BIOMEDICAL COMPOUNDS FROM FRESHWATER CATFISHES *OMPOK BIMACULATUS* (BLOCH, 1797) AND *MYSTUS VITTATUS* (BLOCH, 1797)

Dissertation submitted in partial fulfillment
of the requirements
for the degree of

M. F. Sc. (Inland Aquaculture)

by

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Dedicated to my beloved FAMILY

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CENTRAL INSTITUTE OF FISHERIES EDUCATION
(Deemed University) Indian Council of Agricultural Research

Dated: 1 July 2002

CERTIFICATE

Certified that the dissertation entitled " **BIOMEDICAL COMPOUNDS FROM FRESHWATER CATFISHES *Ompok bimaculatus* AND *Mystus vittatus*** " is a record of independent bonafide research work carried out by **Mr. A. Kathirvelpandian** during the period of study from September 2001 to April 2002 under our supervision and guidance for the degree of **Master of Fisheries Science (Inland Aquaculture)** and that the dissertation has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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DECLARATION

I hereby declare that the dissertation entitled " **BIOMEDICAL COMPOUNDS FROM FRESHWATER CATFISHES *Ompok bimaculatus* AND *Mystus vittatus*** " is an authentic record of the work done by me and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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Aagardian .

CIFE, MUMBAI A.KATHIRVELPANDIAN

सारांश

मुंबई से एकत्रित किए गए मीठा पानी कैटफिश ओमपोक बीमाकुलटस एवं मायस्टस विट्टाटस के बाहयत्वचा स्त्राव के विषाणु का अध्ययन किया गया । कच्चा विषाणु मिथेनाल एवं लाइफोलाइजेशन साथ सीधा निकर्षण से प्राप्त किया गया जिसे क्रोयाइटोग्राफी आयन विनिमय द्वारा आंशिक शुद्धिकरण किया गया । ओ.बीमाकुलटस तथा एम.विटाइटस के लिए प्रोटीन 1.76 मिग्री/मिली एवं 1.94 मि.ग्रा./मिली प्राक्कलित किया गया । म्यूकस विषाणु का कच्चा निष्कर्षण प्राणघातक था । 20 ± 2 कसौली से इस नर एल्बिनो माइस में इंजेक्टेट किया गया । ओ.विमाकुलेटस के कच्चा म्यूकस निष्कर्षण 0.6 एम.एल. खुराक के निम्नतर प्राणघातक था जिसका मृत्युकाल 3 मिनट 55 सेकेण्ड था तथा एम.विटाटस का 0.4 एम.एल. था जिसका मृत्युकाल 3 मिनट 20 सेकेण्ड था । इसमें बैचेनी, हांफना, प्राणघातक, मुंह से फ्रोंथी म्यूकस के ओजिंग, मरोड़ एवं हाथ के अंग में लकवा के लक्षण पाए गए । उतकीय रोग विज्ञान अध्ययन से यह प्रकट होता है उत्तकक्षय, यकृत एवं हृदय की झिल्ली टूटता है तथा हिमोलाईज्ड रक्त का संचयन होता है ।

ओ.विमाकुलेटस एवं एम.विट्टाटस का अपरिपक्व विषाणु को मुर्गी के रक्ताणु में निषेचित किया गया ।

ओ.विमाकुलेटस में हिमोलेटिक टिट्री 8 एवं विशिष्ट हिमोलेटिक क्रिया 4.54 hu/mg पाया गया । इस अंतर में किसी प्रकार या हिमोलेटिक क्रिया नहीं देखा गया । दोनो विषाणु एनायलजेसिक क्रिया को प्रदर्शित कर रहे थे । ओ.बिमाकुलेटस के लिए अधिकतम एनायलेजिक अनुपात 1.32 पाया गया तथा एम.विटाटस के लिए यह 1.65 था । ऐरोनोमास हाइड्रोफिला एवं विब्रियो एंगुलेरम तथा ओ.विमाकुलेटस म्यूकस विषाणु दोनो संवेदनशील था तथा दोनो 5 एम.जी. तथा 10 एम.जी. विषाणु सांद्रित करता है । ए.हाइड्रोफिला 0.4 एम. Nacl तक संवेदनशील था । ए.हाइड्रोफिला एम.विट्टाटस से अधिक संवेदनशील था तथा दोनों 5 एम.जी. एवं 10 एम.जी. विषाणु सांद्रित करता है । 0.2 M Nacl एवं 1.0 M Nacl ए.हाइड्रोफिला के विरुद्ध संवेदनशील प्रतिक्रिया को दर्शाता है । सेल लाइन को परखने हेतु चूहे के मांसपेशी कोशिका में दोनो विष की उपस्थिति कराया गया तथा इसमें रुपात्मक (मोरफोलोजिकल) परिवर्तन जैसे कोशिका की गोलाई, कनिकाएं तथा उनके जीवनकाल में कमी होती पाई गई ।

ABSTRACT

The toxicity of the epidermal secretion of the freshwater catfishes *Ompok bimaculatus* and *Mystus vittatus* from Mumbai were studied. Crude toxin was obtained by direct extraction with methanol and lyophilised. Crude extracts of the mucus toxin was lethal to 20±2g Kasauli strain male albino mice when injected intraperitoneally; crude mucus extract of *Ompok bimacultus* was lethal at the lowest dose of 0.6 ml and the time period was 3 minutes and 55 seconds and for *Mystus vittatus* it was 0.4ml at which death occurred with in 3 minutes and 20 seconds. The symptoms were restlessness, gasping, lethargy, oozing of frothy mucus from mouth, convulsion, and paralysis of hind limbs. Histopathological studies revealed necrosis of kidney, liver and heart accompanied by membrane break down and accumulated with hemolysed blood. The crude toxin of the *Ompok bimacultus* and *Mystus vittatus* induced hemolysis on chicken erythrocytes. In *Ompok bimacultus*the hemolytic titre was found to be 8 and the specific hemolytic activity was 4.54 HU/mg. In *Mystus vittatus*, the hemolytic titre was 16 and the specific hemolytic activity was 8.25 HU/mg. And the fractions did not show any hemolytic activity. Both the toxins exhibited analgesic activity. For *Ompok bimacultus* the maximum analgesic ratio was observed as 1.32, and for *Mystus vittatus* it was 1.65. *Aeromonas hydrophila* and *Vibrio anguillarum* were sensitive to *Ompok bimacultus* mucus toxin at both 5 mg and 10 mg toxin concentration and among fractions *Aeromonas hydrophila* was sensitive to 0.4 M NaCl fraction. *Aeromonas hydrophila* was sensitive to *Mystus vittatus* mucus toxin at both 5 mg and 10 mg toxin concentration, 0.2 M NaCl and 1.0 M NaCl fractions showed sensitive reaction against *Aeromonas hydrophila.* In the cell line assay using mouse muscular cell line, both the toxins produced morphological changes like rounding of cells, granulation and also reduced the survival.

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Introduction

INTRODUCTION

India has inland cultivable areas of about 1.6 million ha, and yield is about 1.5 tonnes. Inland fishery resources of India are broadly divided into two categories, viz. freshwater fishery resources and brackishwater fishery resources. The former includes the great river systems, reservoir, lakes, irrigation canals, tanks, ponds, etc. while the later is constituted by estuaries, lagoons, backwaters, brackishwater lakes, etc. The inland fishery resources of India comprise of 1.99 million ha of tanks and ponds, 1.89 million ha of lakes and reservoirs, 0.69 million km length of rivers and canals, 1.04 million ha of oxbow lakes and derelict water bodies and 1.38 million ha of brackishwater areas for the development of fisheries (Agrawal, 1999)

The catfishes are one of the most distinctive groups of fishes. These are one of the most important cultivable groups of fishes which are considered to have a very high growth and market potential throughout the world. It appears that the catfishes have diversified for being active after being dark or in turbid waters. This diversification has produced over 2,200 species of catfishes. Most catfishes are readily recognizable by their whiskery snout containing one to four pairs of barbels, head that is usually flattened, adipose fin and streamlined body. Most also have a stout spine leading to each pectoral and dorsal fin. Many species have venom glands associated with spines. Many families of the freshwater and marine catfishes have toxic members. The catfish families which are reported to be venomous are Aridae, Bagridae, Clariidae, Doradidae, Heteropneustidae, Ictaluridae, Pimelodidae and Siluridae (Halstead and Courville, 1970).

Biotoxinology is the science of plant and animal poisons and is concerned with a vast number of toxic chemical substances. Biotoxins are specific substances produced by living organisms that are seriously impairing living processes. Atleast 1000 species of fishes are known to be venomous or poisonous (Russel, 1965).

Fish Biotoxinology imbues to have absolute knowledge of any fish to be of that sort by solving a number of complex biological and biochemical conundrums

yielding in several tangible results in terms of human health security and economic benefits. Biomolecules extracted from the aquatic organisms has been proved to have powerful bioactive and biomedical properties. Biotoxinology offers to solve a number of unresolved, complex biological and chemical riddles, which may yield tangible results in terms of health security and economic benefit.

Toxic and poisonous fishes and their effect on public health have always held suspicions among the fishermen and consumers. Even though the fishes are termed toxic and neglected in some parts, they are of greater importance on the other parts of the globe. For example, puffer fish, which can cause poisoning upon ingestion, is surprisingly in great demand in China and Japan as a food fish. The toxin is not associated with a specific organ or portion in all the fishes; rather it varies from species to species. It may be linked with blood (Ichthyohemotoxin), in flesh (Ichthyosarcotoxin), in roe or gonads (Ichthyootoxin), or in epidermal mucus (Ichthyocrinotoxin).

Ichthyocrinotoxic fishes (Russel, 1965; Halstead and Courville, 1970; Concon, 1998) are those which produce their toxin by glandular secretions but lack a true venom apparatus. These fishes produce a toxic substance in their skin and subsequently release this toxin into the environment. Examples of ichthyocrinotoxic fishes are boxfishes, hagfishes, catfishes, toadfishes, etc. the symptoms of poisoning include nausea, tenemus, vomiting, abdominal pain and weakness. Catfishes in general have moderate to high levels of epidermal mucus secretions. Many catfishes have prominent venom glands elaborated in their dorsal and pectoral stings, the contact with which results in painful wounds (Halstead and Courville, 1970). Cameron and Endean (1973) described six species of catfishes which produce crinotoxins, which have been defined by Halstead, and Courville (1970) as toxins in fish epidermal secretions not associated with any venom apparatus. Most of the catfishes are probably crinotoxic. The Arabian Gulf catfish (*Arius thalassinus*) produce a moderate flow of epidermal mucus under normal conditions and they also secrete a thick gel coating from proteinaceous cells when threatened, thermally shocked or injured (Al-Hassan *et al.,* 1982). The skin toxin of the Oriental catfish is proteinaceous nature and is composed of hemolytic, lethal and edema forming factors (Shiomi *et al.*, 1986).

Ompok bimaculatus (Bloch): It is distributed in freshwaters of India, Afghanistan, Pakistan, Ceylon, Burma, Java, Sumatra, China and Bangladesh. It inhibits rivers, lakes, and ponds in plains and sub mountain regions. It mainly feeds on animals like crustaceans (copepods, daphnids), insects (water bugs, beetles, dragonflies, damselflies). It is having elongated body, and strongly compressed, mouth is large and oblique, and having 2 pairs of barbels, color is silvery but varies with habitat, a block spot on the shoulder is present it attains maximum length of 45cm and spawns during monsoon months, and thrives well in confined water it is tasty and highly priced.

Mystus vittatus (Bloch): Distributed in Afghanistan, India, Pakistan, Burma, Nepal, and Bangladesh. It inhabits rivers, canals, ditches, inundated fields, etc.It is having 4 pairs of barbels, and scales are absent, it attains maximum size of 12 cm. It is carnivore and predatory fish, feeds on insects, crustaceans, gastropods, small fishes and prawns.

There have been many reports regarding the toxicity and pharmacological properties of epidermal secretions of marine catfishes of Gulf coast and Oriental coast. However, many freshwater fishes of gastronomic importance are not studied with reference to their potential health hazards or their biomedicinal and neuropharmacological utilities. With demand for bioactive compounds on an exponential phase, and fish being the most diversified in nature, they are definitely a promising bountiful source of such compounds and various related chemicals. Biotoxins extracted from aquatic organisms including fish can be promoted as anti toxins, therapeutic agents and other pharmaceutical products. A few compounds isolated so far have proved to have powerful bioactive and pharmacological properties. It requires a multi pronged approach to establish its possible medicinal and industrial uses.

Review of literature

2. REVIEW OF LITERATURE

2.1 Ichthyocrinotoxicism

Halstead and Courville (1970) coined the term ichthyocrinotoxicism for epidermal secretion of fishes, which are not associated with any venom apparatus. The first report on crinotoxism was on lampreys and hagfishes by Prokhoroff (1884), Kobert (1902), and Crinotoxicism has been reported from different groups of fishes such as toadfishes (Collette, 1956; Nair *et al.,* 1982); soapfishes (Maretzki and Castillo, 1967); sea bass (Liguori *et al.,* 1963); boxfishes (Thomson, 1964); moray eels (Halstead and Courville, 1970; Randall *et al.,* 1981); catfishes (Halstead and Courville, 1970); gobies (Noguchi *et al.,* 1971); flatfishes (Clark and Chao, 1973; Clark, 1974) and clingfishes (Hori *et al.,* 1979).

2.2 Skin Structure

Halstead (1970) described that skin of fish comprised of 3 main layers viz., the outer epidermis, the dermis and the subcutaneous tissue. The epidermis is ectodermal in origin and consists of several layers of flattened cells, the moist cells. The dermis is a complicated structure, mesodermal in origin and is composed of connective tissue, blood vessels, nerve and cutaneous sense organ. Halstead (1970) also quoted Schrrier's histological studies on the skin of hagfish, according to which, there are five different types of cells in the epidermis and among these mucus and thread cells.

The epidermis is ectodermal in origin and consists several types of secretory cells. The dermis is mesodermal in origin and is composed of connective tissue, blood vessels and nerves. The epidermis has got two types of secretory cells.

a) Goblet cells - They are spherical in shape and secrete mucus. The matured ones are saucer shaped and in these nucleus will be displaced to the periphery of the cell by large vacuoles. These vacuoles lodge secretory material in the form of goblets. These cells open to the exterior for discharging their secretions (Cameron and Endean, 1973) or rather burst on the skin surface to release their contents (Whitear, 1970).

b) Club or Clavate cells - These cells secrete proteinaceous substances. They are known to secrete the mucus toxin also (Cameron and Endean, 1973; Al-Hassan *et al.,* 1985a; Shiomi *et al.,* 1986). They never open to the exterior to discharge their contents, but the epidermis will get ruptured to release its secretions.

Reed (1924) and Bhatti (1938) have reported that the catfish epidermal clavate cells produce proteinaceous secretions. Randall *et al.,* (1971) reported the presence of secretory cells in the epidermis of clingfishes. He also revealed the presence of peculiar clubbed cells in addition to the mucus cells, which produce proteinaceous secretions in moray eels. The clavate cells producing mucus are seen more in number on the gill surfaces (Laurent, 1984). Halstead and Courville (1970) quoted Schreiner's studies on the skin of hagfish according to which there are five different types of cells in the epidermis.

2.3 Functions of Skin Secretions

Mucus from goblet cells performs a lot of functions. It is found to reduce the turbulence generated during swimming (Rosen and Cornford, 1971). Antibodies are detected in the mucus (Fletcher and Grant, 1969). Randall (1967) reported that the toxic secretions of soapfishes act as repellent to the predators. It is also involved in the defense against pathogenic epibionts (Nigrelli *et al.,* 1955), parasites (Nigrelli, 1935, 1937) and microorganisms (Kitzan and Sweeney, 1968). In several families (Ostracidae, Grammistidae, Soleidae, Siluridae and Tetraodontidae). The skin secretions act as a chemical defence against predators, and several toxins (pahutoxin, deacetoxypahutoxin, grammistins, povoninins, and tetrodotoxin) have been identified (Boylan and Scheuer, 1967; Randall *et al.,* 1971; Hashimoto and Oshima, 1972; Clark and Charge, 1979; Goldberg *et al.,* 1982; Tachibana, 1985; Kodama *et al.,* 1985,1986). Applications of epidermal gel preparations to the wound of mice, guinea pigs and human beings can reduce the healing time up to 60% (Al-Hassan *et al.,* 1983). Its involvement in blood clotting i.e. thrombosis is established by Al-Hassan *et al.,* (1986).

In small doses tetrodotoxin has been used clinically to relax muscular spasm and as a palliative agent in terminal cancer (Burkholder, 1963). Reed (1924)

suggested that the clavate cell secretions of the epidermis have a deleterious effect on the fungus *Saprolegnia* which infested some catfishes.

2.4 Additional Functions of Skin Secretions

The other functions of mucus according to Shephard (1994) are ion and water regulation, gas exchange, chemical and physical protection, functions related to the nutritional and immune factors of mucus, chemical communication and disease resistance. Fish mucus contains immunoglobulins, agglutins, lectins, and lysins, especially lysozyme (Shepard, 1994).

Mucus coatings partially protect fish from attack by fungi, bacteria, and other small organisms (Liguori *et al.,* 1963; Lewis1970; Pickering, 1974) and also aid in osmotic regulation (Ball, 1969) selective passage of ions (Solanki and Benjamin, 1982) protection from pollutants (Hora, 1934; Van oosten, 1957) and reduction of body friction (Rosen and Cornford, 1971) mucus is also secreted for the construction of nests (Solanki and Benjamin, 1982) and sleeping cocoons (Winn 1955, Byrne, 1970).

Many groups of fishes benefit in other ways from their body slimes. Some fishes produce toxins from the slime, which are highly toxic. Clark and Chao (1973) and Clark (1974) reported that Red Sea flat fishes (Moses sole) *Pardochirus marmoratus* secreted a toxin that was so effective in warding off shark bite that the attacker's jaws are said to be frozen in mid-bite.

It is likely that some of the toxins from the skin of the fishes have marked pharmacological activities. According to Halstead and Courville (1967) puffer poison including the skin toxins is used in Japan as an analgesic and in treatment of many diseases like arthritis, rheumatism, tetanus, asthma, etc. Tetradotoxin has lately been regarded as a skin toxin, since a puffer fish was found to secrete toxin from the skin (Hashimoto, 1979).

The respiratory surface of the fish supports a complex of interactive diffusive and active transport processes (Evans, 1984; Randall and Daxboeck, 1984). The situation for the oxygen transport has been considered, by Ultsh and Gross (1975) for carp. Interactions between carbon dioxide and ammonia excretion and gill

mucus have been considered by Wright *et al.,* (1986,1989) and by Randall and Wright (1989). Mucus may even immobilize carbonic anhydrase near the gill surface.

The African lungfish avoids desiccation during summer and dry seasons by making a shell of its body slime commonly called cocoon (Winn, 1955; Byrne, 1970). Mucus cocoon is secreted by *Protopterus aethiopicus* in combination with mud to prevent the loss of water from the body (Fishman *et al.,* 1984). An interesting use of mucus in defense has been noted by Hildeman (1959); young discus fish (*Symphysodon aequifasciata*) feed from the skin mucus of their parent and this seemed to be necessary for the survival. It is likely that the young discus acquire immunity in the form of antibodies supplied in parental mucus (Hildeman, 1959; Ingram, 1980).

Mucus is also secreted for the construction of nests by some fishes like gouramis, and sticklebacks (Wooton, 1984), in which the males spit eggs and keep young safe until they are able to fare on their own (Solanki and Benjamin, 1982). In some fishes mucus is produced with toxic constituents to serve as a protection for eggs (Moyer and Zaiser, 1981). Randall (1967) suggested that the toxic secretions of soapfishes might be repellant to predators.

2.5 General Aspects of Fish Mucus

The mucus cells are scattered in the epidermis and secretes mucus. The goblet cells of fish are supposed to produce mucus granules, which burst at the cell surface to release mucus (Whitear, 1970). The mucus cells have various names such as clevate cells, club cells and the proteinaceous cells. Fish mucus is a multifunctional material, which plays a major role in communication, resistance to diseases, respiration, ionic and osmotic regulation, feeding, reproduction and excretion (Ellis, 1981). Mucus is the epidermal secretion of fish and of high water content, high molecular weight and gel forming macromolecules. A major portion of epidermal mucus cells is mucin, a mixture of glycoproteins with a molecular weight of 1-2 million subunits, joined by disulphide bonds, a protein core rich in amino acids, threonin, serine, and proline, 70-80% dry weight carbohydrates and considerable structural variability (Pigman, 1977; Allen, 1983). Numerous secretory cells like sacciform cells and acidophilic granular cells also contribute to fish mucus

(Blackstock and Pickering, 1982; Whitear, 1986). Glycosaminoglycans that have been found in fish mucus, also appears in mammalian mucus (van de Winkel, 1986, Reid and Bhaskar, 1989). Other ingredients include immunoglobulins (Fletcher and Grant, 1969), lysozyme (Bullock *et al.* 1978), complement (Harrel *et al.* 1976), carbonic- anhydrase (Wright *et al.* 1986), lectins (Shiomi *et al.* 1988), calmodulin (Filk *et al.* 1984), proteolytic enzymes (Hjelmeland *et al.* 1983), pheromones (Hara, 1986) and C- reactive proteins (Alexander and Ingram, 1992).

2.6 Chemistry of Mucus Toxin

Thomson (1964) isolated a non-protein crude toxin from the boxfish *Ostracion lentiginosus* and named it as Ostraciatoxin and assumed it to be a steroidal saponin. Enomoto *et al.,* (1964) found the presence of β-methylaminobenzaldehyde and acrinol in the mucus of loach. Scheuer (1966) isolated a pure toxin from the Hawaiian boxfish in crystalline form and named it as pahutoxin, after the name of the fish pahu. Pahutoxin is made up of long chain of aliphatic acid ester of choline hydrochloride (3,caetoxy hexadeconoic acid), which is soluble in water, ethanol, chloroform, acetone and ethyl alcohol. (Boylan and Scheuer, 1967). Maretzki and Castillo (1967), based on dialysis and gel filtration, concluded that mucus toxin of the soapfish *Rypticus saponaceous* to be polypeptides of high molecular weight.

The crude toxin was found to be a mixture containing over 60 proteins and a variety of other components including sugars, lipids, nucleotides and nucleosides (Al-Hassan *et al.,* 1982, 1987). Boylan and Scheuer (1967) isolated a toxin from Hawaiian boxfish (*Ostracion lentiginosus*), identified it as choline ester chloride of β-acetoxypalmitic acid and named it as 'pahutoxin'. Peptides known as grammistins were reported from the skin of soapfishes (Maretzki and Castillo, 1967; Randall *et al.,* 1971; Hashimoto and Oshima, 1972).

