td>
<td>75.83 ± 3.76</td>
<td>102.50 ± 6.12</td>
<td>131.67 ± 5.16</td>
</tr>
<tr>
<td>6</td>
<td>63.33 ± 4.08</td>
<td>91.67 ± 4.08</td>
<td>102.50 ± 2.74</td>
</tr>
<tr>
<td>24</td>
<td>40.83 ± 3.76</td>
<td>70.00 ± 4.47</td>
<td>69.17 ± 7.36</td>
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<tr>
<td>48</td>
<td>31.67 ± 2.58</td>
<td>49.17 ± 5.85</td>
<td>59.17 ± 4.92</td>
</tr>
</tbody>
</table>

Fig 7.1: Oedematogenic activity of S. argus venom on mouse hind paw. Different doses of S. argus venom and normal saline were injected into the right footpad of mice [subplantar]. Each point represents mean [± SE] of six mice.
**Fig 7.2**: Effect of antihistamine, pheniramine maleate on oedema formation induced by *S. argus* venom injected into mouse hind paw. Pheniramine maleate [50mg/kg] was administered 30 min before *S. argus* venom injection [25μg in 0.025ml]. Each point represents the mean [± SE] of six mice.
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**Table 7.1. Results of GLM repeated measure for oedematic activity.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilks' Lambda</td>
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<td>618.699</td>
<td>5</td>
<td>16</td>
<td>0.000</td>
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<tr>
<td>TIME * CONCN</td>
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<td>44.6</td>
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**Table 7.2. Results of GLM repeated measure showing the effect of Pheneramine maleate on oedematic activity.**

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<thead>
<tr>
<th>Source</th>
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<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
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<td>20</td>
<td>26.903</td>
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</table>

**Table 7.2. Results of GLM repeated measure showing the effect of Pheneramine maleate on oedematic activity.**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>TIME</td>
<td></td>
<td></td>
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<tr>
<td>Wilks' Lambda</td>
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<tr>
<td>TIME * CONCN</td>
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<td>Wilks' Lambda</td>
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<td>22</td>
<td>0.000</td>
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</table>

**Table 7.2. Results of GLM repeated measure showing the effect of Pheneramine maleate on oedematic activity.**

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
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<th>Sig</th>
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<tr>
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<td>65801.398</td>
<td>1719.050</td>
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</tr>
<tr>
<td>Error</td>
<td>574.167</td>
<td>15</td>
<td>38.278</td>
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</table>
**Fig 7.3**: Estimation of nociceptive inducing activity. Each point represents mean ± SD of six independent experiments.
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Fig 7.4: Haemolytic activity of S. argus venom on [A] Rat erythrocytes [B] Human erythrocytes [C] Mice erythrocytes [D] Goat erythrocytes. The 100% control for cell lysis was determined by addition of distilled water value represents the mean ± SE of six experiments.
<table>
<thead>
<tr>
<th>Group</th>
<th>Dose [μg/ml]</th>
<th>LDH activity [% Response]</th>
<th>Expected response [%]</th>
<th>Probit</th>
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<tr>
<td>5</td>
<td>5</td>
<td>100</td>
<td>99.99</td>
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</table>

*Table 7.3.: Probit analysis for effect of S. argus venom on platelet lysis [LDH activity].*
Fig 7.5: Effect of S. argus venom on platelet lysis [LDH activity]. The release of LDH from washed platelets incubated with different concentrations of S. argus venom results are expressed as percentage of activity in relation to that evoked by 0.1% Triton X-100 in assay medium and represented as mean ± SE of six independent experiments.

Fig 7.6: Effect of S. argus venom on cell viability of HeLa cells. Increasing concentrations of S. argus venom were incubated with HeLa cells for 72 hours at 37°C. The cell viability as estimated by a colorimetric test with MTT and expressed as percentage of viable cells compared with cells cultured with medium alone. Results are represented as mean ± SE of six independent experiments.
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**Fig 7.5:** Effect of *S. argus* venom on platelet lysis [LDH activity]. The release of LDH from washed platelets incubated with different concentrations of *S. argus* venom results are expressed as percentage of activity in relation to that evoked by 0.1% Triton X-100 in assay medium and represented as mean ± SE of six independent experiments.

**Fig 7.6:** Effect of *S. argus* venom on cell viability of HeLa cells. Increasing concentrations of *S. argus* venom were incubated with HeLa cells for 72 hours at 37°C. The cell viability as estimated by a colorimetric test with MTT and expressed as percentage of viable cells compared with cells cultured with medium alone. Results are represented as mean ± SE of six independent experiments.
<table>
<thead>
<tr>
<th>Group</th>
<th>Dose [µg/ml]</th>
<th>Cellviability [%]</th>
<th>Lysis [%]</th>
<th>Expected response [%]</th>
<th>Probit</th>
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<td>71.9</td>
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<td>6</td>
<td>10</td>
<td>16.13</td>
<td>83.9</td>
<td>91.17</td>
<td>0.91</td>
</tr>
</tbody>
</table>

*Table 7.4.* Probit analysis for cell viability of HeLa cells on exposure to *S. argus* venom. The cell viability as estimated by a colorimetric test with MTT and expressed as percentage of viable cells compared with cells cultured with medium alone.
persisted beyond 48 hours [Fig.7.2]. The antihistamine pheneramine maleate did not have any profound influence on the oedematric activity suggesting that histamines were not involved in *S. argus* venom oedema formation. [Table7.2]. Footpad was measured 2hr after injection. Fig.7.3 shows that an intra plantar injection of the venom induced an increase in paw licking duration that reached its maximum with 25 μg.