The skin mucus of *Arius thalassinus* (Arabian Gulf catfish) consists of protein (85%), lipids (10%), small amounts of carbohydrates and nucleic acids (Al-Hassan *et al.,* 1982). Shiomi *et al.,* (1986) determined the concentration of protein in *Plotosus lineatus* to be 18 mg/ml. He also reported the presence of one hemolysin, two lethal factors and two edema-forming factors in the same fish. The lethal and the edema-forming factors were found to be identical. Biological properties of pahutoxin

were found very much similar to certain echinoderm and red tide toxins. Al-Hassan *et al.*, (1985a) reported that the fish mucus is similar to the mucus of mammalian cells. The toxin from the red sea flatfish is hydrophobic, acidic protein (paradaxin) with a molecular weight of 17,000 Da and is composed of 162 aminoacids (Primor *et al.*, 1978,1980). The Indo-Pacific moray eel secretes a skin toxin with an estimated molecular weight greater than 1,00,000 (Randall *et al.*, 1981) the trunk fish (Ostracidae) have been shown to elucidate a variety of choline esters of fatty acids which are responsible for the toxic nature of these skin secretions (Goldberg *et al.*, 1988)

Nair *et al.*, (1982) separated a skin toxin from the toadfish *Opsantus tau*. They found that the toxin was a mixture of 3 aliphatic ketones and the main component was 3-octanone.

2.7 Toxicity of Mucus Toxin

Shiomi *et al.*, (1986) reported that the mucus toxin of *Plotosus lineatus* showed hemolytic and edema-forming activities but not ichthyotoxicity. Intravenous injection of *Arius* sp skin secretions in rabbits was lethal at the levels of 1.5mg proteins/kg body weight. (Al-Hassan *et al.*, 1985b; Alnaqeeb *et al.*, 1989). The lung and liver tissue on autopsy revealed considerable damage (Alnaqeeb *et al.*, 1989). The lethal dose of purified toxin in rabbits was found to be 0.04mg protein/kg. The symptoms showed on injection of the purified toxin were the elevation of both cardiac and liver enzyme (Thomson *et al.*, 1998). According to Shiomi *et al.*, (1988) the LD_{50} of crude mucus toxin of *P. lineatus* on mice was 1.44mg.

The skin toxin of the flatfish *Pardachirus marmoratus* was reported to be ichthyotoxic and it also showed hemolytic activity. Both these activities were found to decrease about 7 fold on lyophilisation of the toxin (Clark and Chao, 1973). The intravenous injection of skin toxin of *Ostracion meleagris* (Pahutoxin) to mice killed it in a few minutes. Autopsy revealed the complete collapse of lungs. The toxin also showed high hemolytic and hemagglutination action on fish erythrocytes (Thomson, 1964). The tropical Indo-Pacific moray eel *Lyncodontis nudivomer* secretes mucus, which has high toxic, hemolytic and hemagglutination properties (Randall *et al.*, 1981). Noguchi *et al.*, (1971) found that environment plays an important role in the

toxicity of *Gobius criniger* as it varies with the season. Skin including the fins and head of the above were found to be highly toxic and the testis was toxic irrespective of the season and also exhibited the maximum toxicity (1000 MU /g).

Gastrointestinal distress characterized by nausea, vomiting, diarrhea, abdominal pain and weakness in human beings due to crinotoxicism was reported by Auerbach (1987). Soapfish skin toxin is found to cause irritant dermatitis in human beings (Hashimoto and Kamia, 1969).

2.8 Therapy of Mucus Toxin

Alnaqeeb *et al.,* (1989) reported that the lethality caused due to *Arius bilineatus* can be prevented by the pretreatment of the victim with indomethacin. Halstead and Courville (1970) reported that the dermatitis caused by the mucus toxin can best be managed with a cool compress of aluminium sulphate and calcium acetate. Al-Hassan *et al.,* (1986a) found atropine and indomethacin to block most of the residual activity of the vasoconstrictor components in the skin secretion of *Arius thalassinus.*

2.9 Bioactivity of Fish Mucus

Fish mucus plays many important roles such as protection, an aid to defense of fish, reduce the turbulence generated during swimming (Rosen and Cornford, 1971), antibiotic function (Fletcher and Grant, 1969), involved towards defence against pathogenic epibionts and microorganisms (Nigrelli *et al.* 1935).

2.9.1 Wound healing activity

The wound healing properties of mucus toxin are regarded as precious for the development of biomedical compounds. Van Oosten (1957) suggested that the mucus might aid in healing of epidermal injuries in fishes. The gel prepared from the epidermal secretion of the Arabian Gulf catfish *Arius thalassinus* accelerated healing of wound due to accidental injury in man in less than 4 days, which was commonly attained in about 10 days while oedema and swelling of wound area subsided and disappeared in a matter of minutes; the application of same also reduced the healing time about 60% when applied to mice, rats and guinea pigs (Al-

Hassan *et al.*, 1983). Al-Hassan *et al.*, (1985a) observed that the vasoactive substances in the skin mucus of catfish concomitant with prostaglandin release and constriction could play a major role in thrombosis, inflammation and stimulation of cell proliferation. Al-Hassan (1986b) reported that the clinical and pharmacological properties like accelerated clotting, vasoconstriction, hemolysis, hemagglutination of RBC, protease and nuclear activity present in the skin secretion of the Arabian Gulf catfish might indicate the wound healing properties of the mucus. Al-Hassan *et al.*, (1990) devised a novel and simple method without atrophy and side effect for injected diabetic foot ulcers in an uncontrolled Diabetis mellitus patient with aqueous extract from the skin of Arabian Gulf catfish *Arius bilineatus,* enriched with different fractions and catfish lipids. The treatment resulted in natural depredation of necrotic tissues and also sensation returned. The rate of healing was proportional to the amount of healing materials.

2.9.2 Antimicrobial activity

Liguori *et al.*, (1963) observed the inhibition of growth of *Escherichia coli*, assayed by paper disk method on plate cultured at 37^0C and zone of inhibition increased with concentration of crude skin mucus toxin of the golden striped bass, *Grammistes sexlineatus.* Takahashi *et al.*, (1986) observed plural biological substances to be exhibited in the skin mucus of yellowtail during their study of the enzymatic properties of the bacteriological substances extracted from the skin mucus, using lyophilized cells of *Micrococcus lysideikticus* and *Pasteurella pisicida* substrates. Etami *et al.* (1987) examined the bacteriolytic activity of skin mucus collected from the fish Ayu against *Micrococcus lysodeikticus* and *Vibrio anguillarum,* the etiological agent of vibriosis of Ayu. Bhuyan (1998) also examined the antimicrobial activity of skin mucus collected from the fish *Boleopthalmus dentatus.*

Antibacterial ion and channel forming proteins from skin mucus of carp *Cyprinus carpio* has been characterized (Leimaitre *et al.*, 1996). Pickerling (1974) suggested that continuous replacement of mucus in fish prevents colonization by parasites, fungi and bacteria.

Hjelmeland *et al.*, (1983) reported that *Vibrio anguillarum* was not inhibited when exposed to protease extracted from rainbow trout skin mucus in a

standard medium. However the bacteria lost their viability faster than normal. The mucus of cleaner-wrasse (*labroides dimidiatus)* protects the fish from parasite settlement and the possibility of antibiotic properties has been predicted by Lenke (1991).

2.9.3 Hemolytic activity

The mucus secretion of moray eel (*Lycodontis nudivomer)* has a hemolytic activity as high as 150 saponin units per g of mucus. This value is much higher than those determined for the mucus of soapfishes, 0.8 to 3.2 units per g of mucus (Randall *et al.,* 1971). Of the skin toxins produced by the sole *Pardachirus marmotatus* (Soleidae) was believed to be a protein with strong ichthyotoxic and hemolytic activities. The toxin was found to be rather unstable and lost most of its activity even when freeze dried (Primer and Zlotkin, 1975) Hemolytic activity of mucus of oriental catfish (*Plotosus lineatus*) was enhanced in the presence of the divalent cations except for Hg^{2-}. In the presence of Mn^{2-} rabbit erythrocytes have shown more sensitivity to the crude toxin, followed by red cells from chicken and guinea pig, while no measurable activity obtained from horse, cow or sheep erythrocytes (Shiomi *et al.,* 1986). Kamiya *et al.*, (1988) found agglutinins in fish mucus and Nair (1998) pointed out that most skin released ichthyotoxins causes hemolysis.

2.9.4 Cell line bioassay

Ichthyotoxins are cytotoxins that makes pores on cellular membranes, as in the case of Ichthyotoxic and hemolytic protein, named paradaxin, isolated from skin secretions of *P. marmoratus* (Lazarovici *et al.,* 1988). Aune *et al.,* (1991) used a mammalian cell based bio assay for okadoic acid by freshly preparing rat hepatocytes to monitor toxicity, evaluated by observing morphological changes. Amzil *et al.,* (1992) used kb carcinoma cell as a means of measuring okadoic acid cytotoxicity, reflected in morphological changes.

The two non-transformed intestinal epithelial cell lines (RTE-1and IEC – 6) and three intestinal carcinoma cell lines (HT29, T84 and MCA-38) have shown sensitivity to okadoic acid in mussel homogenates (Blay and Poon, 1995).

2.10 Other Activities

According to Halstead (1967), puffer fish toxin is used as an analgesic in Japan and in treatment of many diseases like arthritis, rheumatism, enuresis, tetanus, impotence, asthma, headache, etc. A tetradotoxin - like skin toxin has been used clinically to relax muscular spasms and as a pain relieving compound in case of patients suffering from the neurogenic form of Hasen's disease (leprosy) (Burckholdr, 1963; Bhakuni and Silva, 1975).

Material and methods

3. MATERIAL AND METHODS

3.1 Location

The present study and the experiments were carried out in the Biotoxinology and Aquatic Environment laboratories at Central Institute of Fisheries Education, Mumbai.

3.2 Specimen collection

The specimens of *Ompok bimaculatus* and *Mystus vittatus were* collected from the fish market at 4-Bungalows, Mumbai in fresh condition. The average sizes of the fishes were 28.5 cm and 10 cm respectively and the average weights of the fishes were 153.8 g and 20.9 g respectively. The fishes were identified following Talwar and Jhingran (1991), and stored in deep freezer at -20 ^{0}C until use.

3.3 Extraction of Crude Toxin

Extraction of crude mucus toxin was done by following the method of Noguchi and Hashimoto *et al.,* (1971). Each fish was dipped in 25 ml of methanol and swirled for about 30 minutes, the fishes were removed and the methanol was filtered through Whatman No.1 filter paper. Then the filtrate was evaporated till the methanol evaporates in serological water bath at 30 ^{0}C, lyophilised and then stored at -20 ^{0}C.

3.4 Ion-Exchange Chromatography

Separation of the different fractions from the crude toxin was carried out by Ion exchange chromatography using DEAE cellulose following the procedure of Shiomi *et al.*, (1987).

3.4.1 Regeneration of DEAE cellulose

25 g of DEAE cellulose powder was swelled in 250 ml of distilled water for 3 hrs and the supernatant was discarded and the sediment was mixed with 1l of 1M sodium hydroxide for about 30 minutes with intermittent stirring and allowed to stand for 30 minutes. The supernatant was discarded and the sediment was stirred

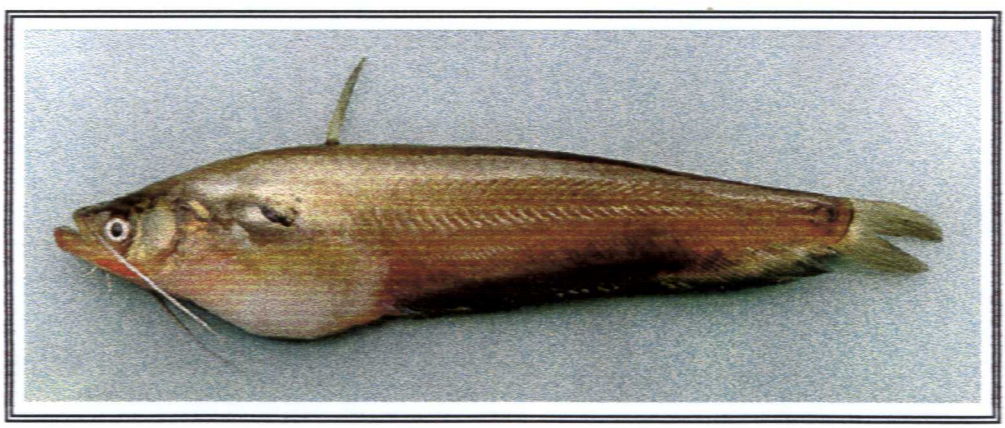

PLATE 1: Showing the specimen of *Ompok bimaculatus* (Bloch, 1797)

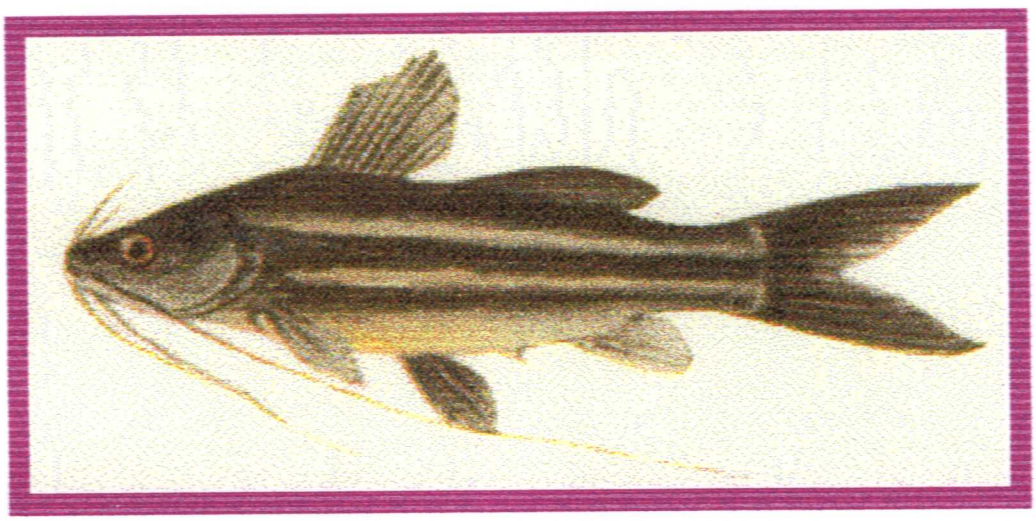

PLATE 2: Showing the specimen of *Mystus vittatus* (Bloch, 1797)

with 1 l of 1M sodium chloride for 30 minutes. The supernatant was discarded again. The mixture was allowed to stand for 30 minutes and the supernatant was removed. The sediment was stirred with 1l of 1N HCl for 30 minutes and soaked with 0.02M EDTA for 30 minutes and washed with distilled water till the pH become 7 and then washed with 0.01M Tris-HCl buffer (pH 7.4) for column packing.

3.4.2 Packing of Column

The column (2x45cm) was mounted vertically onto a stand and filled to about 10cm from the bottom with 0.01M Tris-HCl buffer (pH 7.4) and the outlet was closed. Care was taken to remove air bubbles from below the sintered glass disc and from the flow-regulating device. Thick slurry of DEAE cellulose in 0.01M Tris-HCl buffer (pH 7.4) was poured into the column, by carefully avoiding entrapment of air in the column. It was allowed to settle under gravity until about 5cm of cellulose was packed. The outlet was opened to give a moderate flow rate and more slurry was added until the height of packed level reached the required level of 22 cm. The flow rate was adjusted to 45ml/hr and washing with the 0.01M Tris-HCl buffer (pH 7.4) was continued until the pH of resin pack was the same as that of the buffer.

3.4.3 Sample Application and Elution

The sample was dialyzed thoroughly against 0.01M Tris-HCl buffer (pH 7.4). The level of buffer in the column was allowed to run down to the level of packed DEAE cellulose and the outlet was closed. A circular disc of filter paper was placed on the cellulose.10ml of sample was applied carefully on the bed of the column and the outlet opened till the entire sample had entered the bed. A few ml of Tris-HCl buffer was used to wash down the sample from sides of the column into the bed. Different protein fractions of crude mucus toxin was eluted with a salt gradient of 0.2M, 0.4M, 0.6M, 0.8M and 1M concentration of sodium chloride in 0.01M Tris-HCl buffer at a flow rate of 15 ml/hr. Eluted fractions were collected in the sterile screw capped vials and were stored at 4°C till use.

3.5 Estimation of Protein

Estimation of protein was done by the method of Peterson (1977), using Bovine Serum Albumin at the rate of 1 mg/ml as the standard. The standard of different concentrations ranging from 0.1 to 1.0 mg/ml were taken and made up to 1ml with distilled water and 1ml of reagent A was added, prepared by mixing equal parts of copper-tartarate carbonate (CTC), 10% Sodium Dodecyl Sulphate (SDS), 0.8N sodium hydroxide and triple filtered distilled water and allowed to stand for 10 minutes at room temperature. After 10 minutes 0.5ml of reagent B (Folin Ciocalteu Phenol) was also added. The test tubes were shaken gently to mix the solution and incubated for 10 minutes in the dark. The absorbance at 750nm was read spectrophotometrically. A standard graph was obtained by plotting absorbance against concentration. From the absorbance of the unknown samples (crude mucus toxin at the rate of 5 mg/ml and fractions @ 1 ml) the concentration of the protein was estimated using the standard graph.

3.6 Mice Bioassay

Clinically healthy male albino mice (Kasauli strain) of 20±2 g weight were procured from M/S Haffkines Biopharma Ltd., Mumbai, and were maintained in the laboratory.

5 mg of lyophilized samples were taken and diluted in 1 ml of phosphate buffer saline having the pH of 7.5. The samples were then injected intraperitonially to the mice in various doses varying from 0.2 to 1ml. Triplicate sets were maintained for each dose. The injected mice were kept under observation in cages and their activities were noted. The time of injection and the time of death were recorded. A control was maintained in each case by injecting an equal volume of 0.15M phosphate buffer saline (pH 7.5).

Convulsions, vigorous jumping, palpitation, urination, scratching of face and mouth, paralysis of hind limbs, foaming with blood from mouth, hiding on corners, arching the back of body were taken as indications of toxicity.

3.7 Histopathological Studies

The tissue of heart, liver, kidney, spleen and brain were taken out from mice that died upon envenomation and were fixed in 10% formalin for a minimum of 24 hours. Then they were washed thoroughly in three changes of 70%(v/v) alcohol to remove the excess fixative. The tissue samples were dehydrated in ascending grades of alcohol, 50%, 70% and 90%, for 1 hour each and three times in 100% alcohol for 45minutes each separately. The samples were then cleared in xylene for 2 hours, and embedded in paraffin wax thrice, each time kept for 45minutes.The samples were then blocked .The blocks were cooled and cut on a rotary microtome at 7µm thickness. The 'ribbon' thus obtained were kept with its shiny side downwards onto the surface of warm water in the water bath where the temperature was maintained 2-3°C lesser than the melting point of paraffin wax.

The sections were then picked up on a microscopic slide. The excess water was removed using a blotting paper. Dewaxing of the sections was done by drying the slides in hot plate for 2-3 hours and by clearing them in xylene. Samples were then hydrated in descending grades of alcohol (90%, 70% and50%). The staining was done by using Delafield's haemotoxylene for 7 minutes. Stained sections were washed in tap water followed by Scott's tap water for 3 minutes. These washed sections were subjected to Eosin stain for 5 minutes. The sections were then washed in water and dehydrated through 70%, 90%, 100% alcohol for 1 minute each. Finally stained sections were cleared in xylene and mounted on slide with DPX. Prepared sections were examined and photographed on microscope.

3.8 Hemolytic Assay

Following micro hemolytic method (Paniprasad and venkateshvaran, 1997), the hemolytic activity of the crude mucus extract and the DEAE cellulose fractions on chicken RBC was tested

3.8. 1 Collection of Blood

Chicken blood was collected From a nearby slaughterhouse using 2.7% Ethylene diamine tetra acetic acid (EDTA) as an anticoagulant at the rate of 1ml for every 20ml blood and stored at 4°C until further use.

3.8.2 Preparation of one percent RBC

The collected blood was centrifuged at 5000 rpm for 5 minutes, the supernatant was discarded and pellet was suspended in 0.9% normal saline (pH 7.2). The mixture was centrifuged again at 5000 rpm for 5 minute, the supernatant was discarded and pellet resuspended in 0.9% normal saline. This procedure was repeated thrice and finally 1%RBC was prepared for the test. A 1% erythrocyte suspension was prepared by adding 99ml of 0.9% normal saline to 1ml of packed RBC. Increasing hemolytic effect with increasing dose of toxin was taken as criteria for the toxicity.

3.8.3 Procedure

Hemolytic assay was followed according to Pani Prasad and Venkateshvaran (1997) in U shaped Laxbro microtitre plates. One row was selected and, 100 µl of PBS (phosphate buffer saline) was transferred to each well. 100 µl of toxin was added in to the first well, mixed thoroughly and from this 100 µl was transferred to the next well. This process was repeated till the last well and form the last well 100 µl was discarded. 100 µl of 1% erythrocytes was added to all wells. Appropriate control wells were included in the plate. Erythrocyte suspension to which 100 µl of distilled water was added served as a blank. Equal amount of PBS added to 100 µl of 1% erythrocytes served as a negative control. Formation of button in wells were taken as negative. The plates were incubated at room temperature for 2 hours before reading the results. Reciprocal of the highest dilution of the mucus toxin showing the hemolytic pattern was taken as 1 Hemolytic Unit (HU). Specific hemolytic activity was also calculated as number of hemolytic Unit per mg of protein (HU/mg). Same procedure was followed to estimate both hemolytic activity and specific hemolytic activity for different fractions.

3.9 Assay For Analgesic Activity

Analgesic activity was measured following the D'Amour and Smith test. This was done using a tail flick analgesia meter (Harvard, USA, 50-9495, 230V, 50 Hz) with a variable 150W, 25v lamp as the heat source.

The mouse was held in a holder in such a way that it's tail covers the photocell of the meter. When the heat source is turned on, the mouse feels the pain and it flicks the tail thereby uncovering the photocell. This action switches off the bulb and stops the counter simultaneously. The reaction time of the animal is the displayed and noted down.

Mice were injected with 0.25 ml of toxin intraperitoneally and were subjected to various light intensities (3.0, 3.5, 4.0, 4.5 Amperes) and the time was noted. Mice without administration of any toxin, injected with 0.25 ml PBS were used as controls while those injected with paracetamol (Paracetamol @ 0.25 ml/20 g) served as reference standards. The tail flicking time of the mice injected with toxin was compared with the tail flicking time of the mice injected with normal saline as also with that of paracetamol.

Analgesic activity was expressed as a ratio of reaction time of the envenomated mice against those of the control and also those mice injected with paracetamol.

3.10 Antibacterial Assay

The antibacterial activity of the crude mucus extract and purified fractions were assayed on selected bacterial cultures using the disc diffusion method.

3.10.1 Bacterial cultures

The bacterial species selected for the study viz. *Escherichia coli, Salmonella typhi, Streptococcus aureus, Klebsiella pneumonia* were procured from Institute of Microbial technology (IMTECH) Chandigarh, and *Aeromonas hydrophila, Pseudomonas fluorescence, Vibrio parahemolyticus* and *Vibrio anguillarum* were drawn from the stock cultures maintained in the Aquatic Environment laboratory of CIFE.

3.10.2 Media

Nutrient broth was used for culturing the bacteria and nutrient agar was used for plates to prepare bacterial lawn. Additional 0.5% sodium chloride was added

to the above medium to grow marine bacteria like *Vibrio parahemolyticus, V. anguillarum* etc. The composition of the media were as follows:

1.Nutrient broth:

Composition	(gm/l)
Peptone	5.0
Sodium chloride	10.0
Yeast	2.0
Beef extract	1.0
pH	7.2±0.2

The above ingredients were dissolved in the distilled water. This solution was poured in the test tubes and sterilized in autoclave at 121°C for 15 minutes.

2.Nutrient Agar:

Composition	(g/l)
Peptone	5.0
Sodium chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5
Agar	15
pH	7.4±0.8

The above ingredients were dissolved in one liter dissolved water and sterilized in an autoclave at 121°C for 15 minutes.

3.10.3 Preparation and sterilization of Discs

Discs of 5-6 mm diameters were punched from Whatman No.1 filter paper using a hand punch. The discs were sterilized in petridishes at 121°C in an autoclave for 15 minutes.

3.10.4 Impregnation of Discs with Toxins

The lyophilized toxin was diluted in 0.9% normal saline at the rate of 5 mg/ml, 10 mg/ml and were filter sterilized with 0.2 μ syringe filters. Similarly all the absorbed fractions of both the species were also filter sterilized in order to know its antibacterial activity. Impregnation was done by soaking the sterilized discs in the filtered crude toxin extract and also in the fractions. Then the soaked discs were dried in a vacuum desicator. The dried discs were packed in screw-capped bottles and stored in refrigerator. Commercially available dry discs were used as control.

3.10.5 Disc sensitivity test

Bacterial lawns were prepared by uniformly swabbing the overnight grown bacterial cultures in nutrient broth on the surface of the Nutrient agar plates. Toxin impregnated discs and control discs were placed aseptically on bacteria inoculated agar surface, at least one inch apart using a sterile forceps. Then the plates were incubated at 37°C for 24 hours in upright position.

The diameter of the zone of inhibition around the impregnated discs was measured using an antibiotic zone reader and recorded.

3.11Study On Cell lines

3.11.1Maintenance of Cell Lines

3.11.1.1 Preparation of Glasswares

The glasswares used in the experiment were of neutral glass of Borosil made and nontoxic to the cell cultures. They were soaked overnight in a mild acid solution (HCl). Later, they were washed thoroughly with tap water, and again they were soaked in mild detergent solution. Next day, they were washed thoroughly with

tap water several times, then with demineralized water for 3-4 times and finally with triple distilled water 6-7 times. After drying, they were properly wrapped in Aluminum foil and sterilized in hot air oven at 180°C for one and half-hour.

3.11.1.2 Preparation of Plastic wares

All the plastic wares used were nontoxic to cell cultures having good optical quality and were recommended suitable for 'tissue culture" by manufactures (Tarsons, India). A large quantity of disposable sterile plastic wares was used for the cell cultures like tissue culture flask, petridishes, multiwell plates, syringes, filters, etc.

3.11.1.3 Procedure

The mouse muscular cell line was procured from the National Center for Cell Sciences (NCCS), Pune.

The cells were sub cultured into a fresh flask containing growth medium. It involve rinsing the flask in serum free medium such Eagle's minimum essential medium (EMES) or L-15 (Leibovitz) medium and adding trypsin (0.1%), which removes the cells from the substratum. When the monolayer had detached from the flask the activity of trypsin was stopped by adding serum-containing medium (serum contains the trypsin inhibitor α-1 antitrypsine). The cells were sedimented by centrifugation washed in medium, counted and seeded into fresh medium at 10^4-10^5 cells /ml depending on the depending time.

3.11.2 Tissue culture assay

96 well microtitre plates were seeded with 200 µl of culture medium containing $1x10^5$ cells/ml. The cells were incubated at 37°C for three days or until a monolayer was formed. 500µg/ml of toxin was prepared and added to each well. The pH of toxin solution was adjusted to 7.4, sterilized by syringe filter through 0.22µ membrane filter and used to infect the cell culture. Serial ten fold dilution of the toxin was made in sterile L-15 (Leibovitz) maintenance medium (0.2% serum) and used for infecting the cells. For quantitative investigations, triplicate wells were prepared for each sample. The inoculated plates were kept at 37°C for kept for 48hrs in an incubator. At least 200cells in each concentration were randomly chosen and

observed under the inverted microscope. Those which had swollen or lost morphological rigidity, were judged to be dead. The number of viable cell in each well was quantified by MTT cell viability assay.

3.11.3 MTT Cell viability Assay

The toxin treated plates after 48 hrs of incubation were taken for MTT cell viability test. MTT stock solution was prepared (5mg/ml) and filter sterilized in 0.2 μ membrane filter. 50 μl of MTT stock solution was added to each well. The plates were incubated in a humidified incubator at 37°C for 4 hrs. Then the medium was replaced by 200μl of DMSO and incubated for 10 minutes on a microplate shaker to dissolve the formazan crystals of MTT. 25 μl of Sorensen's glycine buffer was added to each well. The plates were then read at 570 nm against a reference of 690 nm in an ELISA reader. Plates were read within 30 minutes after addition of DMSO.The viable cells were quantified using statistical methods.

Results

4. RESULTS

4.1 Extraction of crude mucus toxin

A total amount of 230 mg and 610 mg of crude mucus toxin was extracted in three sets of methanol extraction from 75 and 60 specimens of *Ompok bimacultus* and *Mystus vittatus* repectively.

4.2 Protein estimation

The amount of protein in the crude mucus extract of *Ompok bimacultus* was found to be 1.76 mg/ml and it varied between 0.172 to 0.332 mg/ml in purified fractions; and for *Mystus vittatus* it was 1.94 mg/ml for crude mucus and for the fraction varied between 0.134 to 0.414 mg/ml. The absorbance of standard protein (BSA) is given in Table 1 and that of crude and purified mucus fractions of *Ompok bimaculatus* and *Mystus vittatus* are given in Tables 2 and 3.

4.3 Mice bioassay

The lowest lethal dose of crude mucus extract of *Ompok bimacultus* was found to be 0.6 ml and the time period was 3 minutes and 55 seconds and for the mucus extract of *Mystus vittatus* it was 0.4ml at which death occurred with in 3 minutes and 20 seconds; When injected with the highest dose of 1 ml of crude mucus extract, death occurred with in I minute and 4 seconds and 50 seconds for *Ompok bimacultus* and *Mystus vittatus* repectively. The results of mice bioassay are shown in Tables 4 and 5.

4.3.1 Behavioral changes

The symptoms recorded were restlessness, gasping, and lethargy, oozing of frothy mucus from mouth, convulsion, and paralysis of hind limbs.

TABLE 1

Absorbance of standard protein (BSA) samples at 750 nm

Sl no	BSA (ml)	Protein (mg)	Distilled water (ml)	Reagent (A) ml	Reagent (B) ml	Absorbance 750 nm
1	0.00	0.00	1	1	0.5	0.000
2	0.1	0.1	0.9	1	0.5	0.823
3	0.2	0.2	0.8	1	0.5	0.848
4	0.3	0.3	0.7	1	0.5	1.03
5	0.4	0.4	0.6	1	0.5	0.981
6	0.5	0.5	0.5	1	0.5	1.243
7	0.6	0.6	0.4	1	0.5	1.128
8	0.7	0.7	0.3	1	0.5	1.634
9	0.8	0.8	0.2	1	0.5	1.256
10	0.9	0.9	0.1	1	0.5	1.402
11	1	1	0.00	1	0.5	1.564

TABLE 2

Amount of protein in crude mucus extract of *Ompok bimaculatus* and *Mystus vittatus.*

Species	Amount of toxin taken (ml)	Distilled water (ml)	Reagent A (ml)	Reagent B (ml)	Absorbance 750 nm	Protein mg/ ml
Ompok bimaculatus	0.1	0.9	1	0.5	2.72	1.76
Mystus vittatus.	0.1	0.9	1	0.5	3.04	1.94

TABLE 3

The amount of protein in purified fractions of mucus extract of *Ompok bimaculatus* and *Mystus vittatus.*

Sl no	Fractions	*Ompok bimaculatus*		*Mystus vittatus*	
		Absorbance 750nm	Protein mg/ml	Absorbance 750nm	Protein mg/ml
1	UN1	0.281	0.172	0.198	0.134
2	UN2	0.293	0.186	0.234	0.158
3	UN3	0.384	0.255	0.293	0.212
4	UN4	0.227	0.148	0.214	0.146
5	UN5	0.410	0.236	0.384	0.255
6	0.2M	0.374	0.251	0.428	0.291
7	0.4M	0.484	0.324	0.467	0.304
8	0.6M	0.498	0.332	0.583	0.414
9	0.8M	0.467	0.304	0.427	0.294
10	1M	0.534	0.362	0.473	0308

TABLE4

The toxicity of crude mucus extract of *Ompok bimaculatus* injected to male albino mice (20±2 g)

Sl no	Toxic solution	Dose (ml)	Death time		Remarks
			Minutes	Seconds	
1	Crude extract	0.2	-	-	Non lethal
2	Crude extract	0.4	-	-	Non lethal
3	Crude extract	0.6	3	55	Lethal
4	Crude extract	0.8	2	35	Lethal
5	Crude extract	1.0	1	4	Lethal

TABLE 5

The toxicity of crude mucus extract of *Mystus vittatus* injected to male albino mice (20±2 g)

Sl no	Toxic solution	Dose (ml)	Death time		Remarks
			Minutes	Seconds	
1	Crude extract	0.2	-	-	Non lethal
2	Crude extract	0.4	3	20	Lethal
3	Crude extract	0.6	2	35	Lethal
4	Crude extract	0.8	1	50	Lethal
5	Crude extract	1.0	-	50	Lethal

4.4 Histopathological studies

4.4.1 *Ompok bimaculatus* crude toxin

In heart changes were in the hemolytic form, the myofibrils got fragmented and in the brain, damage was mainly in the cell wall, areas of liquefaction were also seen in the cerebrum, and gliosis could be seen in focal areas. In kidney scattered hemorrhages were seen throughout the kidney parenchyma mostly containing hemolysed blood. In lung the alveoli moderately emphysemated and pulmonary blood vessel were engorged with hemolysed blood. The Branchioles were also distended. Marked necrotic changes were observed in the liver and the normal architecture was severely damaged. The results are given in Plates 3 to 12.

4.4.2 *Mystus vittatus* crude toxin

In the liver, mild to moderate degenerative changes were seen, and the hepatic cells lost their structure and appeared granular with pleomorphic nuclei and aggregation of lymphocytes were also seen. The cerebrum of the brain revealed large areas of vacuolation and glial cell reaction. In kidney scattered hemorrhages were seen through out the kidney parenchyma. In the lung blood vessels were seviourly congested with hemolysed blood, alveoli emphysemated, while heart did not show any significant changes. The results are given in Plates 13 to 22.

4.5 Hemolytic activity

The crude toxin of the *Ompok bimaculatus* and *Mystus vittatus* induced hemolysis on chicken erythrocytes. In *Ompok bimaculatus* the hemolytic titre was found to be 8 and the specific hemolytic activity was 4.54 HU/mg. In *Mystus vittatus* the hemolytic titre was 16 and the specific hemolytic activity was 8.25HU/mg. However fractions of both species did not show any hemolytic activity. The results are presented in Table 6 and Plate 23.

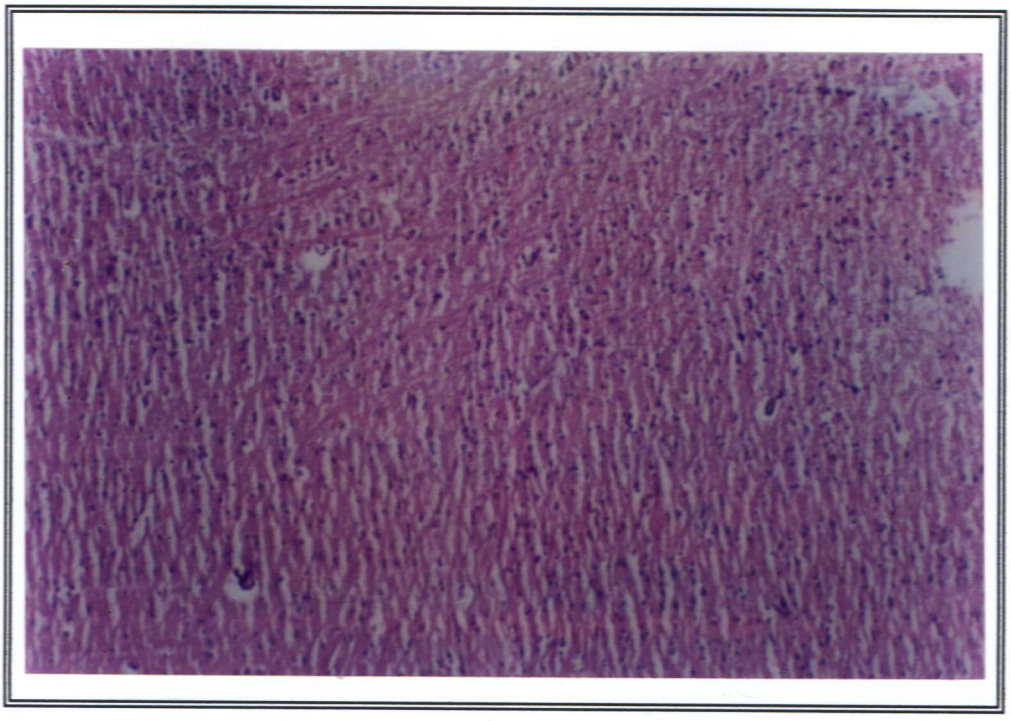

PLATE 3. Showing Cross section of Brain of normal mouse (control) (Hematoxylin- Eosin) 100x

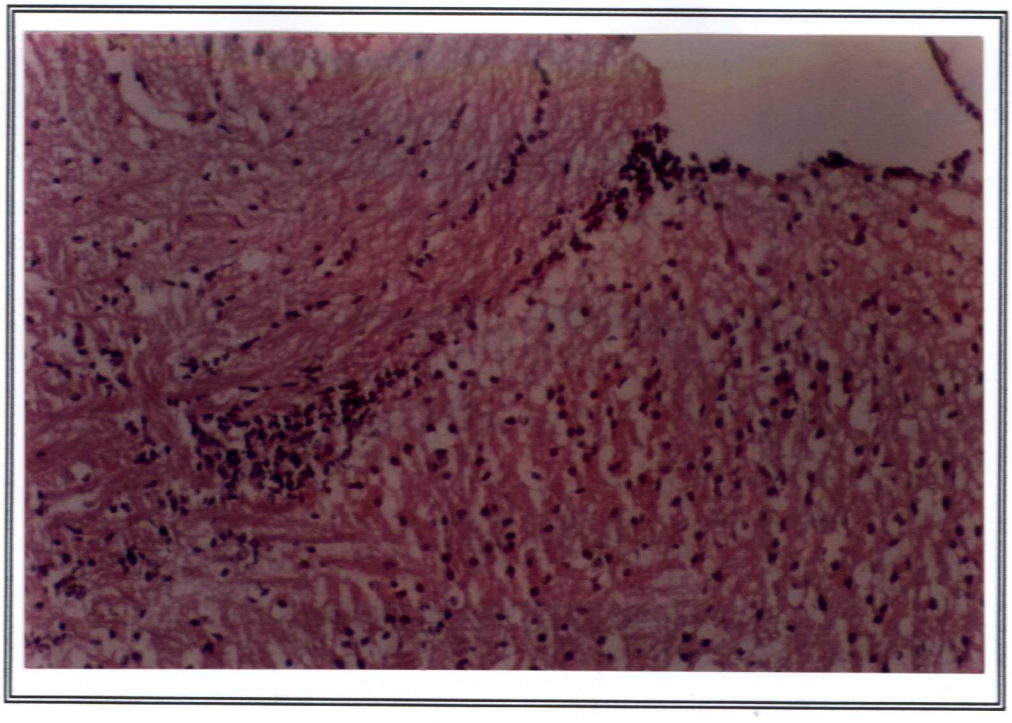

PLATE 4. Showing cross section of Brain of mouse envenomed with crude mucus toxin of *Ompok bimaculatus* (Hematoxylin- Eosin) 200x

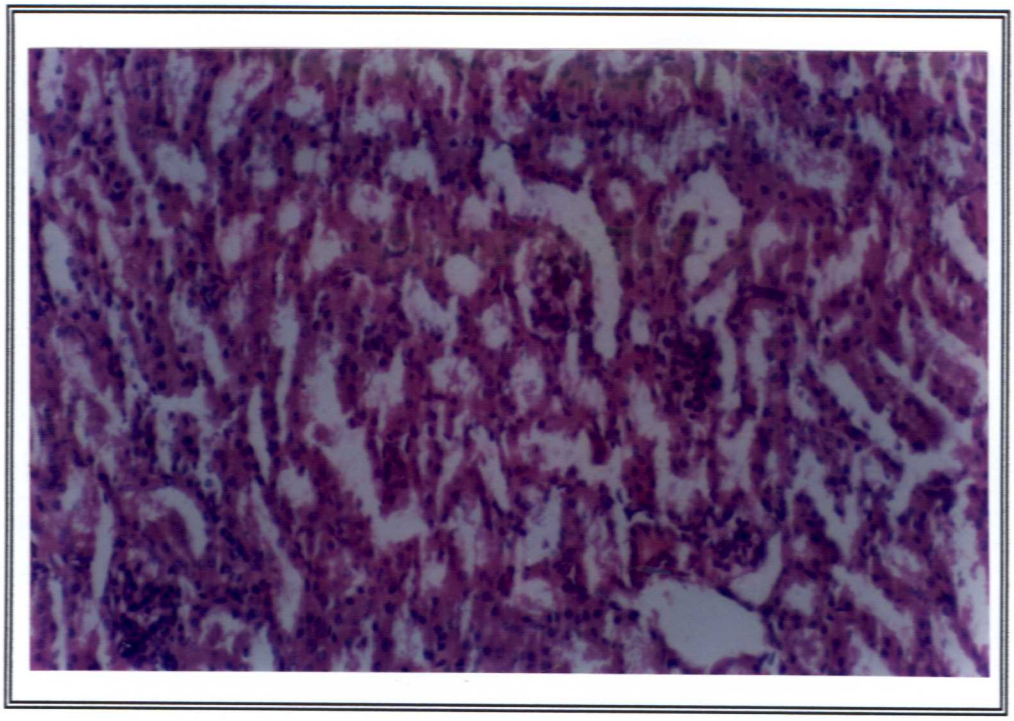

PLATE 5. Showing Cross section of kidney of normal mouse (control) (Hematoxylin- Eosin) 200x

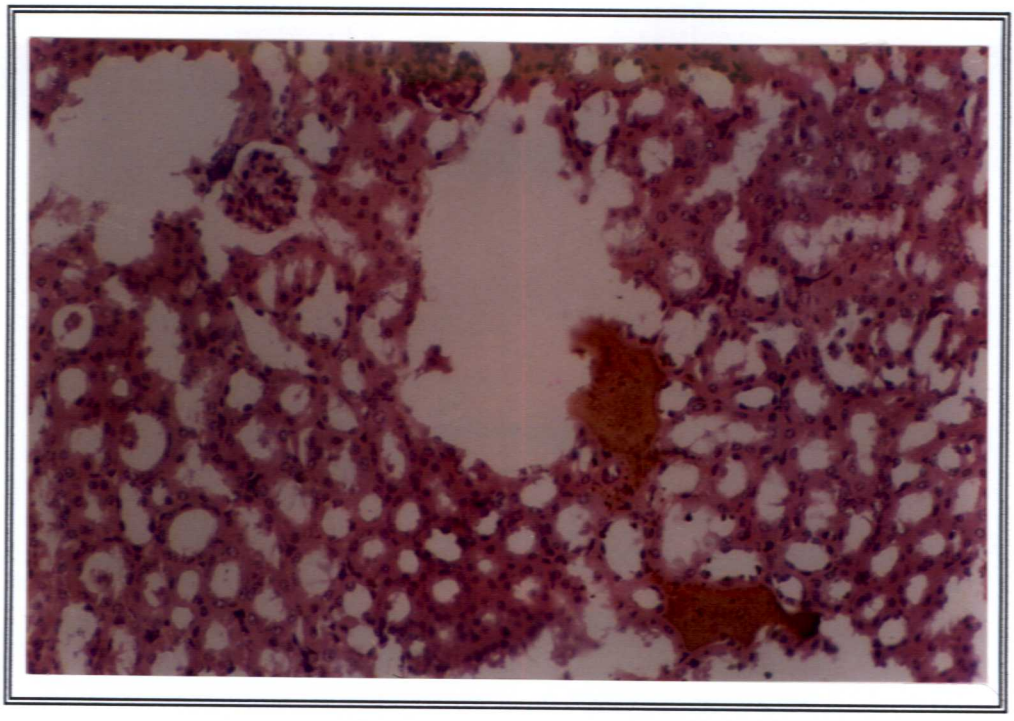

PLATE 6. Showing cross section of Kidney of mouse envenomed with crude mucus toxin of *Ompok bimaculatus* (Hematoxylin- Eosin) 200x