*S. argus* venom exhibited haemolytic activity on washed erythrocytes of human, rat, mice, and goat. Lysis of erythrocytes was dose dependent. The activity was found to be species specific showing a high activity for rat erythrocytes followed by human > mice and finally goat erythrocytes which showed mild activity. The ED₅₀ for human erythrocytes was 1.97 μg/ml [Fig: 7.4 A], ED₅₀ for rat erythrocytes was [1.28 μg/ml] [Fig:7.4 B], ED₅₀ for mice and goat erythrocytes were 2.2μg/ml [Fig:7.4 C] and [2.7μg/ml] [Fig :7.4 D] respectively. No clotting was observed when blood plasma was incubated with different doses of venom nor did it cause any lysis of clot formed.

Incubation of platelets with venom resulted in their rapid clearance from the suspension. A dose dependent lysis of platelets as shown in Fig.7.5 was observed. At a dose 0.59 μg venom/ml, 50% of platelet lysis occurred, while 5.8μg/ml venom resulted in almost complete lysis of platelets [Table7.3].

To study the cytotoxic activity of *S. argus* venom HeLa cells were incubated with different doses of venom [0.1, 0.5, 1, 2.5, 5 and 10μg/protein of venom/ 100μl/ well]. Results obtained are shown in [Table7.4]. The crude extract of *S. argus* venom adversely affected the viability of HeLa cells when compared with the control [with PBS] [Fig.7.6]. Phase Contrast Microscope studies of HeLa cells
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incubated with different doses of venom showed rounding, distension and lysis of cells.

7.5. DISCUSSION

The discoveries of toxins from venoms, especially from marine resources, are racing ahead because of their extremely complex and unique action upon various mammalian physiological systems. After all, the venom secretion is part of an organism's defense and/or predatory mechanism, whose specificity is horned over a million years of evolution.

The oedematogenic response observed for S. argus venom in mice footpad was persistent even after 24 hours and the oedema formed was intense suggesting the potentiality of S. argus venom in producing oedematic activity. The role of histamines released from mast cells causing vasodilation and increasing vascular permeability is ruled out as pheneramine maleate, an antihistamine was unable to block or reduce the oedematic activity. Studies on stonustoxin have shown that the increase in vascular permeability is not mediated by histamine release as the antihistamine diphenylhydramine, did not inhibit the oedema effect in mice [Poh et al., 1991]. The inflammatory cellular influx into intraplantar region of mice induced by T. nattereri venom have shown to be due to a consequence of an impaired blood flow in venules at injured tissue and the cytotoxic effect of the venom on inflammatory cells contribute to this impairment [Lima et al., 2003].

The pretreatment of mice with either indomethacin [a cyclo-oxygenase inhibitor] dexamethasone [a steroid anti-inflammatory inhibitor] or L-NAME [inhibitor of nitric oxide synthase] did not affect the T. nattereri venom induced oedematogenic responses [Lopes-Ferriera et al., 2004]. These studies suggest an alternate mechanism of inflammation. Such venom-induced modifications of vascular permeability may account for the potent hypotension associated with
envenomation [Low et al., 1993]. Significant nociceptive response was observed for S. argus venom injected into the mouse right hind paw. The maximum nociceptive response was observed for 25 µg venom.

S. argus venom exhibited haemolytic activity for human, mice, goat, and rat erythrocytes. The haemolytic activity for chicken erythrocytes was mild. This suggest that the haemolytic activity was species specific being selective for erythrocytes of each species. Studies have shown that most of the piscine venom exhibit species specific haemolysis. The stone fish S.trachynis venom show potent haemolysis which is lytic-in vitro for guinea pig [Weiner, 1959a] rabbit, dog, rat and pig, while activity is less against sheep, cow, human, monkey, mouse, goat, horse, burro and cat erythrocytes [Kreger, 1991]. The verrucotoxin and stonustoxin are potently haemolytic being specific for rabbit erythrocytes [Garnier et al., 1995; Khoo et al., 1992]. Dracotoxin, the lethal factor from T.draco is species specific being very potent on rabbit erythrocytes, less potent on rat erythrocytes and weakly effective against mouse and bovine erythrocytes [Chhatwal and Dreyer, 1992a; b]. No phospholipase activity was found in S. argus venom [Chapter 8] suggesting an alternate mechanism for lysis. Similarly though P. volitans, T. natterei, T. maculosa and S. plumeiri and N. robusta possess haemolytic activity, no phospholipase activity is detected in the extracts of the venoms of these fishes supporting the present findings [Carrijo et al.2005; Hahn and Oconnor, 2000;Lopes-Ferriera et al., 1998; Shiomi et al., 1989]. Studies on cytolytic activity of SNTX found that it does produce haemolysis by forming hydrophilic pores in cell membranes, which then result in haemolysis [Chen et al., 1997].
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No clotting was observed when blood plasma was incubated with different doses of venom nor does it cause any lysis of the clot formed. The findings show that the amount of bleeding from the puncture wound appears to be similar to that from a similar non-venomous injury. The S. argus venom does not have any anticoagulant activity as observed for other vertebrate venoms. This is well in relation to the effects observed in envenomated patients where no reports of bleeding have been reported after stung by venomous spine of S. argus.