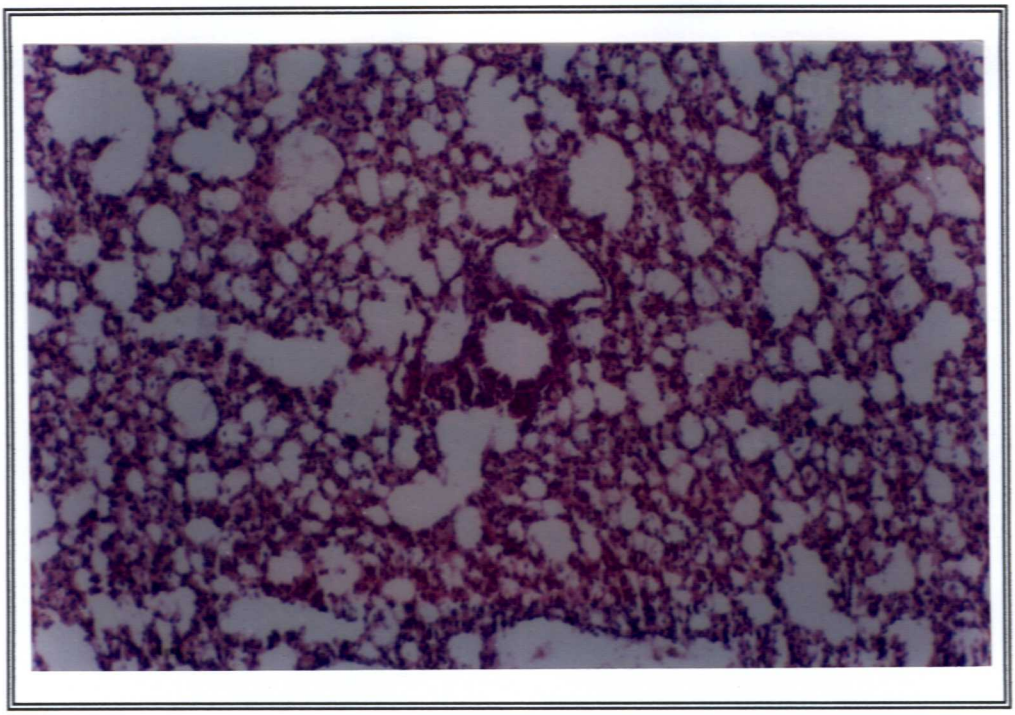

PLATE 7. Showing Cross section of Lung of normal mouse (control) (Hematoxylin- Eosin) 200x

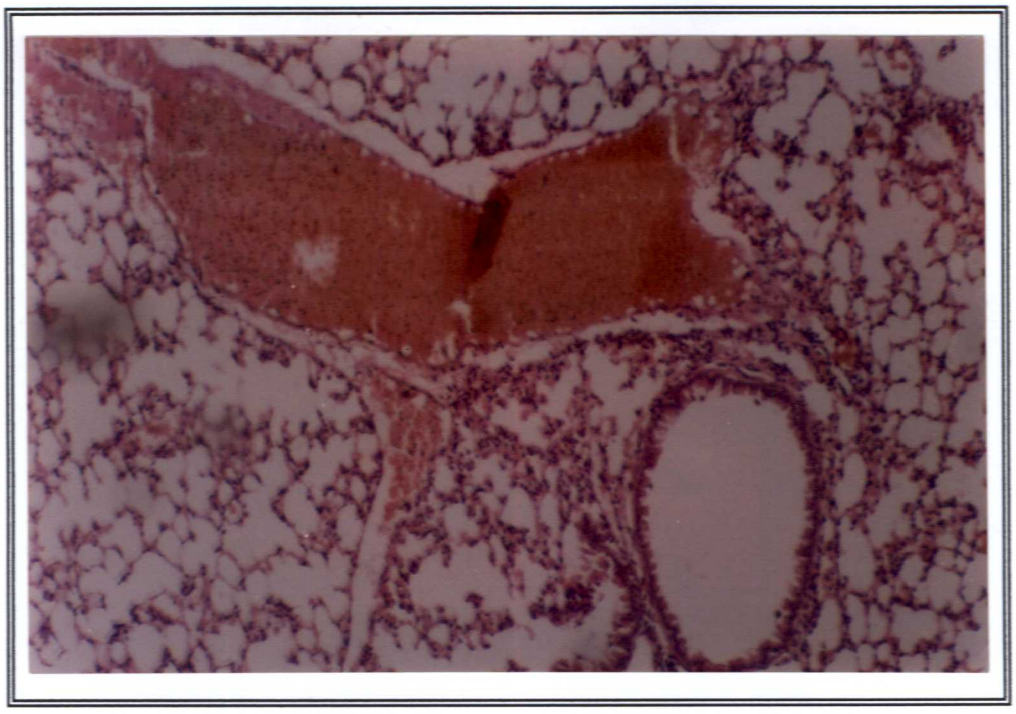

PLATE 8. Showing cross section of Lung of mouse envenomed with crude mucus toxin of *Ompok bimaculatus* (Hematoxylin- Eosin) 200x

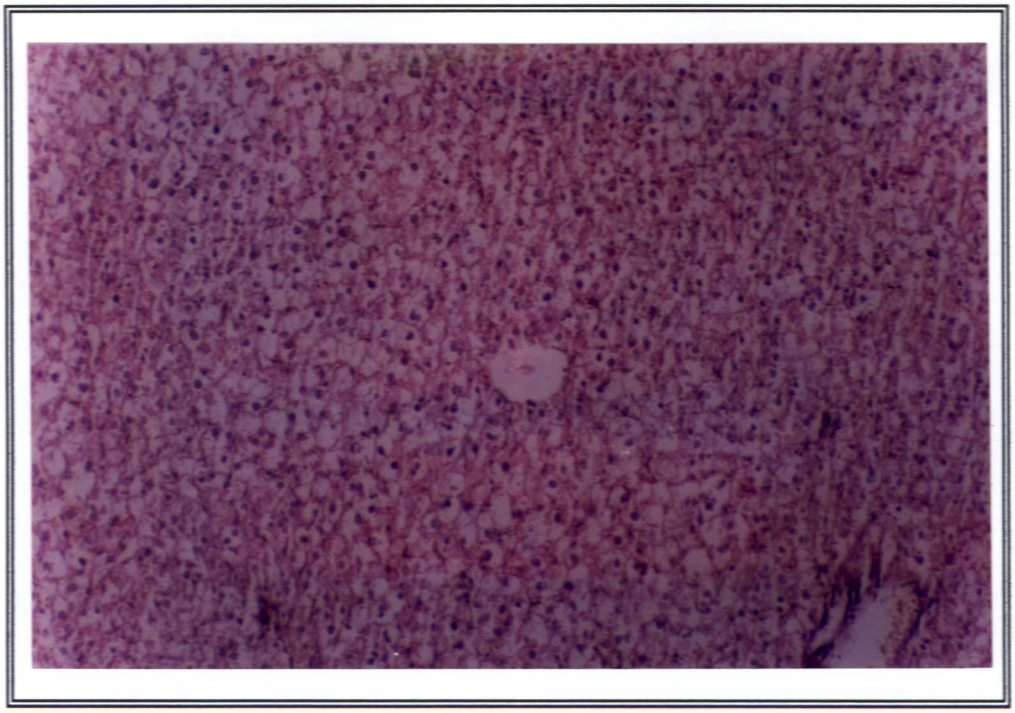

PLATE 9. Showing Cross section of Liver of normal mouse (control) (Hematoxylin- Eosin) 100x

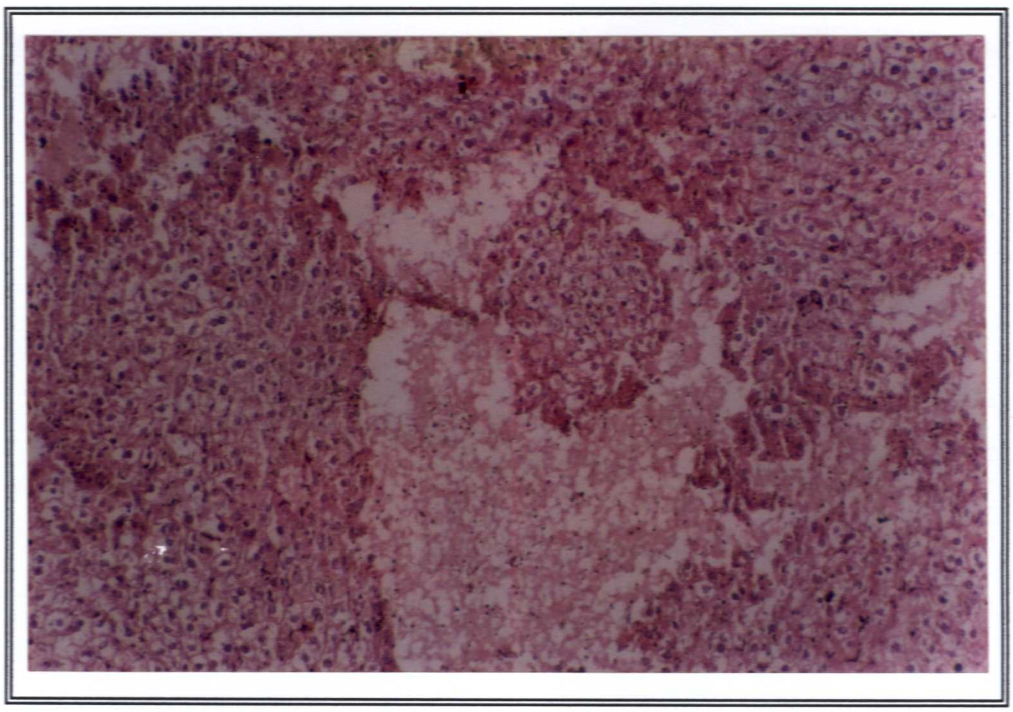

PLATE 10. Showing cross section of Liver of mouse envenomed with crude mucus toxin of *Ompok bimaculatus* (Hematoxylin- Eosin) 100x

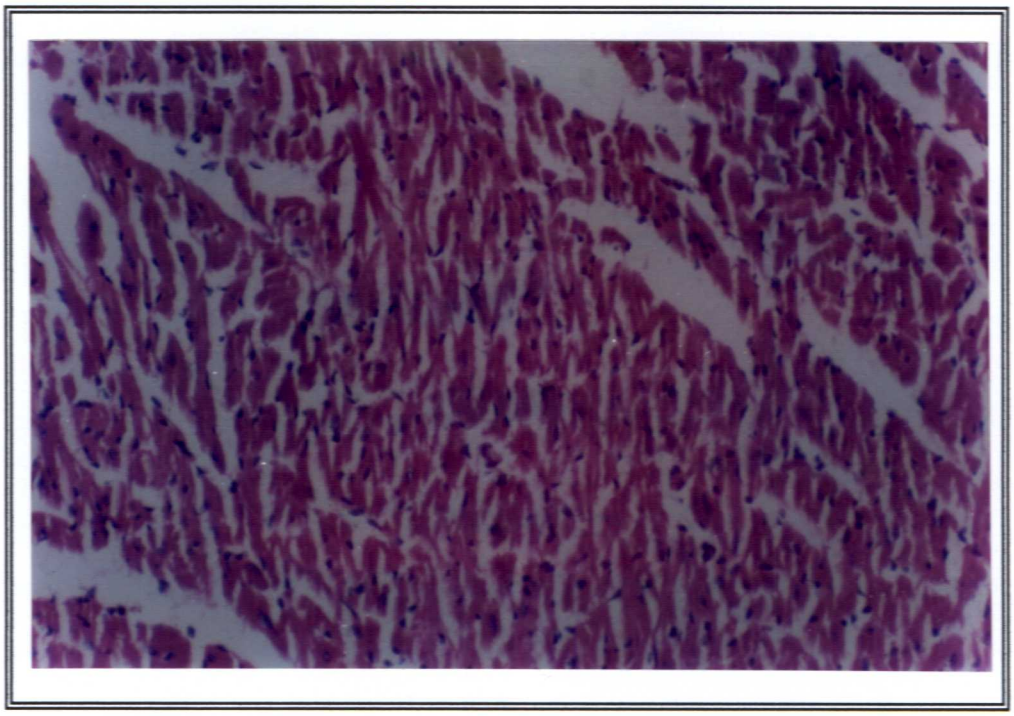

PLATE 11. Showing Cross section of Heart of normal mouse (control) (Hematoxylin- Eosin) 200x

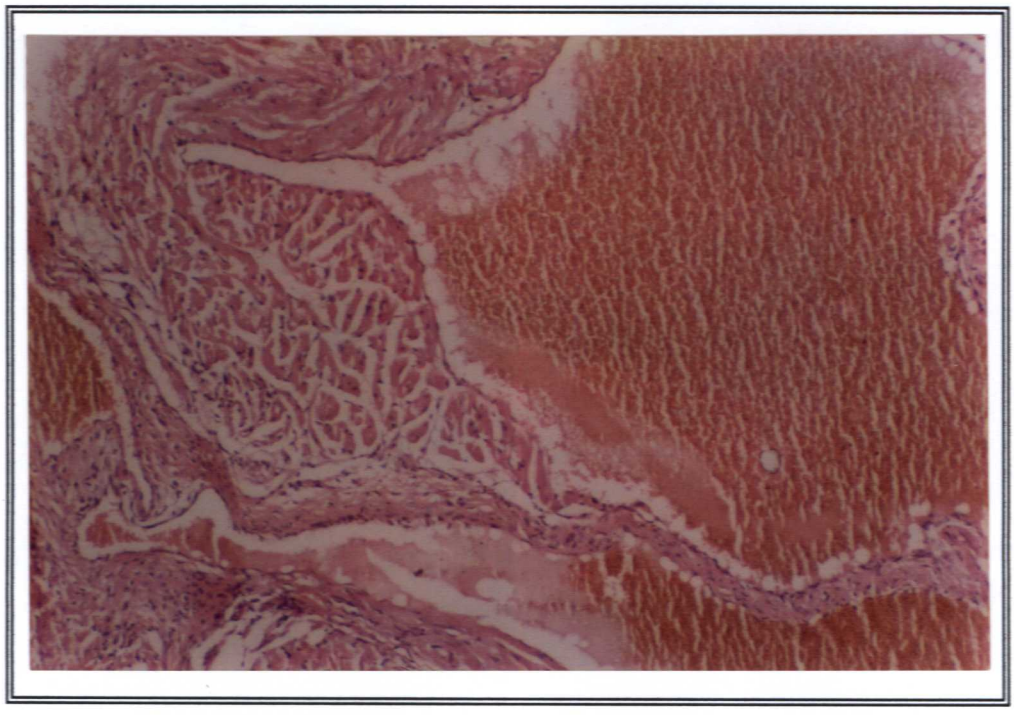

PLATE 12. Showing cross section of Heart of mouse envenomed with crude mucus toxin of *Ompok bimaculatus* (Hematoxylin- Eosin) 100x

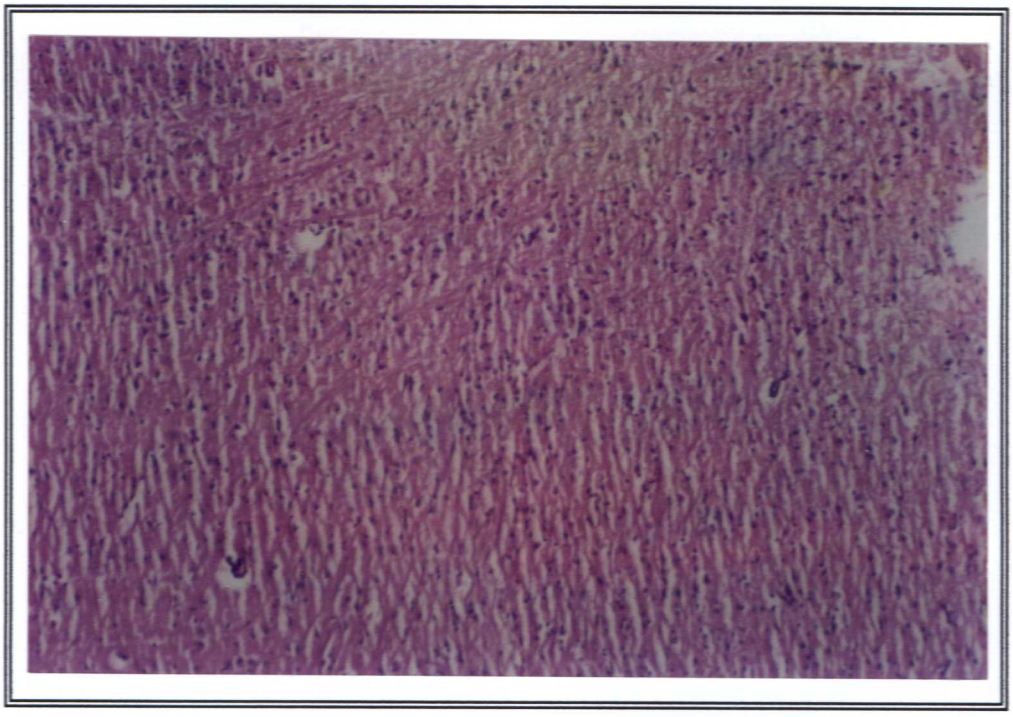

PLATE 13. Showing Cross section of Brain of normal mouse (control) (Hematoxylin- Eosin) 100x

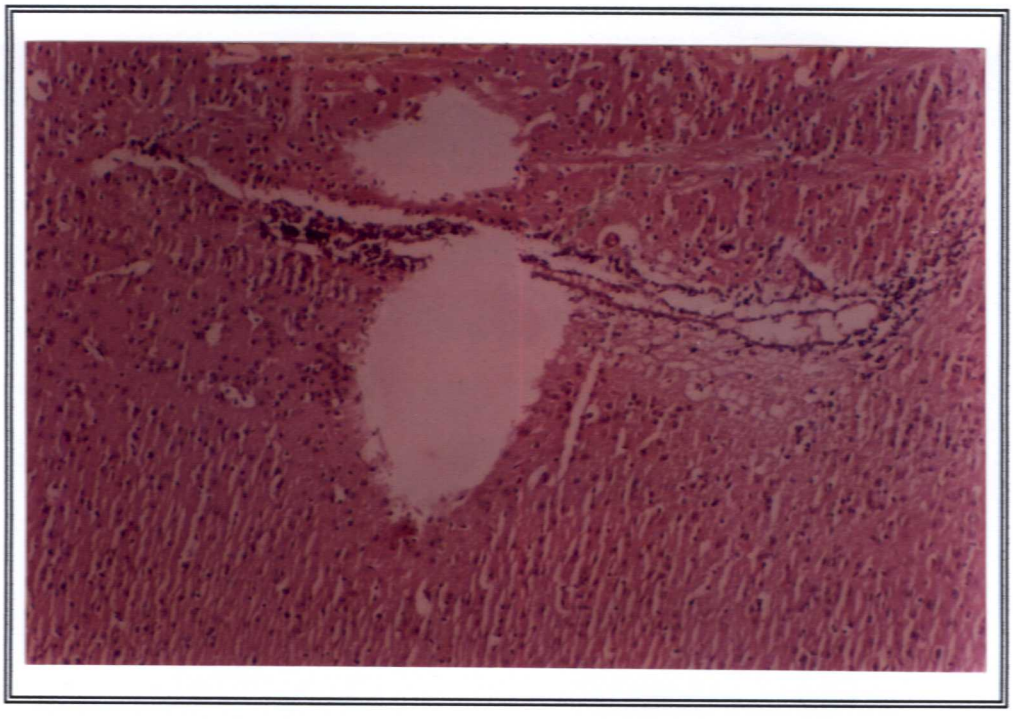

PLATE 14. Showing cross section of Brain of mouse envenomed with crude mucus toxin of *Mystus vittatus* (Hematoxylin- Eosin) 100x

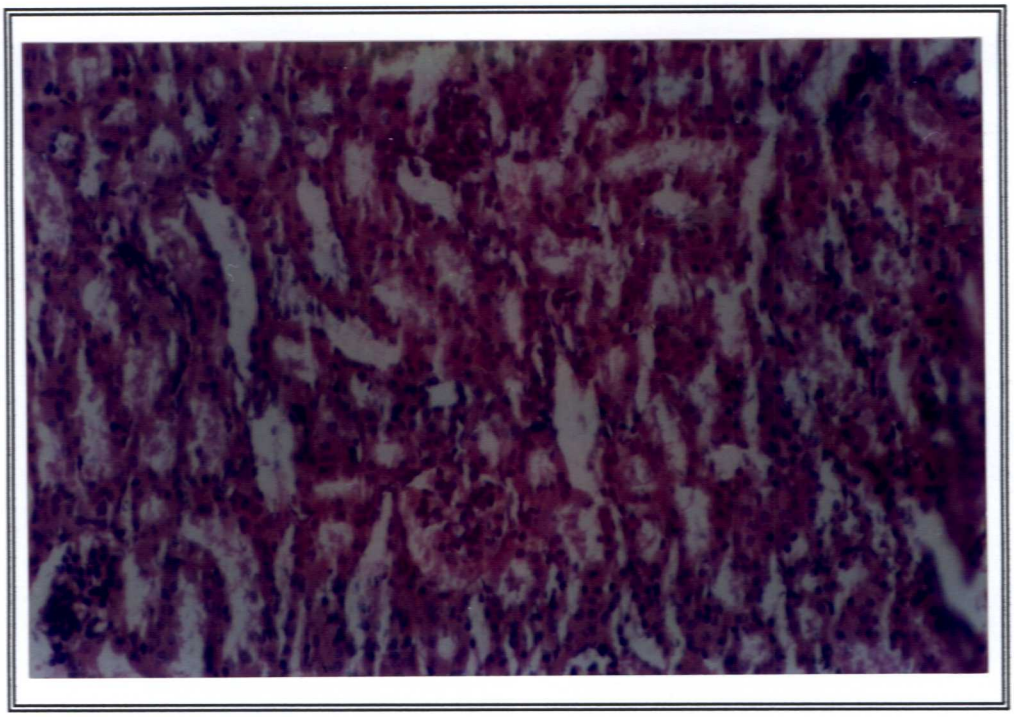

PLATE 15. Showing Cross section of kidney of normal mouse (control) (Hematoxylin- Eosin) 200x