LDH is a cytosolic enzyme present in platelets that are intact under normal activation and release reactions of platelets. Lysis of platelets results in release of this enzyme. Hence LDH assay served as an induction of platelet lysis. 2% Triton X-100 caused complete platelet lysis. Studies have shown that S. argus venom induces platelet lysis. It has an ED$_{50}$ of 0.7 µg/ml. S. argus venom presented a marked lytic effect towards platelets. Adhesion, activation, and aggregation of platelets are integrated phenomena that trigger thrombus formation [Kristensen et al., 2000]. The lytic effect of S. argus on platelets leads to the liberation of potent products contained in their granules [Ca$^+$, ADP, Thromboxane A$_2$] that induce aggregation, thrombus formation and further contribute to vasoconstriction effects. However, the significance of the observed platelet lysis in the pathogenesis of envenoming is still unclear. Lysis in vivo may occur but only in a limited number of platelets or since the number of platelets are more in in vivo condition and the haemolytic ED$_{50}$ observed is more than for platelets, RBC’s were lysed more than platelets. Studies have shown that T.nattereri venom induced total lysis of platelet at a concentration of 5µg/ml and the effect was dose dependent [Lopes-Ferriera et al., 2002]. SNTX was shown to induce a dose dependent [ED$_{50}$ = 0.038 µg/ml] lysis of platelet in rabbit platelet rich plasma [Khoo et al., 1992].
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Nearly 4 hours after incubation of HeLa cells with S. argus venom of different concentration, the cells were distended [a morphological feature common to necrosis [Moldrich et al, 2000] when compared to preexposure on examination under phase contrast microscope. Degradation or lysis of the cell had taken place. Vehicle [PBS] had no effect on the morphology of cells. Studies on HeLa cells have shown that S. argus venom have potent cytolytic activity. The viability of cells was dose dependent. When cell death was measured in cultured neurons using propidium iodide fluorescence, G.marmoratus venom [30μg protein/ml], S.trachynis venom [4μg protein/ml] and P.volitans venom [20μg protein/ml] all produced an increase in fluorescence to 35,50 and 55% of pretreatment fluorescence respectively [Church et al., 2003].

The flow cytometric analysis for S.trachynis [stonefish] and Hypodytes rubripinnis [redfin velvette fish] have shown that the venom are capable of differentiating between normal and tumor cells as mitogenic effects were observed towards cell lines. There was significant cytotoxicity induced by crude venom as well as certain isolated fractions [Satoh et al., 2002]. The incubation of murine endothelial cell line of capillary origin with T.nattereri venom resulted in a dose dependent increment of LDH [Lopes-Ferrieria, 2002]. T.nattereri venom also affects the viability of mononuclear cells [J774Al] [Lopes-Ferrieria, 2003]. Studies on TLY have found evidence of an ability to form pores in the membrane of neuroblastoma cells [Ouanounou et al., 1999] suggesting that the cytolitic activity of the venoms is also dependent on extra-cellular Ca^{2+}, and most likely due to ionic imbalances caused by the entry of extra cellular Ca^{2+} through pores in the cell membrane [Church et al., 2003]. The ionic imbalances in the cells produce an influx of fluid, cell swelling, and necrosis.

The haemolytic activity, platelet lysis and cell line studies have all pointed out the cytolitic activity of the venom. Further studies are required to trace the
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pathway of oedematic activity of S. argus venom. The present results open up new vistas for research on the effects of S. argus venom on cytolytic activities. Scatophagus argus venom stands out as an extremely encouraging material for future studies.

Findings

- S. argus venom showed oedematic activity in mouse hind paw which was peak during 1-3 hours and persisted for more than 24 hours.
- The antihistamine Pheneramine maleate did not have any profound influence on the oedematic activity suggesting that histamines were not involved in S. argus venom oedema formation.
- S. argus venom induced an increase in paw licking duration.
- The haemolytic activity exhibited by S. argus venom was species specific.
- No clotting was observed when blood plasma was incubated with different doses of venom.
- The ED$_{50}$ for the platelet lysis induced by S. argus venom was 0.75 $\mu$g / ml.
- S. argus venom showed cytolytic activity towards HeLa cells.