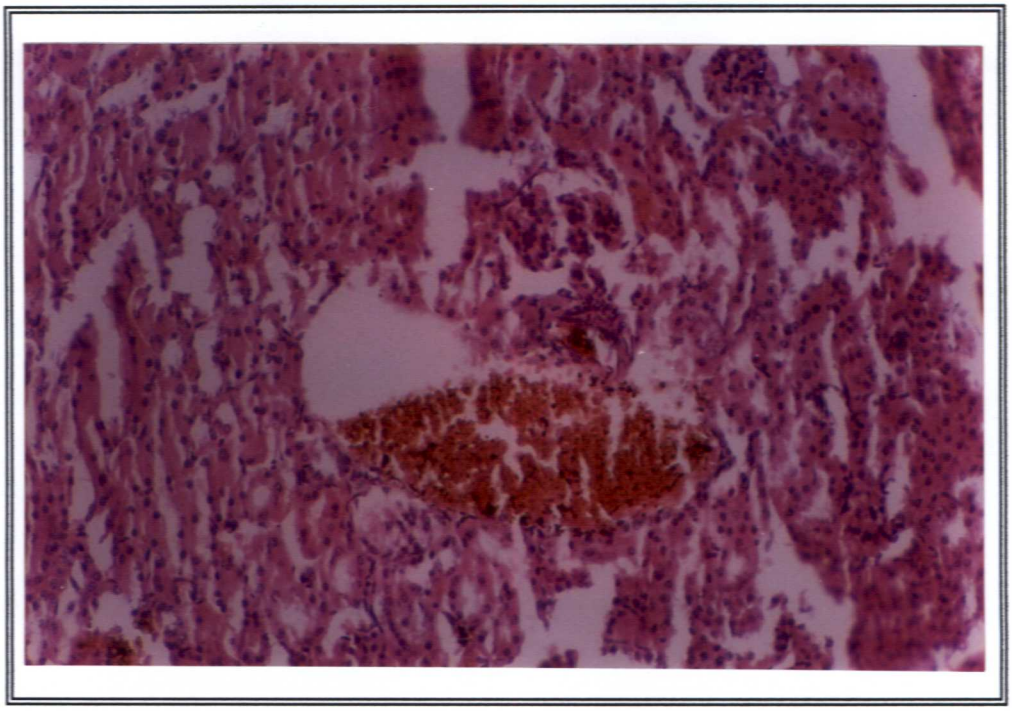

PLATE 16. Showing cross section of kidney of mouse envenomed with crude mucus toxin of *Mystus vittatus* (Hematoxylin- Eosin) 200x

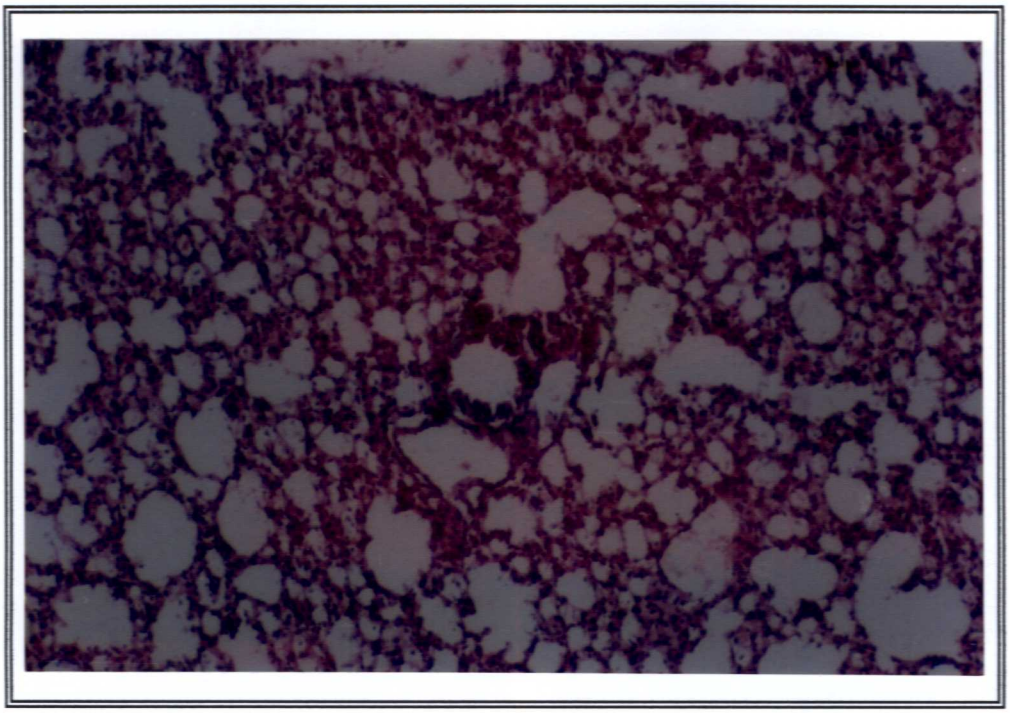

PLATE 17. Showing Cross section of Lung of normal mouse (control) (Hematoxylin- Eosin) 200x

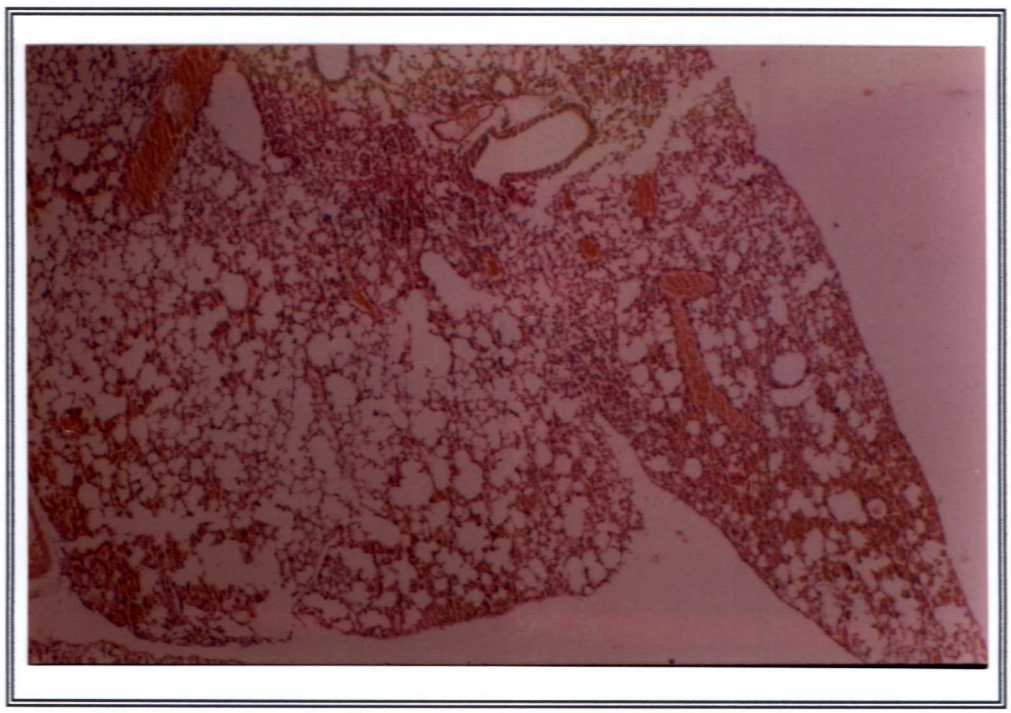

PLATE 18. Showing cross section of Lung of mouse envenomed with crude mucus toxin of *Mystus vittatus* (Hematoxylin- Eosin) 100x

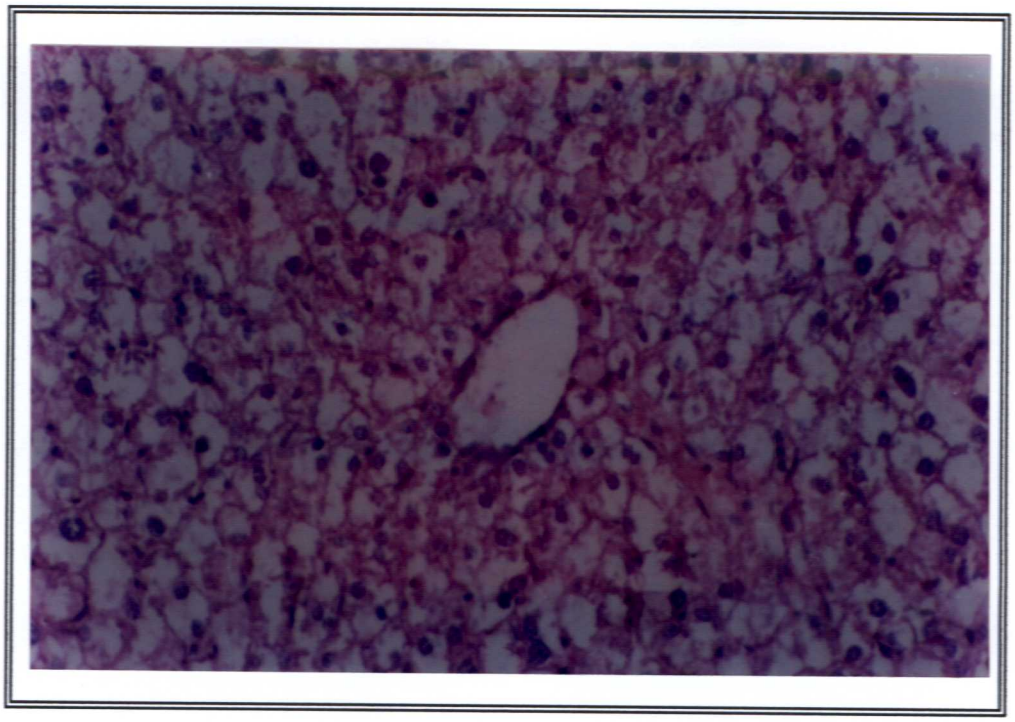

PLATE 19. Showing Cross section of Liver of normal mouse (control) (Hematoxylin- Eosin) 200x

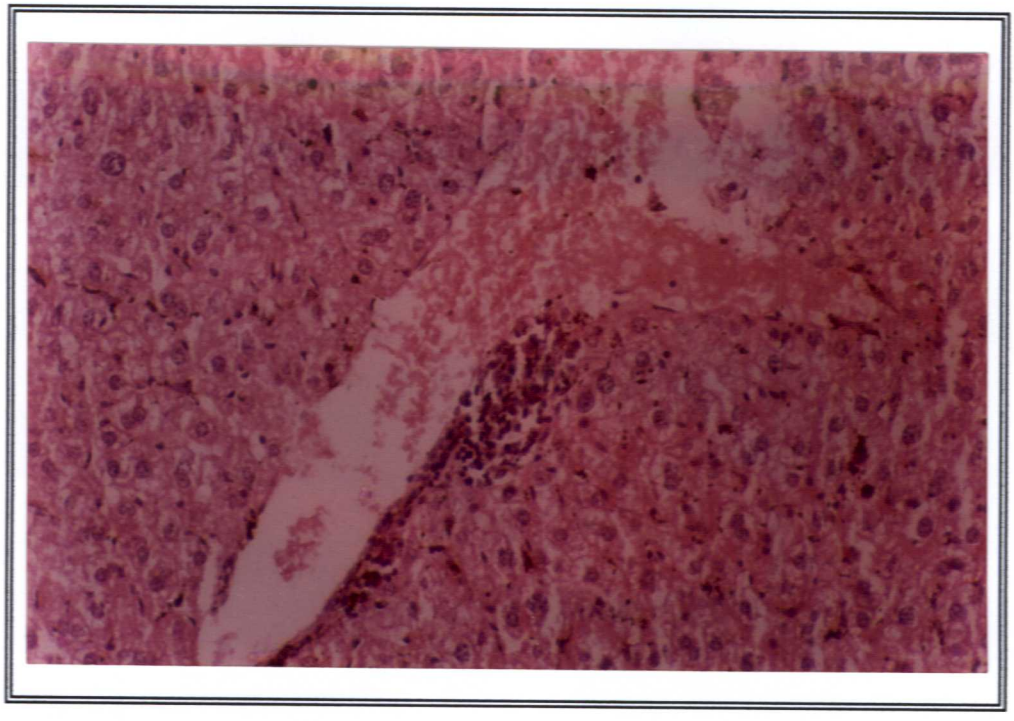

PLATE 20. Showing cross section of Liver of mouse envenomed with crude mucus toxin of *Mystus vittatus* (Hematoxylin- Eosin) 200x

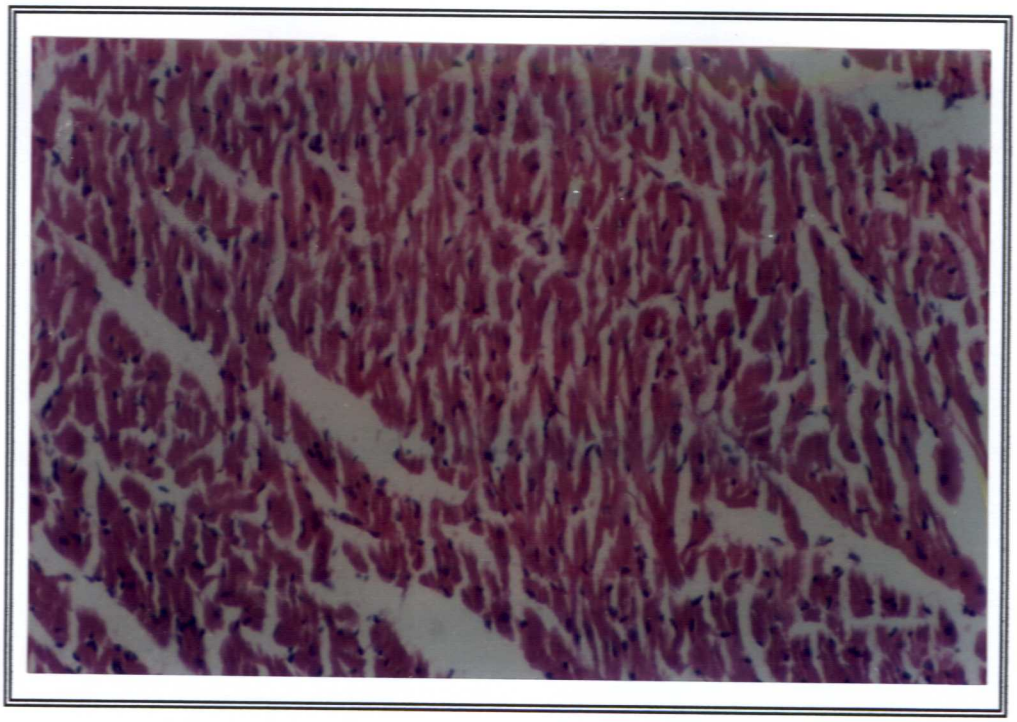

PLATE 21. Showing Cross section of Heart of normal mouse (control) (Hematoxylin- Eosin) 200x

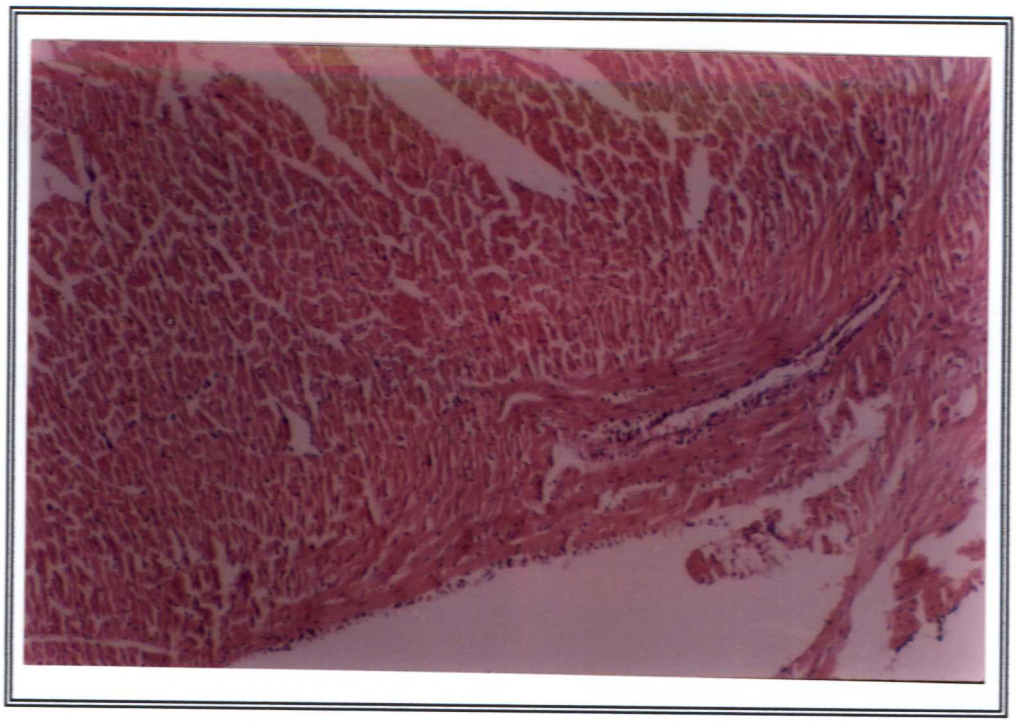

PLATE 22. Showing cross section of Heart of mouse envenomed with crude mucus toxin of *Mystus vittatus* (Hematoxylin- Eosin) 100x

TABLE 6

Hemolytic activity of crude mucus extract of *Ompok bimaculatus* and *Mystus vittatus.*

Species	Blood	Protein (mg)	Total hemolysis upto dilution	Hemolytic titre	Specific hemolytic activity HT/ mg
Ompok bimaculatus	Chicken	1.76	3	8	4.54
Mystus vittatus.	Chicken	1.94	4	16	8.25

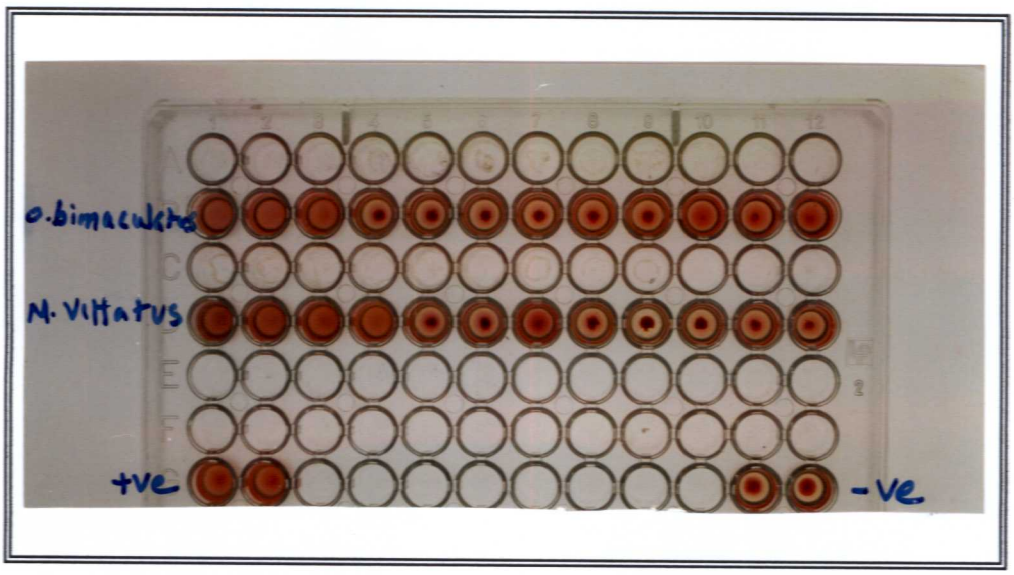

PLATE 23. Showing the hemolytic activity of crude mucus toxin of *Ompok bimaculatus* and *Mystus vittatus* on chicken erythrocytes

4.6 Analgesic activity

4.6.1 *Ompok bimaculatus* crude toxin

The crude mucus toxin exhibited analgesic activity against control with AR values between 1.05 to 1.32 but at lesser extent compared to paracetamol whose AR values varied between 0.44 to 0.59. The results are presented in Table 7.

4.6.2 *Mystus vittatus* crude toxin

The crude mucus toxin exhibited analgesic activity against control with AR values between 1.21 to 1.65 but at lesser extent compared to paracetamol whose AR values varied between 0.45 to 0.84. The results are presented in Table 8.

4.7 Antibacterial activity

4.7.1 *Ompok bimaculatus* crude toxin

Out of the 8 bacterial species tested, *Aeromonas hydrophila, Vibrio anguillarum, Vibrio parahaemolyticus*, were sensitive to crude mucus extract of *Ompok bimacultus* and remaining were resistant. *Aeromonas hydrophila* and *Vibrio anguillarum* were the most sensitive to both 5 mg and 10 mg toxin concentration, and the *Vibrio anguillarum* has shown maximum sensitivity with a zone of inhibition 13 mm at 10 mg concentration. Among fractions the 0.4M fraction had shown the sensitivity reaction with 5 mm inhibition zone against *Aeromonas hydrophila.* The results are presented in Tables 9 and 11, Plates 24, 25, 26 and 30.

4.7.2 *Mystus vittatus* crude toxin

Out of the 8 bacterial species tested, 3 were sensitive to crude mucus extract of *Mystus vittatus* namely *Aeromonas hydrophila, Pseudomonas flourescens, Vibrio parahaemolyticus,* and remaining were resistant. Among the three sensitive bacterial species, *Aeromonas hydrophila* showed highest sensitivity in both 5 mg and 10 mg toxin concentration. Among the fractions the 0.2M fraction had shown sensitivity against *Aeromonas hydrophila* with 6 mm inhibition zone, and 1M fraction also sensitive to the above bacteria with 9 mm inhibition zone. The results are given in Tables 10 and 11, Plates 27, 28, 29 and 31.

TABLE 7

Analgesic activity of crude mucus extract of *Ompok bimaculatus*

Sl no	Light intensity (AMP)	Reaction time of the mice injected with			Ratio of C and A	Ratio of C and B
		A	B	C		
1	3	66.2	126.1	74.5	1.13	0.59
2	3.5	51.2	110.8	53.8	1.05	0.49
3	4	37.4	98.6	43.4	1.16	0.44
4	4.5	27.8	82.4	36.6	1.32	0.45

A : Phosphate buffer saline (PBS)
B : Paracetamol
C : Mucus toxin

TABLE 8

Analgesic activity of crude mucus extract of *Mystus vittatus.*

Sl no	Light intensity (AMP)	Reaction time of the mice injected with			Ratio of Cand A	Ratio of C and B
		A	B	C		
1	3	66.2	126.1	106	1.60	0.84
2	3.5	51.2	110.8	84.3	1.65	0.76
3	4	37.4	98.6	45.2	1.21	0.46
4	4.5	27.8	82.4	37.7	1.36	0.45

A : Phosphate buffer saline (PBS)
B : Paracetamol
C : Mucus toxin

TABLE 9

Antibacterial activity of crude mucus extract of *Ompok bimaculatus.*

Sl no	Bacteria	Diameter of inhibition zone (mm)		Remarks
		a	b	
1	*Aeromonas hydrophila*	8	9	Sensitive
2	*Pseudomonas flourescens*	nil	nil	Resistant
3	*Vibrio anguillarum*	5	13	Sensitive
4	*Vibrio parahaemolyticus*	nil	6	Sensitive
5	*Escherichia coli*	nil	nil	Resistant
6	*Staphylococcus aureus*	nil	nil	Resistant
7	*Salmonella typhi*	nil	nil	Resistant
8	*Klebsiella pneumoniae*	nil	nil	Resistant

a : 5 mg/ml toxin concentration
b : 10 mg/ml toxin concentration

TABLE 10

Antibacterial activity of crude mucus extract of *Mystus vittatus.*

Sl no	Bacteria	Diameter of inhibition zone (mm)		Remarks
		1	2	
1	*Aeromonas hydrophila*	7	10	Sensitive
2	*Pseudomonas flourescens*	nil	9	Sensitive
3	*Vibrio anguillarum*	nil	nil	Resistant
4	*Vibrio parahaemolyticus*	nil	6	Sensitive
5	*Escherichia coli*	nil	nil	Resistant
6	*Staphylococcus aureus*	nil	nil	Resistant
7	*Salmonella typhi*	nil	nil	Resistant
8	*Klebsiella pneumoniae*	nil	nil	Resistant

1: 5 mg/ml toxin concentration
2: 10 mg/ml toxin concentration

TABLE 11

Antibacterial activity of crude mucus extract fractions of *Ompok bimaculatus* and *Mystus vittatus*

Species	Fraction	Bacteria	Inhibition zone (mm)
Ompok bimaculatus	0.4M	*Aeromonas hydrophila*	5
Mystus vittatus	0.2M	*Aeromonas hydrophila*	6
	1M	*Aeromonas hydrophila*	9

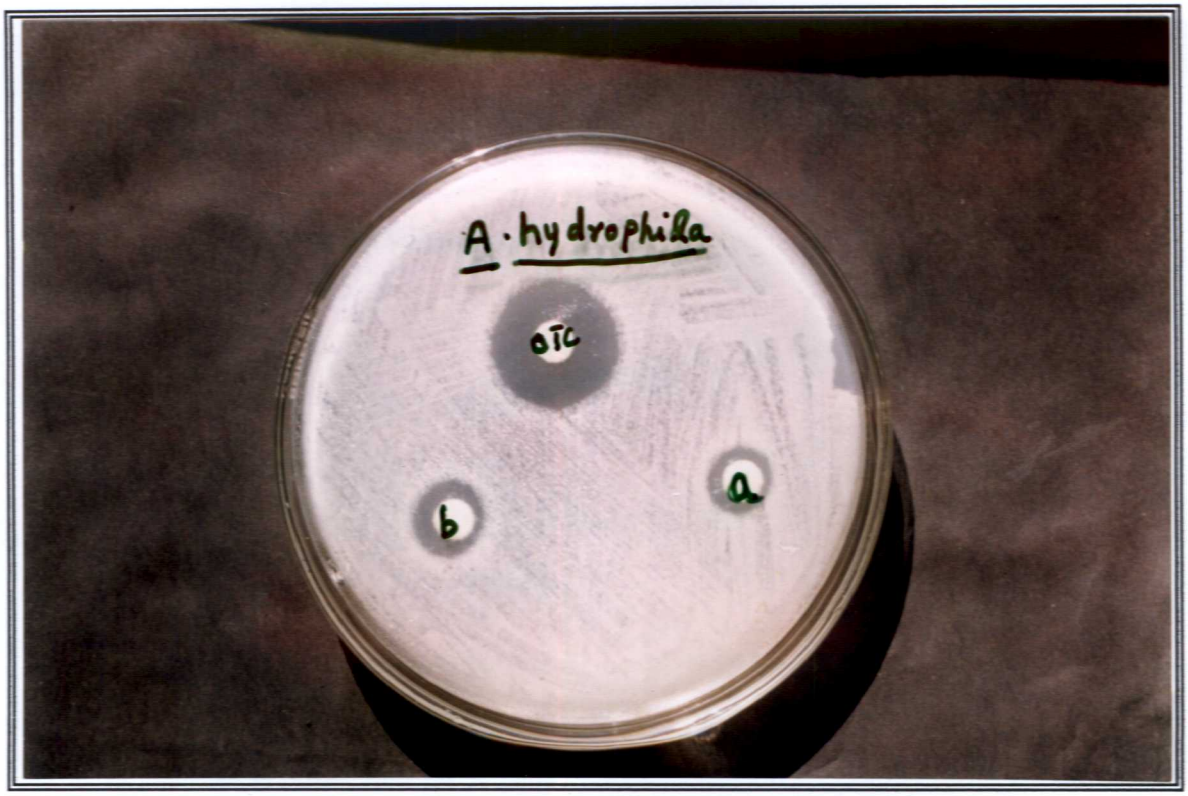

Plate 24. Crude mucus extract of *Ompok bimaculatus* showing inhibition zone against *Aeromonas hydrophila*

Plate 25. Crude mucus extract of *Ompok bimaculatus* showing inhibition zone against *Vibrio parahemolyticus*

Plate 26. Crude mucus extract of *Ompok bimaculatus* showing inhibition zone against *Vibrio anguillarum*

Plate 27. Crude mucus extract of *Mystus vittatus showing* inhibition zone against *Vibrio parahemolyticus*

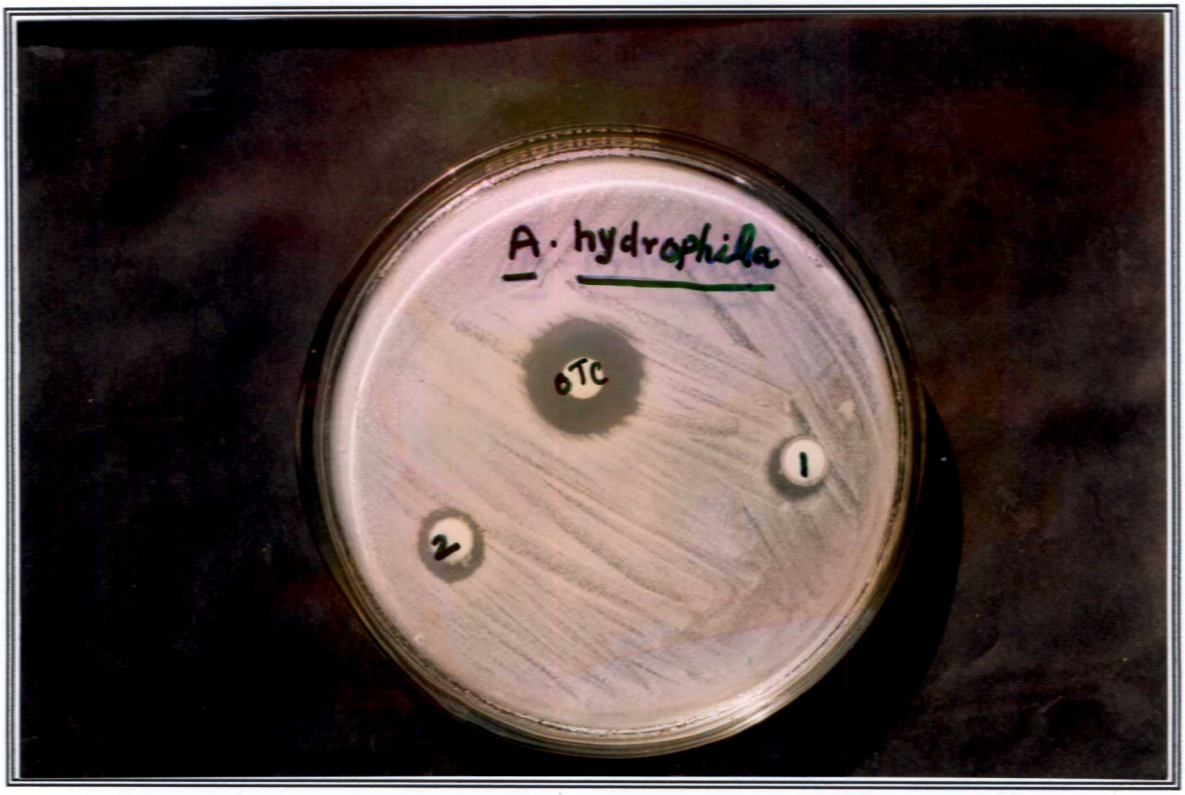

Plate 28. Crude mucus extract of *Mystus vittatus* showing inhibition zone against *Aeromonas hydrophila*

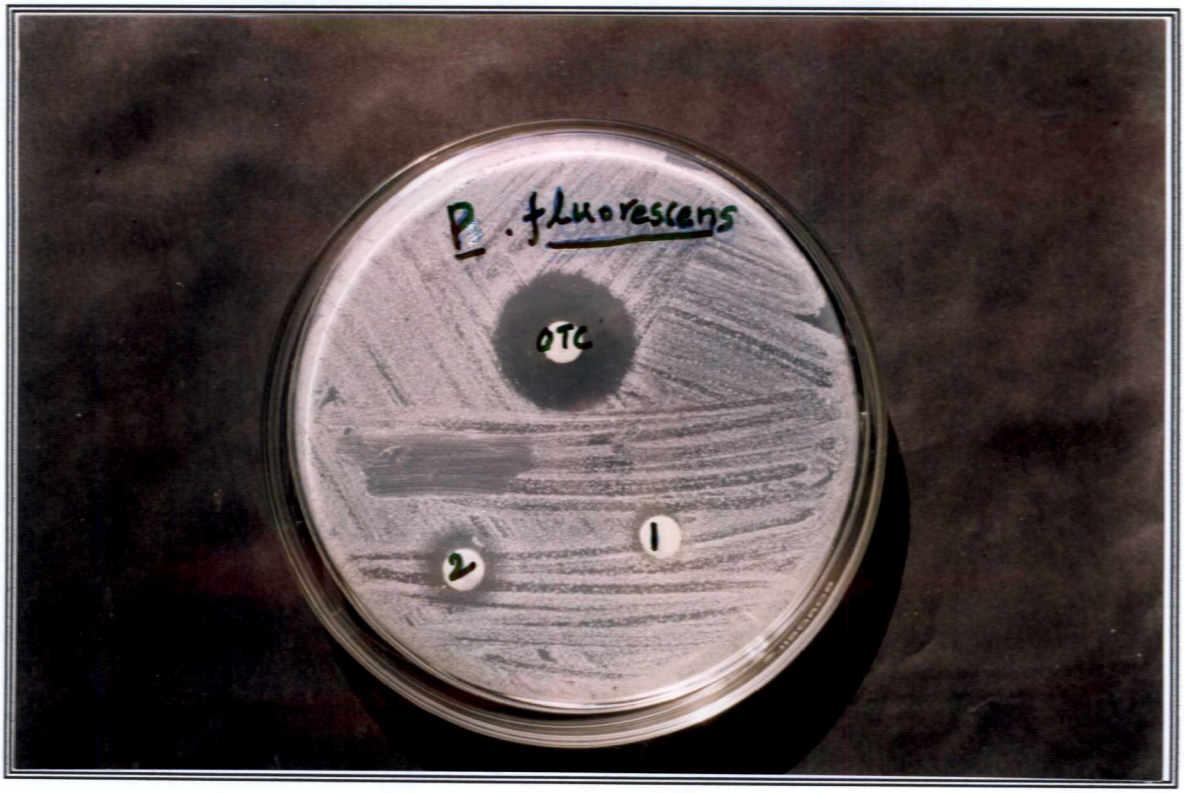

Plate 29. Crude mucus extract of *Mystus vittatus* showing inhibition zone against *Pseudomonas flourescens*

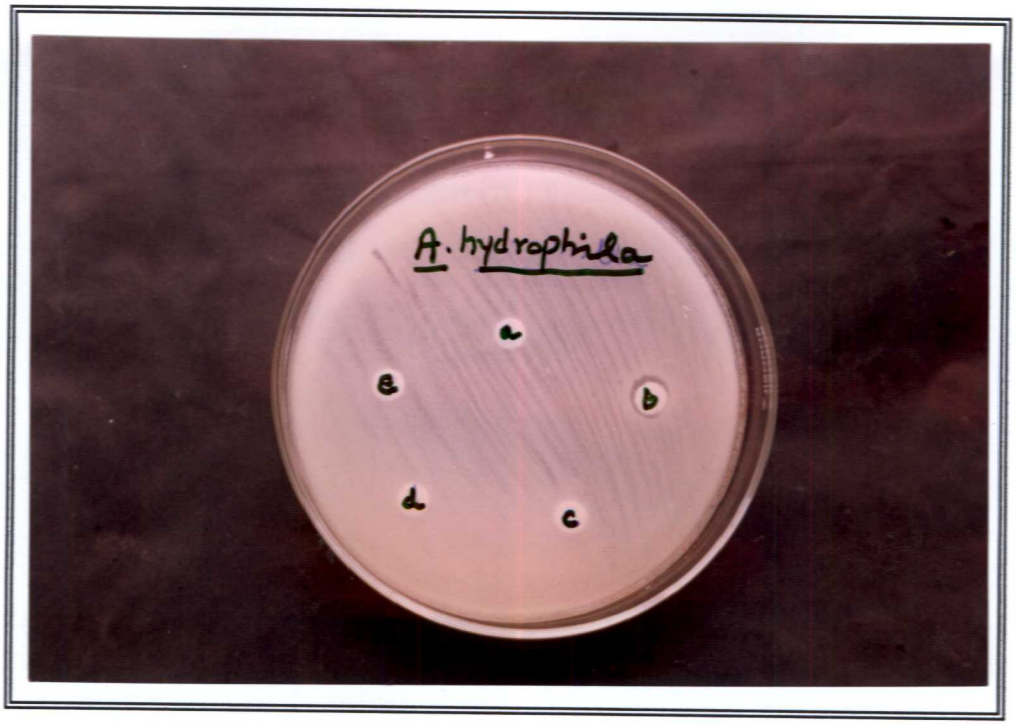

Plate 30. Purified mucus fractions of *Ompok bimaculatus* showing inhibition zone against *Aeromonas hydrophila*

Plate 31. Purified mucus fractions of *Mystus vittatus* showing inhibition zone against *Aeromonas hydrophila*

4.8 Cell line assay

As the calculated t value were greater than that of t table value at 95% level of confidence, the both the toxins exhibited significance changes in the tested the mouse muscular cell line. The results are given in Plates 32 to 39.

The mucus toxin of *Ompok bimaculatus* produced more cellular changes at various dilutions ($10^{-1}$to 10^{-7}) when compared to *Mystus vittatus*. The OD values of MTT cell viability test are shown in Table12 and Figure 1.

TABLE 12

MTT cell viability test on mice muscle cell line showing mean ± standard error

S.No	Toxin dilutions/ Control	*Ompok bimaculatus* mucus toxin on L929	*Mystus vittatus* mucus toxin on L929	Control
1.	10^{-1}	0.9611*±0.110	0.997*± 0.050	1.2538 ± 0.027
2.	10^{-2}	0.6966*±0.132	0.996*± 0.04	1.244 ± 0.022
3.	10^{-3}	0.7955*±0.168	1.006*± 0.088	1.256 ± 0.023
4.	10^{-4}	0.977*±0.067	1.012*± 0.078	1.3171 ± 0.025
5.	10^{-5}	1.039*±0.117	1.016*± 0.07	1.2552 ± 0.034
6.	10^{-6}	1.064*± 0.031	1.060*± 0.039	1.2465± 0.028
7.	10^{-7}	0.961*± 0.113	1.062* ± 0.04	2553 ± 0.021

* Denotes significant difference between control and treatment group ($P < 0.05$)

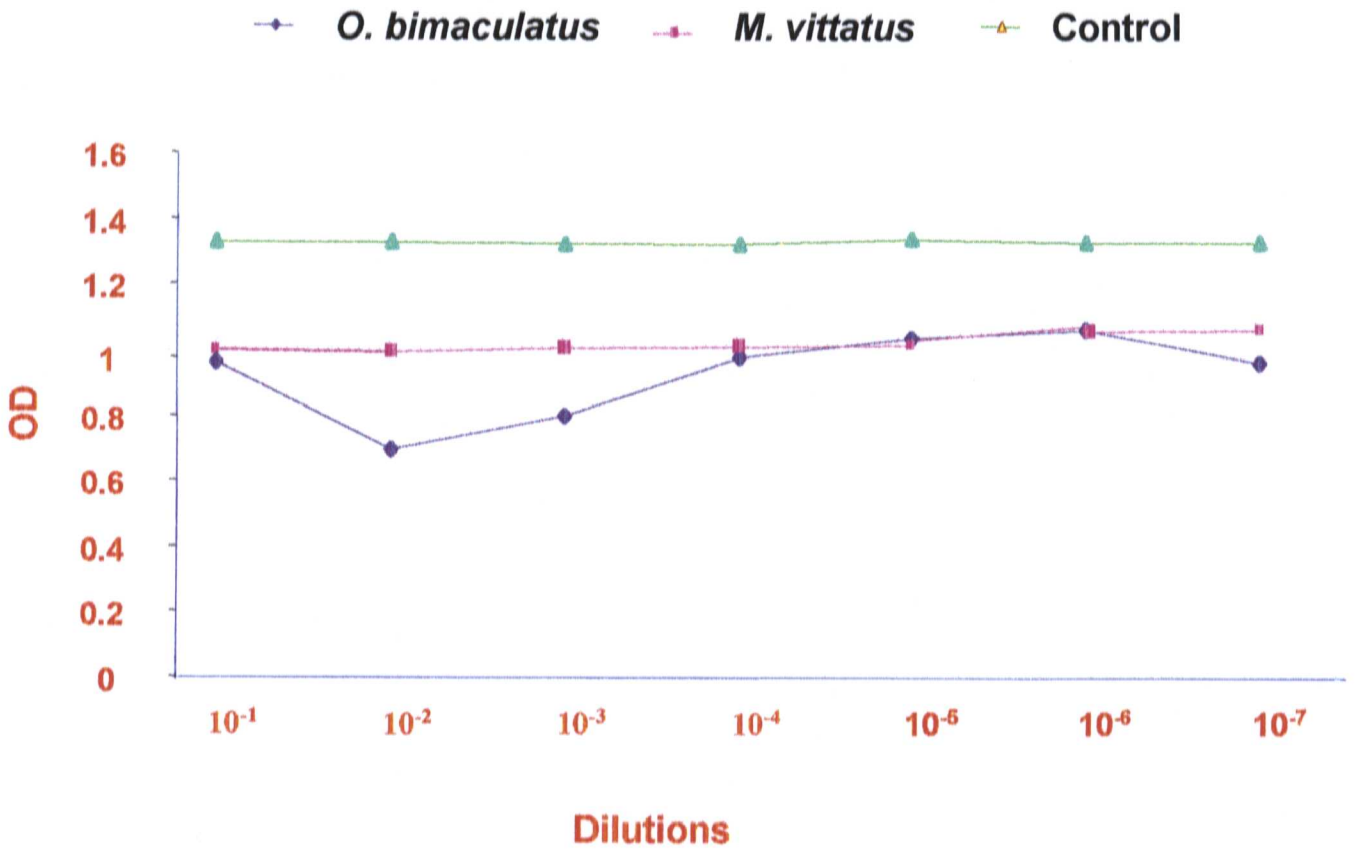

Fig. 1 Effect of Mucus Toxins on mice muscle cell line

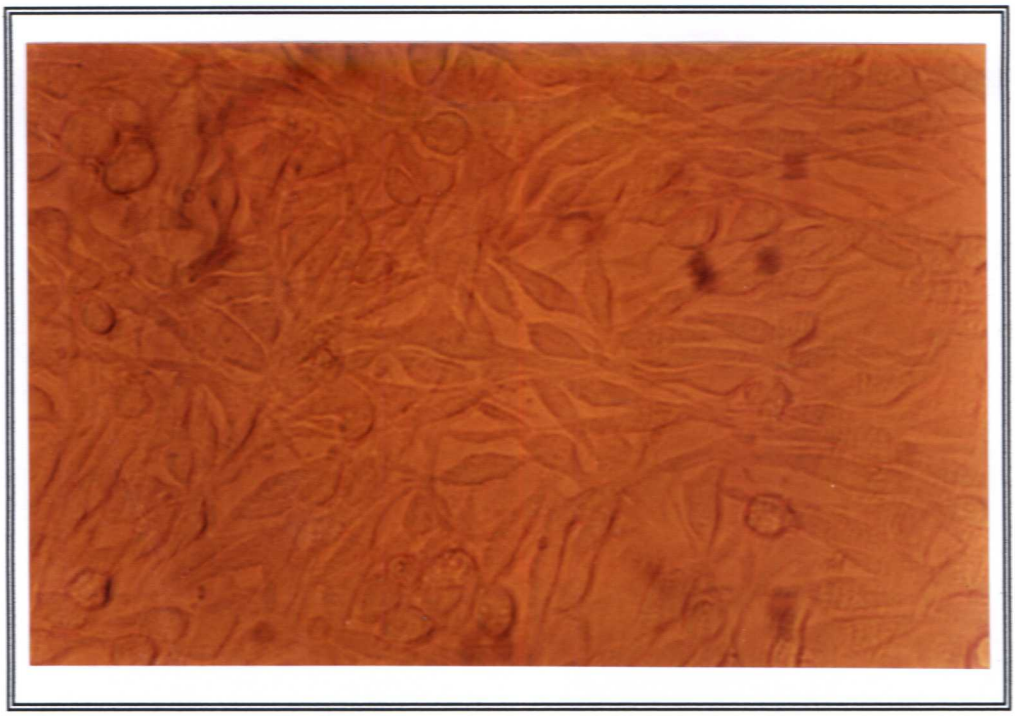

Plate 32. Showing control mouse muscle cell line (L929)

Plate 33. Showing crude mucus toxin treated cell line (L929) of *Ompok bimaculatus* (10^{-2} dilution) 200x

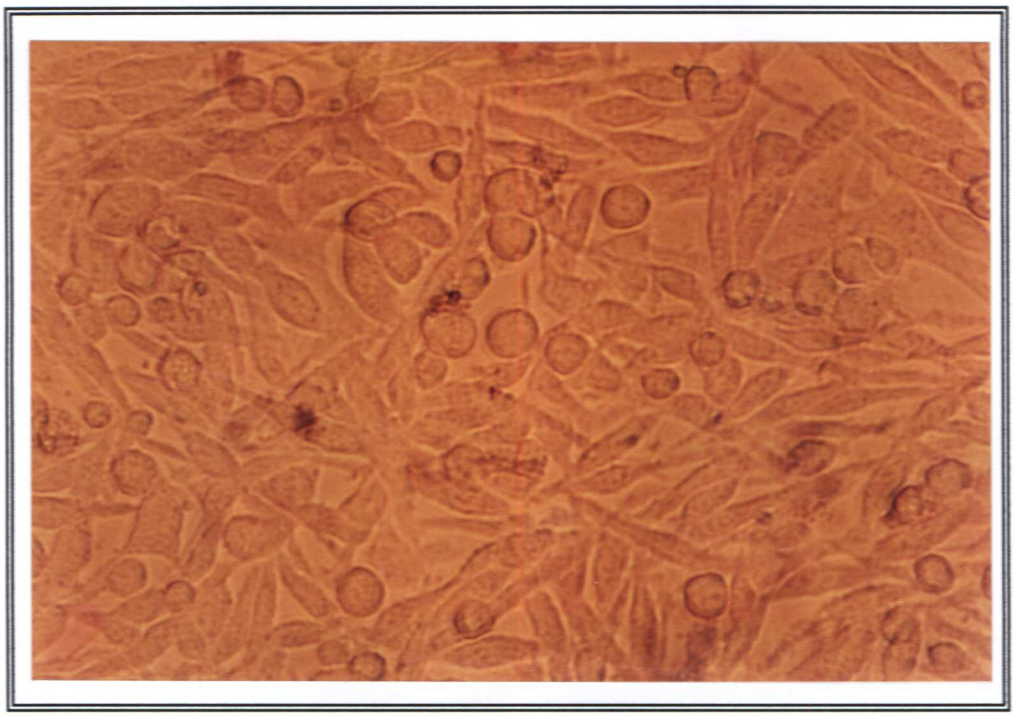

Plate 34. Showing crude mucus toxin treated cell line (L929) of *Ompok bimaculatus* (10^{-4} dilution) 200x

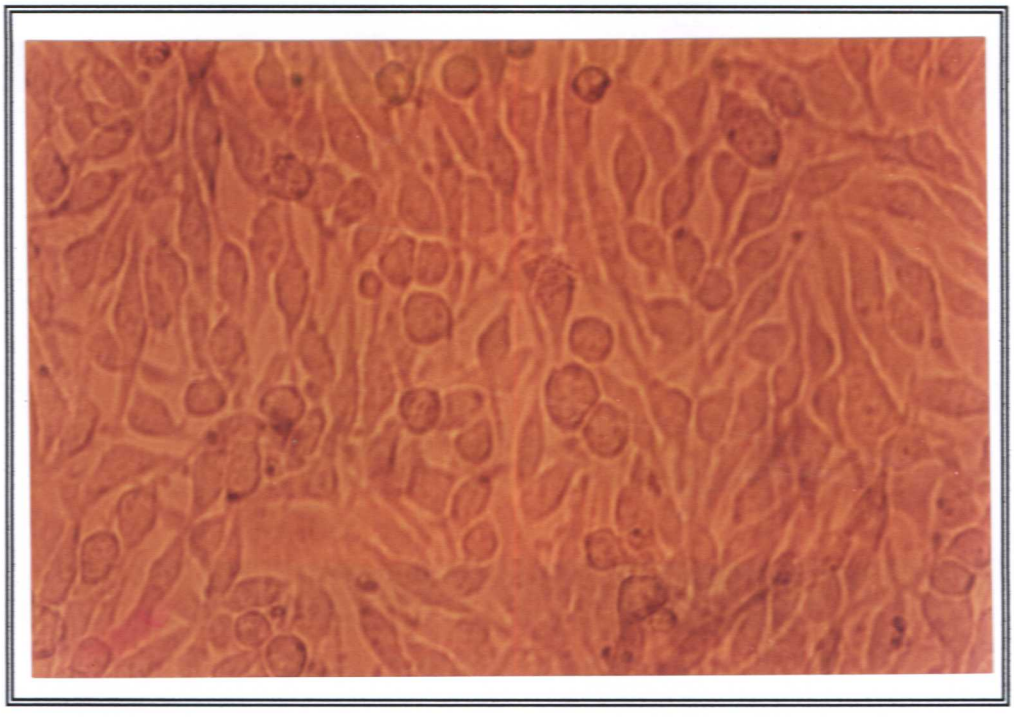

Plate 35. Showing crude mucus toxin treated cell line (L929) of *Ompok bimaculatus* (10^{-6} dilution) 200x

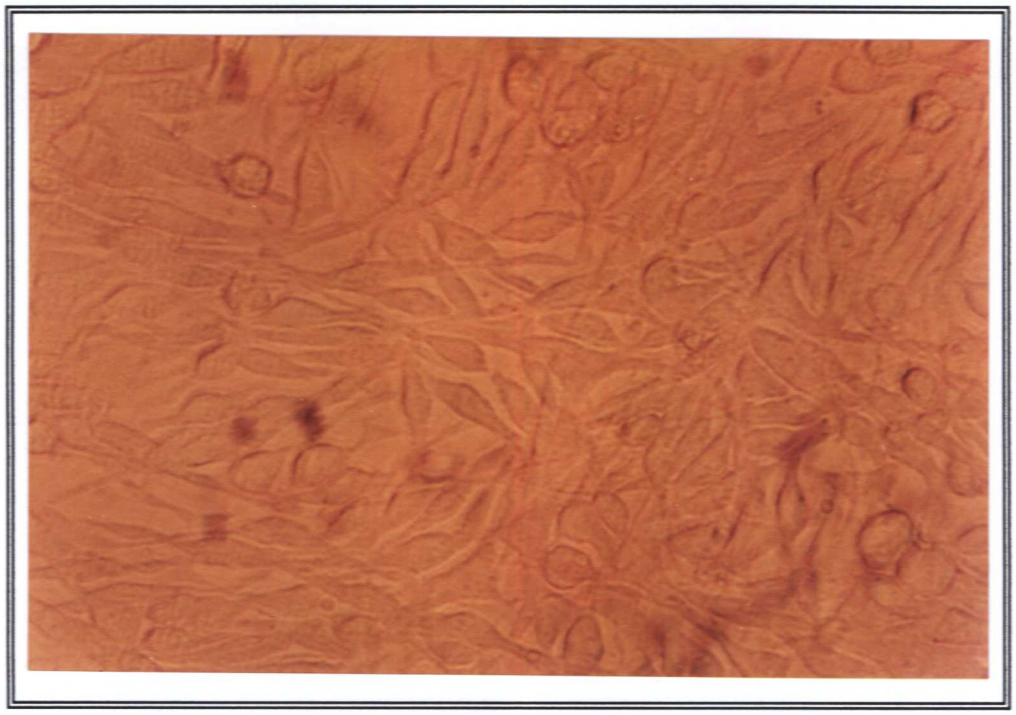

Plate 36. Showing control mouse muscle cell line (L929)

Plate 37. Showing crude mucus toxin treated cell line (L929) of *Mystus vittatus* (10^{-1} dilution) 200x

Plate 38. Showing crude mucus toxin treated cell line (L929) of *Mystus vittatus* (10^{-3} dilution) 200x

Plate 39. Showing crude mucus toxin treated cell line (L929) of *Mystus vittatus* (10^{-5} dilution) 200x

Discussion

5. DISCUSSION

5.1 Crinotoxicity

The crinotoxic nature of the epidermal secretions of *Ompok bimaculatus* and *Mystus vittatus* are clearly evident from the present study. The crude mucus extract of *Mystus vittatus* was found to be lethal to mice and a dosage of 0.4 ml was able to kill the mice in 3 minutes and 40 seconds and for *Ompok bimaculatus* it was 0.6 ml and the time taken was 3 minutes and 55 seconds. At a dose of 1 ml the animals showed pronounced symptoms like jumping, escaping tendency, severe paralysis and death. The mice when injected with lower doses showed stress symptoms, hyperactivity, and then collapsed. Similar behavior of mice to mucus toxin of catfishes has been reported earlier by Vidya (1997), Reddy (2001) and Samantaray(2001). The crude mucus toxin extracts from *Boleophthalmus dentatus* (Absuma, 1997) and *Heteropneustes fossilis* (Deo, 1995) have been reported earlier to be lethal to mice (20 ± 2 g body weight) at levels of 1 ml, and the mice died in 2 hours. Similar toxicity of catfish mucus secretions have been reported by various authors (Shiomi *et al.,* 1986; Shiomi *et al.*, 1988 and Alnaqeeb *et al.*, 1989). But the result differs in case of Ravi (1999), where the skin extract of *Heteropneustes fossilis* was not lethal to the mice of 20 ± 2 grams at levels of 1 ml. The method of extraction also differs in case of Bhuyan (1998) where he used diethyl ether for the extraction where methanol was used in the present study and also the quantity of toxic factor was not measured in the study of Absuma (1997) since lyophilisation was not done.

The observed symptoms indicate that the crude mucus extract of the epidermal secretions of *Mystus vittatus* and *Ompok bimaculatus* affected the central nervous system, cardio vascular system, urinary system and also the muscular co-ordination. Mucus of Arabian Gulf catfish, *Arius thalassinus* caused respiratory distress, agitated behavior and increased heart rate in rabbits envenomated intraperitoneally. Shiomi *et al.*, (1988) reported agitated behavior and increased heart rate in mice injected with a lethal dose of crude extract of *Plotosus lineatus.* Alnaqeeb *et al.*, (1989) observed an affect on lungs and liver tissues of rabbit injected with mucus extract of *Arius bilineatus.* Thompson (1964) reported ataxia, labored

breathing, coma and death in mice injected with Ostracitoxin. Dragging of hind limbs, sluggishness, lethargy and apathy in mice injected with crude mucus extract of *Arius caelatus, A. dussmeieri and Osteogeneiosus militaris* had been reported by Vidya (1997). Absuma (1997) observed adverse effects on the cardiovascular system, central nervous system and respiratory system of mice injected with crude mucus extract of *Boleophthalmus dentatus*, based on gross anatomical and histopathological changes.

5.2 Histopathological Changes

Autopsy revealed dark discolouration of heart, liver and kidney in the mice envenomated with the crude mucus extract of *Ompok bimaculatus* and *Mystus vittatus.* Accumulation of fluid in the visceral cavity was the other symptom noticed. In liver the entire central vein contained hemolised blood, completely blocking of lumen or hepatic cells near the capsules and showed pyknotic nuclei Histopathological observations revealed extensive cell damage and necrosis due to membrane breakdown in case of kidney and liver. This proves that the mucus toxin of *Ompok bimaculatus* and *Mystus vittatus* has got a toxic effect on liver and kidney. Chan *et al.,* (1985) observed similar membrane breakdown to be caused by mucus toxins of Ictalurid and Ariid catfishes. Weissman (1965) postulated that the toxin may cause the release of acid hydrolases from the lysosomes, which may lead to the disruption of all biological membranes. The present work indicates that a similar activity could have been caused by the toxic components in the mucus extract under study.

5.3 Hemolytic Activity

The crude extract of *Mystus vittatus* and *Ompok bimaculatus* exhibited hemolytic activity, this is however at variance from Baskara Doss (1999) in *Mystus gulio,* according to whom no such activity was discernible. Hemolytic activity has been exhibited by the mucus of many other fishes are reported by Hashimoto *et al.*, (1976) in *Gobiodon*, Thomson (1964) in Hawaiian boxfish *Ostracion meleagris*, Bhimachar (1944) in the fresh water catfish *Heteropneustes fossilis* and Shiomi (1986) in *Plotosus lineatus*.Primor and Zlotkin (1975) assumed that the hemolytic action was related to and involved in the ichthyotoxicity of the epidermal secretion of

Pardachirus marmoratus. Results similar to those of the present study were obtained by Reddy (2001) and Samantaray (2001) from freshwater cat fishes.

The present results indicate that the proteins might be responsible for the hemolytic activities. In soapfishes, the ichthyotoxicity was supposed to be a tertiary or quarternary amine which demonstrated a strong hemolytic action (Hashimoto and Oshima, 1972). In case of mucus extract of the flatfish *Pardachirus marmoratus*, the same protein factors were responsible for hemolytic and ichthyotoxic action.

5.4 Analgesic Activity

The analgesic activity observed in the mucus of both the fishes in the current study may be due to the selective modulation of the neuronal nicotinic receptors in the spinal cord and brain (Marwick, 1998). It is also possible that the toxin might have blocked the calcium channels responsible for synaptic transmissions that link pain sensitive nerve cells and pain transmitting nerve cells (Marwick, 1998).

5.5 Antibacterial Assay

The results of the antibacterial assay clearly indicate the biomedical potential of the epidermal secretions of *Ompok bimacultus* and *Mystus vittatus* in that they inhibited 4 bacterial species, which are potential pathogens to fish. Similar results were reported by other workers also; Takahashi *et al.,* (1986) reported bacteriolytic enzymes in the skin mucus of yellowtail able to inhibit growth of *Micrococcus lysodeikticus* and *Pasteurella piscida.* Etami *et al.,* (1987) observed bacteriolytic enzyme activity of skin mucus of Ayu against *Micrococcus lysodeikticus* and *Vibrio anguillarum.* The different case was also found in case of *Aeromonas hydrophila* (Bhuyan, 1998), where the *Aeromonas hydrophila* is resistant to the crude mucus toxin.

In *Ompok pabda,* at the crude mucus toxin concentration of 6mg/ml, *Proteus mirabilis* was the most sensitive and recorded the largest inhibition zone of 30 mm while *Klebsiella pneumoniae* was the least sensitive with the smallest inhibition zone of 10 mm. (Samantaray, 2001).

The same kind of result obtained from *Mystus cavasius* at a mucus toxin concentration of 8 mg/ml, *Psuedomonas putida* was the most sensitive and recorded the largest inhibition zone of 34 mm while *Edwardsiella tarda* was the least sensitive with the smallest inhibition zone of 12 mm (Reddy, 2001).

Normally the antibacterial activity is attributed to bacteriolytic enzymes such as lysozymes present in the mucus. Our results are in that all the bacteria that were sensitive to the mucus extract were Gram negative bacteria and it is a established fact that the Gram negative bacteria are refractive to lysozymes (Pelczar *et al.,* 1993). The most significant difference between Gram positive and Gram-negative bacteria is the presence of an outer membrane rich in lipids that surrounds a thin underlying layer of peptidoglycan. The outer membrane serves as a barrier to various external chemicals and prevents the entry of enzymes that could damage the cell. Thus the walls of the Gram-positive bacteria can be destroyed by treatment with lysozyme, however Gram-negative bacteria is refractory to this enzyme because large protein molecules cannot penetrate the outer membrane. Only if the outer membrane is first damaged, as by removal of stabilizing magnesium ions by a chelating agent, can the enzyme penetrate and attack the underlying peptidoglycan layer (Pelczar *et al.,* 1993).

Therefore it is evident from the results that the antibacterial activity exhibited by the mucus extract of *Ompok bimacultus* and *Mystus vittatus* could not be due to lysozyme but attributable to a chelating agent, whose identity is yet to be ascertained.

5.6 Cell line assay

MTT assay have been used to detect the changes in the cell line by theapplication of crude mucus toxin of *Ompok bimaculatus* and *Mystus vittatus.* This was based on the cytotoxicity assay by the MTT colorimetric method evaluated for the detection of okadoic acid in mussels. (Tubaro et *al.*, 1995).

After 24 hours of application of the mucus toxin to the cell line, the morphological changes like the rounding of cells and in granulation of the cells were observed similar changes were reported by Amzil *et al.,* (1992) in mussels contaminated by okadoic acid.

In general, a progressive increase in cell viability was observed as the dilutions of mucus toxin increased and lower in the case of *Ompok bimaculatus* when compared to *Mystus vittatus* which exhibited a little variation to different concentrations of crude mucus toxin.

The present study thus opens up the avenues for further exploration, viz.,

1. Analysis of epidermal secretions of *Mystus vittatus and Ompok bimacultus* on HPLC.
2. Identification of different fractions from the mucus and their toxicity.
3. Application of the toxin as a wound healing agent.
4. Application of the toxin, as a medicine in the form of an antibiotic and antibacterial agent.

Summary

SUMMARY

The present study was conducted with the aim of understanding the toxicity and the biomedical activity of the freshwater catfishes, *Ompok bimaculatus* (Siluridae) and *Mystus vittatus* (Bagridae). The specimens were obtained in fresh condition from the fish market at 4 Bungalows, Mumbai. In the case of *Ompok bimaculatus,* the average size and weight of the fishes were 28.5 cm and 153.8 g while in the case of *Mystus vittatus* the average size and weight of the fishes were 10 cm and 20.9 g. Using methanol extraction, 250 mg of crude mucus was extracted from *Ompok bimaculatus* and 610 mg of mucus was extracted from *Mystus vittatus.* The extracted mucus toxin was lyophilized and stored at –20°c for further use.

Fractions of mucus toxin were obtained by ion exchange chromatography, using DEAE cellulose. Protein estimation was carried out by using Peterson (1977) method and the amount of protein in the mucus of *Ompok bimaculatus* was estimated as 1.76 mg/ ml and in the fractions it varied between 0.172-0.332 mg/ml; the amount of protein in the mucus of *Mystus vittatus* was 1.94 mg/ml I and in fractions, it ranged from 0.134-0.414 mg/ ml.

Male albino mice 20±2 g were used for mice bioassay. The lowest lethal dose for the mice injected with the crude mucus toxin of *Ompok bimaculatus* was found to be 0.6 ml at which death occurred in 3 minutes and 55 seconds, and for *Mystus vittatus* it was 0.4 ml and death occurred with in 3 minutes and 40 seconds. Restlessness, lethargy, frothy mucus from mouth, convulsion, and paralysis of hind limbs were the symptoms observed.

In the histopathogical studies, heart of the mice envenomated with crude mucus toxin of *Ompok bimaculatus* had shown fragmentation of myofibrils, areas of vacuolation and gliosis in the brain. Distended bronchiole in the lungs, necrotic changes in the liver and scattered hemorrhages in kidney were observed. Granular appearance of liver with pleomorphic nuclei, gliosis in the brain, scattered hemorrhages in the kidney, congested vessels with hemolysed blood in the lungs were observed in the mice envenomated with the crude mucus toxin of *Mystus vittatus.* Both the toxins showed hemolytic activity, and for the *Ompok bimaculatus*

the specific hemolytic activity was 4.54 and for *Mystus vittatus* it was 8.25. The fractions did not show any hemolytic activity.

Analgesic activity was also tested using tail flick analgesia meter; the crude mucus toxin of *Ompok bimaculatus* exhibited analgesic activity against control with AR values between 1.05 to 1.32, and for the crude mucus toxin of *Mystus vittatus* it was 1.21 to 1.65.

In the antibacterial activity, the mucus toxin of *Ompok bimaculatus* had shown sensitive reaction against *Aeromonas hydrophila, Vibrio anguillarum* and *Vibrio parahemolyticus* and among fractions 0.4M fraction had shown sensitive reaction against *Aeromonas hydrophila.* The crude mucus toxin of *Mystus vittatus* had shown sensitive reaction against *Aeromonas hydrophila, Pseudomonas florescens* and *Vibrio parahemolyticus.* Among fractions 0.2M and 1M fractions had shown sensitive reaction against *Aeromonas hydrophila.*

In the cell line bioassay, mouse muscular cell line (L929) obtained from National Centre for Cell Science (NCCS), Pune was used. In the cell line assay the morphological changes like granulation and rounding of the cells were observed. MTT assay was used to find the number of viable cells. The calculated t value were greater than that of t table value at 95% level of confidence, both the mucus toxins produced cellular changes at various dilutions (10^{-1} to 10^{-7})

References

REFERENCES

Absuma, V., 1997. Biotoxicity of Epidermal Secretions of *Heteropneustes fossilis* and *Boleophthalmus dentatus*. Unpublished M. F. Sc. Dissertation, Central Institute of Fisheries Education, and Mumbai: 50pp.

Agrawal, V. P., 1999. Aquatic resources. *In*: Recent Trends In Aquaculture. Society of Biosciences, India. 22-24 pp.

Alexander, J. B. and Ingram, G. A., 1992. Non-cellular, non-specific mechanisms in fish. *Rev Fish. Dis.* **2:** 249-279.

Al-Hassan, J. M., 1990. Diabetic ulcer healing preparation from the skin of Arabian Gulf Catfish (*Arius bilineatus*): A novel and effective treatment. *Int. J. Tissue React.* **12 (2):** 121-135.

Al-Hassan, J. M., Thomson, M. and Criddle, R. S., 1982. Composition of the proteinaceous gel secretion from the skin of the Arabian Gulf catfish *(Arius thalassinus). Marine Biology,* **70:** 27-33.

Al-Hassan, J. M., Thomson, M. and Criddle, R. S., 1983. Accelerated wound healing by a preparation from skin of the Arabian Gulf catfish. *Lancet,* **1- 83:** 1043-1044.

Al-Hassan, J. M., Thomson, M., Criddle, K. R., Summers, B. and Criddle, R. S., 1985a. Catfish epidermal secretions in response to threat or injury, a novel defense response to threat or injury; a novel defense response. *Marine Biology,* **88:** 117-123

Al-Hassan, J. M., Ali, M., Thomson, M., Fatima, T. and Gubler, C. J., 1985b. Toxic effects of the soluble skin secretion from the Arabian Gulf catfish (*Arius thalasinus*, Ruppell) on plasma and liver enzyme levels. *Toxicon,* **23:** 532.

Al-Hassan, J. M., Thomson, M., Ali, M., Fayad, S., Elkhawad, A., Thulesius, O. and Criddle, R. S., 1986a. Vasoconstricter components in the Arabian Gulf catfish *(Arius thalassinus*, Ruppell) proteinaceous skin secretion. *Toxicon*, **24:** 1009-1014.

Al-Hassan, J. M., Thomson, M., Al-Lahham, A. and Criddle, R. S., 1986b. * A hemolytic factor secreted from the skin of the Arabian Gulf catfish Arius *thalassinus. Fed. Proc.* **45:** 1795.

Al-Hassan, J. M., Thomson, M., Ali, M and Criddle, R. S., (1987)* Toxic and pharmacologically active secretions from the Arabian Gulf catfish (*Arius thalassinus*, Ruppell). *J. Toxicol. - Toxin Rev.* **6.** 1.

Allen, A., 1983. * Mucus, a protective secretion of complexicity. *TIBS*, **8:** 169-173

Alnaqeeb, M. A., Al-Hassan, J. M., Ali, M., Thomson, H. and Criddle, R. S., 1989. Histological observations on organs from rabbits injected with the skin toxin of the Arabian Gulf catfish (*Arius bilineatus*, Valenciennes). *Toxicon,* **27:** 789-795.

Amzil, Z., Pouchus, Y. F., Le Boterff, J., Roussakis, C., Verbist, J. F., Marcailou – Lebaut, C. and Masselin, P., 1992. Short- term cytotoxicity of mussel extracts: a new bioassy for okadoic acid detection. *Toxicon*, **30:** 1419-1425

Auerbach, P. S., 1987. Natural microbiological hazards of the aquatic environment. *Cln. Dermatol.* **5:** 52-61.

Aune, T., Yasumato, T. and Engeland, E., 1991. Light and scanning electron microscopic studies on effects of marine algal toxins toward freshly prepared hepatocytes. *J. Toxic environ. Hlth.* **34:** 1-9.

Ball, J. N., 1969. Prolactin and osmoregulation in teleost fishes – a review. Gen.Endocrinol. Suppl, **2:**10-25.

Bhakuni , D. S. and Silva , M., 1975. Biodyanamic substances from marine fauna. *J. Scient. Indust. Res.* **34:** 36-53.

Bhaskardoss, K., 1999. Acanthotoxicity of *Mystus gulio* from Mumbai area. Unpublished M.F.Sc. Dissertation, Central Institute of Fisheries Education, Mumbai: 38pp.

Bhatti, H. K., 1938. The integument and dermal skeleton of Siluroidea. *Trans. Zoo. Soc. Lond.* **24:** 1.

Bhimachar, B. S., 1944. * Poison glands in the pectoral spines of two catfishes *Heteropneustes fossilis* (Bloch) and *Plotosus arab* (Forsk), with remarks on the nature of their venom. *Proc. Indian Acad. Sci.C.* **19:** 65–70.

Bhuyan, S., 1998. Bioactivity of the Epidermal Secretions of the Mudskipper *Boleophthalmus dentatus.* Unpublished M.F.Sc. Dissertation, Central Institute of Fisheries Education, and Mumbai: 50pp.

Blackstock, N. and Pickering, A. D., 1982. * Changes in the concentration and histochemistry of epidermal mucus cells during the alevin and fry stages of the brown trout, *Salmo trutta. J. Zool. Lond.* **197:** 463-71.

Blay, J. and Poon, S. L., 1995. Use of cultured permanent lines of intestinal epithelial cells for the assy of okadoic acid in mussel homogenates. *Toxicon,* **33:** 739-746.

Boylan, D. B. and Scheuer, P. J., 1967. Pahutoxin: a fish poison. *Science.* **155:** 52.

Bullock, A. M., Marks, R. and Roberts, R. J., 1978. * The cell kinetics of teleost fish epidermis, epidermal mitotic activity in relation to wound healing at varying temperatures in plaice (*pleuronectus platessa*). *J. Zool. Lond.* **185:** 197-204.

Burkholder, P., 1963. Drugs from the sea. *Armed Forces Chem. J.* **27:** 1-8.

Byrne, J. S., 1970. Mucus envelope formation in two species of Hawaiian parrotfishes. *Pacif. Sci.* **24:** 490 – 493.

Cameron, A. M. and Endean, R., 1973. Epidermal secretions and the evolution of venom glands in fishes. *Toxicon,* **11:** 401-410.

Chan, D. W., Young, C. K. and Chan, M. K., 1985. Acute renal failure after eating raw fish gall bladder. *British Medical Journal*, **290:** 897.

Clark, E., 1974. The Red Sea's shark proof fish. *Natl. Georg. Mag.* **146:** 719-729.

Clark, E. and Chao, S., 1973. A toxic secretion of the Red Sea flat fish *Pardachirus marmoratus* . *Bull. Sea Fish Res.Stn. Israe*l, **60:** 53-56.

Clark . E. and George, A., 1979. Toxic soles, *pardachirus marmoratus* from the Red Sea and *p.pavonius* from Japan, with notes on other species, *Envir. Biol. Fish.* **4:** 103-123.

Collette, B. B., 1956. A review of the venomous toadfishes, subfamily Thalassophyninae. *Copeia,* **4:** 846-864.

Concon, J. M., 1998. Food Toxicology. Principles and concepts, Part A. Marcel Dekkar, INC, New York: 603pp.

D' Amour and Smith., 1941. *J. Pharmac. Exp. Therapy*, 72: 74.

Deo, D. A., 1995. Biotoxins from the Freshwater Catfish *Heteropneustes fossilis* (Bloch, 1794*).* Unpublished M.Sc. Dissertation, Central Institute of Fisheries Education, Mumbai: 53pp.

Ellis, A. E., 1981. Non-specific defence mechanism in fish and their role in disease process. *Dev. Biol.Stand.* **49:** 337-352.

Enomoto, N. and Nakagaiwa, H., 1964. Studies on the external mucus substances of fishes, preparation of crystalline-N-actylneuraminic acid from the external mucus substances of loach. *Bull. Jap. Soc. Sci. Fish.* **30 (6):** 495-499.

Etami, T., Takahashi, Y and Sato K., 1987. Bacteriolytic activities in the skin mucus of Ayu. *Nippon Suisian Gakkaishi*, **53 (3):** 273-278.

Evans, D. H., 1984. The roles of gill permeability and transport mechanisms in euryhalinity. *In*: Fish physiology (ed. Hoar, W. S and Randall, D. J.). Vol. XB. Orlando, FL: Academic Press, 239 – 84 pp.

Filk, G., Van Rijs, J. S., and Weneluar Bonga, S. E., 1984. * Evidence for the presence of Calmodulin in fish mucus. *Eur. J. Biochem.* **138:** 651-654.

Fishman, A. P., Pack, A. I., Delaney, R. G and Galante, R, J., 1984. Estivation in *Protopterus. In:*The Biology and Evoluation of Lungfishes (ed: Inbemis, W. E., Burgyren, W. W and Kemp, N. E.). NY; Liss, 237-248pp.

Fletcher, T. C. and Grant, P. T., 1969. * Immunoglobins in the serum and mucous of plaice, *Pleuronectes platessa. Biochem.* J. **115:** 65.

Goldberg, A. S., Duffield, A. M. and Barrow, K. D., 1988. Distribution and chemical composition of the toxic skin secretions from trunkfish (*Ostracidae*). *Toxicon*, **26**: 651-663.

Goldberg, A. S., Wacylyk, J., Renna, S., Reisman, H. and Nair. M.S.R., 1982. Isolation and structural elucidation of an ichthyocrinotoxin from the smooth trunkfish, *Lactophrys triguesfer. Toxicon,* **20:** 1069-1074.

Halstead, B. W. and Courville, D. A., 1967. Poisonous and Venomous Marine Animals of the World. Vol. 2. U.S. Govt. Printing Ofiice, Washington, D.C: 1070 pp.

Halstead, B.W. and Courville, D. A., 1970. Poisonous and Venomous Marine Animals of the World. Vol. 3. U.S. Govt. Printing Office, Washington, D.C: 1006 pp.

Hara, T. J., 1986. * Role of olfaction in fish behavior. *In:* The Behavior of Teleost Fishes (ed. Pitcher, T. J.). Croom Helm, London. 152-176pp.

Harrel, I. W., Etlinger, H. m., and Hodgins, H. O., 1976. Humoral factors important in resistance of salmonid fish to bacterial disease. II. Anti *Vibrio anguillarum* activity in mucus and observation on complement. *Aquaculture, 7:* 363-370.

Hashimoto, Y., 1979. Marine Toxins and other Bioactive Marine Metabolites. Japan Scientific Societies Press. Tokyo: 369pp.

Hashimoto, Y. and Kamiya, H., 1969. * Occurrence of toxic substances in the skin of sea bass, *Pogonoperca punctata. Toxicon,* **7:** 65.

Hashimoto, Y. and Oshima, Y., 1972. * Separation of grammistins A, B and C from soapfish *Pogonopore punctata. Toxicon*, **10:** 279.

Hashimoto, Y., Kawasaki, M. and Hatano, M., 1976. Occurrence of a toxic phospholipid in Cabezon Roe. *Toxicon*, **14:** 141–143.

Hildeman, W. H., 1959. A cichlid fish, *Symphysodon discus* with unique nature habits. *AM. Nat.* **93:** 27-34.

Hjelmeland, K., Christie M. and Raa, J., 1983. Skin mucus protease from rainbow trout *Salmo gairdneri* (Richardson) and its biological significance. *J. Fish. Biol.* **23:** 13-22.

Hori, K., Fusetani, N., Hashimoto, K., Aida, K. and Randall, J. E., 1979. Occurence of a grammistin like mucous toxin in the cling fish *Diademichthys lineatus. Toxicon,* ***17:*** 418-424.

Ingram, G. A., 1980. Substances involved in the natural resistance of fish to infection – a review *J. Fish Biol.***16:** 23-60.

Kamiya, H., Muramoto, K and Goto, R. 1988. Purification and properties of agglutinins from Conger eel *Conger myriaster* (revoort), skin mucus, *Devl.Comp.Immun.* **12:** 309-318.

Kitzan, S. M. and Sweeney, P. R., 1968. * A light electron microscope study of the structure of *Protopterus annectens* epidermis Mucus production. *Can. J. Zool.* **46:** 787.

Kobert, R., 1902. * Ueher giftfische und fischgifte. *Med. Woche* (**19**): 199-201.

Kodama, M., Ogata, T and Sato, S., 1985. External secretion of tetrodotoxin from puffers stimulated by electric shock. *Mar.Biol.* **87:** 199-202.

Kodama, M., Ogata, T., Sato, S., Suzuki, Y., Kaneko, T. and Aida, k., 1986. tetadotoxin secreting glands in the skin of puffer fishes. *Toxicon* **24:** 819-829.

Laurent, P., 1984. Gill internal morphology. p: 73-183. *In:* Fish Physiology (ed. Hoar, W. S. and Randall, D. J). Vol XA, Academic Press, Orlando, Fl.

Lazarovici, P., Primor, N., Caratsch., C. G., Munz, K., Lelkes, P., Loew, L. M., Shai, Y., Mcphie, P., Louini, A., Contreras, M. L., Fox, J., Shih, Y. L. and Edwards, C.,1988. Action on artificial and neuronal membranes of pardaxin, a new presynaptic excitatory polypeptide neurotoxin with ionophore activity. *In:* Neurotoxins in Neurochemistry (ed. Dolly, J. O.). Halsted Press, 219-240pp.

Leimaitre, C., Orange, N., Saglio, P., Saint, N., Gagnon, J, z., 1996. Characterization and ion channal activities of novel antibacterial proteins from the skin mucosa of carp (*Cyprinus carpio*) *European Journal of Biochemistry*, **240:** 143-149.

Lenke, R., 1991. The opercular gland of the cleaner – wrasse *Labroides dimodiatus* (labridae), a light electron and scanning electron microscope investigation. *Journal of Fish Biology,* **38:** 383-392.

Lewis, R. W., 1970. Fish cutaneous mucus: new source of skin surface lipids. *Lipids,* **5**: 947-949.

Liguori, V. R., Ruggieri, G. D., Baslow, M. H., Stempien, M. F. and Nigrelli, R. E. (1963)* Antibiotic and toxic activity of the mucous of the Pacific golden striped bass *Grammistes sexlineatus. Am. Zool.* **3:** 546.

Maretzki, A., Del Castillo J., 1967. * A toxin secreted from soapfish, *Ripticus saponaceus.* A preliminary report. *Toxicon* **4:** 245.

Marwick, C., 1998. Nature's Agents Help Humans- some now take steps to Reciprocate. JAMA. *Medical News and Perspective*: 279. No.21, 1679 –1681.

Moyer, J. T. and. Zaiser, M. J., 1981. Social organization and spawning behavior of the pterione fish, *Dendrchirus zebra* at Miyake-Jima Japan. *Jpn. J. Ichtyol.* **28**, 52 – 69.

Nair, M. S .R., 1988. Fish skin toxins. *In*: Handbook of Natural Toxins, Marine Toxins fand Venoms. Vol.3 (ed. Tu, A.T). New York, Marcel Dekker, 211-226pp.

Nair, M. S. R., Leong, I. and Nayar. M. S. B., 1982. Ichthyotoxins from the oyster toad fish *Opsantus tau* (Linnaeus). *Toxicon,* **20:** 933-935.

Nigrelli, R. F., 1935. * On the effect of fish mucus on *Epibdella melleni*, a monogenetic trematode of marine fishes.*J. Parasit.* **21:** 438.

Nigrelli, R. F., 1937. * Further studies on suceptability and acquired immunity of marine fishes to *Epibdella mellehi*, a monogenetic trematode. *Zoologica.* **22:** 185.

Nigrelli, R. F., Jakowska, S. and Padnos, M., 1955. Pathogenecity of epibionts in fishes. *J. Protozool.* **2:** 7.

Noguchi, T., Kao, H. and Hashimato, Y., 1971. Toxicity of the goby, *Gobius criniger. Bulletin of the Japanese Society of Scientific Fisheries,* **37:** 642-647.

Paniprasad, K. and Venkateshvaran,K, (1997) Microhemolytic assay, pp 41-42. *In:* Laboratory Manual on Advanced Techniques in Marine Biotoxinology, (ed. Venkateshvaran, K., Paniprasad, K). CAS in Fishery Science, Central Institute of Fisheries Education, Mumbai, India: 76pp.

Pelczar, Jr. M.J., Chan, E.C.S. and Krieg, N. R., 1993. Microbiology. Tata McGraw Hill Publishing Co. Ltd. New Delhi, India: 918 pp.

Peterson, G. L., 1977. A simplification of the protein assay method of Lowry et al., which is more generally applicable. *Anal. Bioc hem.* 346-356.

Pickering, A. D., 1974. The distribution of mucus cells in the epidermis of the brown trout *Salmo trutta (*L.) and the char *Salvelinus alpinus* (L.). *J.Fish Biol.* **6:** 11-118.

Pigman, W., 1977. * Mucus glycoproteins. *In:* The Glycoconjugates, (ed. Horowitz, M. I. and Pigman, W.). Academic Press, New York. 131-137pp.

Primor , N., Parness, J and Zlotkin, E., 1978. Paratoxin; the toxic factor from the skin secretion of the flatfish *Pardachirus marmoratus* (soleidae) *In:* the Toxins Animal, Plant, Microbial, (ed. p. Rosenberg.). Pergamon press, NewYork. 539-547pp.

Primor , N., Sabnay, I., Lavie, V. and Zlotkin, E., 1980. Toxicity to fish, effect on gill ATPase and gill ultra structure changes induced by *Pardachirus marmoratus* (soleidae) secretion and it's derived toxin paratoxin. *J. Exp. Zool.* **211:** 33-40.

Primor, N. and Zlotkin, E., 1975. Ichthyotoxic and hemolytic action of the skin secretion of flatfish, *Paradachirus marmoratus* (Soleidae). *Toxicon,* **13:** 227-231.

Prokhoroff, P., 1884. * On the poisonous character of certain lampreys. (In Russian) *Vrach. St. Petersb.* **5:** 54-55.

Randall, J. E. 1967. * Food habits of reef fishes of the West Indies. *Stud. trop. Oceanogr. Miami.* **5:** 665.

Randall, D. J. and Daxboeck, C., 1984. Oxygen and carbon dioxide transfer across the gills. *In*: Fish Physiology (ed. Hoar, W. S. and Randall, D. J.). Vol. XA. Orlando, FL: Academic press, pp. 263 – 314.

Randall, D. J., Wright, P. A., 1989. The interaction between carbon dioxide and ammonia excretion and water pH in fish *Can. J. Zool.* **67:** 2936-2942.

Randall, J. E., Aida, K., Hibiya, T., Mitsuura, N., Kamiya, H. and Hashimoto, Y., 1971. * Grammistin, the skin toxin of soapfishes, and its significance in the classification of the Grammistidae. *Publs. Seto. Mar. Bio. Lab.* **19:** 157.

Randall, J. E., Aida, K., Oshima, Y., Hori. K., Hashimoto. Y., 1981. Occurrence of a crinotoxin and hemagglutination in the skin mucus of the moray eel *Lyncodontus nudivomer. Marine Biology.* **62:** 179-184.

Ravi, R., 1999. Bioactivity of Epidermal Secretions of *Heteropneustes fossilis.* Unpublished M.F.Sc. Dissertation, Central Institute of Fisheries Education, Mumbai: 38pp.

Reddy, P., 2001. Bioactivity of Herbicidal natural products from the skin secretions of *Mystus cavasius.* Unpublished M.F.Sc. Dissertation, Central Institute of Fisheries Education, and Mumbai, 54 pp.

Reed, H. D., 1924. * The morphology and growth of the spines of siluroid fishes. *J. Morphol.* **38:** 431-451.

Reid, L. M. and Bhaskar, K. R., 1989. * Macromolecular and lipid constituents of bronchial epithelial mucus. *Symp. Soc. Exp. Bio.* **43:** 201-219.

Rosen, M. W. and Cornford N.E., 1971. Fluid friction of fish slimes. Nature. Lond. **234:** 49-51.

Russel, F. S., 1965. Marine toxins and venomous and poisonous marine animals. *Advances in Marine Biology.* **3:** 255-384.

Samantaray, B., 2001. Biomedical activity of the epidermal secretions of *Ompok pabda,* Unpublished M.F.Sc. Dissertation, Central Institute of Fisheries Education, Mumbai: 60 pp.

Scheuer, P. J., 1966. * Presented at the 151st ACS Meetings, Pittsburgh, Pennsylvania, Abstract 19.

Shephard, K. L., 1994. Functions for fish mucus. *Reviews in Fish Biology and Fisheries*, **4:** 401-429.

Shiomi, K., Takamiya, M., Yamanaka, H., Kikuchi, T., 1987. Purification of a lethal factor in the skin secretion from the oriental catfish *Plotosus lineatus, Bull. Jpn. Soc. Sci. Fish,* **53:** 1275-1280.

Shiomi. K., Takamiya, M., Yamanaka, H., Kikuchi, T. and Konno, K. 1986. * Hemolytic, lethal and edema forming activities of the skin secretion from oriental catfish (*Plotosus lineatus). Toxicon,* **24:** 1015.

Shiomi, K., Takamiya, M., Yamanaka, H., Kikuchi, T. and Suzuki, Y., 1988. Toxin in the skin secretion of the oriental catfish *(P.lineatus*). Immunological properties and immuno-cytochemical identification of producing cells. *Toxicon*, **26:** 353-361.

Solanki, T. G. and Benjamin, M., 1982. Changes in the mucus cells of the gills, buccal cavity and epidermis of the nine-spined stickleback. *Pungitius pungitius* L, induced by transferring the fish to sea water. *J.Fish Biol.* **21:** 563-575.

Tachibana, K., Nakanishi, K., and Gruber, S. H., 1985. * Shark repellents of Red Sea Moses sole. Unpublished results. Presented at 24th Symposium of Natural Products, Hiroshima (in Japanese). Symposium Papers: 545 – 552.

Takahashi, Y., Kajiwaki, T. and Itam, T., 1986. Enzymatic properties of the bacteriolytic substances in the skin of yellow tail. *Nippon Suisian Gakkaishi*, **52 (7):** 526-532.

Talwar, P. K and Jhingran, A. G., 1991. Inland fishes of India and adjacent countries. Vol 2. Oxford IBH publishing co. pvt. Ltd. New Delhi: 1158pp.

Thomson, D. A., 1964. Ostracitoxin: An ichthyotoxic stress secretion of the boxfish *Ostracion lentiginosus, Science,* **146:** 244-255.

Thomson, M., Al-Hassan, J. M., Fayad, S., Al-Saleh, J. and Ali, M., 1998. Purification of a toxic factor from Gulf catfish epidermal secretions. *Toxicon,* **36:** 859-865.

Tubaro, A., Florio, C., Luxich, E., Vertua, R., Della loggia, R.D. and Yasumato, T.,1995. Suitability of the MTT based cytotoxicity assay to detect okadoic acid contamination of mussels. *Toxicon,* **34:** 965-974.

Ultsh, G. R. and Gross, G., 1975. Mucus as a diffusion barrier to oxygen: possible role in oxygen uptake at low pH in carp (*Cyprinus carpio*) gills. *Comp. Biochem. Physio.* **62A:** 685-689.

Van de Winkel, J. S. J., Van Kuppevelt, T. H. M. S. M., Janseen, H. M. S. and Lock. R. A. C., 1986. Glycasaminoglycons in the skin mucus of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.* **68 (C):** 234-237.

Van Oosten, J., 1957. * The skin and scales, 207-247. *In* : Physiology of Fishes (ed. Brown, M. E.). Vol. I. Academic Press. New York: 460 pp.

Vidya, V., 1977. Bioactivity of Epidermal Secretions of Three Arid Catfishes of Mumbai Waters. Unpublished M.F.Sc. Dissertation. Central Institute of Fisheries Education, Mumbai, India: 50 pp.

Weissman, G., 1965. Hemolysis and augmentation of hemolysis by neutral steroid and bile acids. *Biochem. Pharmac.* **14:** 537.

Whitear, M., 1970. The skin surface of bony fishes. *J. Zool. Lond.* **160:** 437-454.

Whitear, M., 1986. * The skin of fishes including Cyclostomes. *In:* Biology of the Integument, Vol. 2. (ed. Bereiter-Hahn, J., Matallsy, A. G. and Richards, K. S.). Springer, Berlin. 8-64.

Winn, H. E., 1955. Formation of mucus envelope at night by parrot fishes. *Zoologica.* **40:** 145-48.

Wooton, R. J., 1984. A Functional Biology of Sticklebacks. Croom Helm. London: 265 pp.

Wright, P. A., Hemin, T and Randall, D. J., 1986. Downstream pH changes in water flowing over the gills of rainbow trout. *J. Exp.Biol.* **126:** 499-512.

Wright, P. A., Randall, D. J and Perry, S.F., 1989. Fish gill water boundary layer: a site of linkage between carbon dioxide and ammonia excretion. *J. Comp Physiol.* **158 B:** 627-635.

* REFERENCES NOT SEEN IN ORIGINAL