INFLAMMATORY RESPONSE IN *ETROPLUS SURATENSIS* (BLOCH) EXPOSED TO SUBLETHAL LEVEL OF ORGANOPHOSPHORUS PESTICIDE (NUVAN)

DISSERTATION SUBMITTED
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BY

MAYA R.J.

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D.256

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(INDIAN COUNCIL OF AGRICULTURAL RESEARCH)
COCHIN - 682 014
INDIA.

JULY 2000

DEDICATED
TO MY
PARENTS

CERTIFICATE

Certified that the dissertation entitled **"Inflammatory Response in *Etroplus suratensis* (Bloch) Exposed to Sublethal level of Orgnophosphorus Pesticide (Nuvan)"** is a bonafide record of work done by **Maya .R.J.** under our guidance at the Central Marine Fisheries Research Institute during the tenure of her **M.F.Sc. (Mariculture)** Programme of 1998-2000 and that it has not previously formed the basis for the award of any other degree, diploma or other similar titles or for any publication.

Dr.K.C. George,
Senior Scientist,
CMFRI,Kochi,
Major Advisor ,Advisory Committee.

Dr. (Mrs.) K.S. Sobhana,
Scientist,
CMFRI, Kochi,
Co-Chairman & Member,
Advisory committee.

Mr.N.K. Sanil,
Scientist (Sr.Scale),
CMFRI,Kochi,
Member,Advisory committee.

DECLARATION

I hereby declare that this dissertation entitled "**Inflammatory Response in *Etroplus suratensis* (Bloch) exposed to sublethal level of organophosphorus pesticide (Nuvan)**" is based on my own research and not formed the basis of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Maya.R.J.

Cochin

July 2000

सारांश

एट्रोप्लस सुराटेन्सिस की शोथ प्रतिक्रिया (इनफ्लमेटरी रेस्पोन्स) में नुवान (ओरगनोफोसफेट पेस्टिसाइड) की अवघातक सान्द्रता (0.02 पी पी एम) के प्रभाव का अध्ययन किया गया. 0.1 मि लि फ्रॉयन्ड्स कंप्लीट एड्जुएन्ट का अंतःक्षेपण (इन्जेक्शन) करके शोथ को प्रेरित किया गया. अंतःक्षेपण के पश्चात् 3 घंटों से 1,2,3,9,13 और 15 दिनों के अंतराल में शोथित पेशी ऊतकों को काटकर ऊतकीय अध्ययन के लिए संसाधन किया गया. नियंत्रण और उपचार वर्गों की प्राथमिक शोथ प्रतिक्रिया में समानता देखी गई. नियंत्रण वर्ग में 13 वां दिन न्यूट्रोफिलिक इनफिल्ट्रेशन देखा गया और ग्रानुलोमाटस अभिक्रिया तीसरे दिन से शुरू की गई और 15 वां दिन तक जारी रही. 15 वां दिन महाकोशिका (जयन्ट सेल) दिखाई पडी. उपचार वर्ग में तीसरे दिन ग्रानुलोमाटस अभिक्रिया शुरू नहीं हुई और 15 वां दिन भी महाकोशिका नहीं दिखाई पडी. नियंत्रण वर्गों की तुलना में उपचार वर्ग में अभिक्रिया अपचित थी.

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CONTENTS

INTRODUCTION

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Aquaculture is developing into a prime industry to tap the enormous turnover of bioenergy for the benefit of mankind. Many countries including India have fertile bays, estuaries and intertidal zones, which are ideal locations for taking up aquaculture. In the early part of 20^{th} century most of these areas were not polluted. But unfortunately today they face the major problem of environmental degradation.

Toxic pollutants released into the environment by diverse human activities interact with the living organisms and interferes with the metabolic processes. Consequently the problem of persistence, bio-magnification, selective toxicity and sub-lethal effects arise. Among pollutants pesticides are the most dangerous and they create much havoc.

Pesticides, which drain into water bodies through rain, agricultural run off and disposal of industrial effluents affects the aquatic fauna especially fish. Pesticides or the biocidal agricultural chemicals include insecticides, acaricides, nematocides, rodenticides, herbicides and fungicides. Of these insecticides, organochlorines and organophosphates are the most widely used.

The inland aquatic environment and coastal areas are more affected than the open sea. As a result of this, fishes in these areas are exposed to frequent stresses. Environmental stress can trigger the outbreak of infectious diseases in fish populations (Wedemeyer, 1970; Snieszko, 1974). There are lacunae on the knowledge about stress response in fish and the subsequent increase in its susceptibility to infections. Many of these insecticides are known stress factors and immunotoxins in mammals; however little information is available on the immunotoxic response of fish to these pollutants. It is felt that a study on the effect of some of the insecticides on the non-specific tissue reactions will be useful. Hence we planned a programme to assess the inflammatory response in fish.

The present study is done on banded pearl spot, *Etroplus suratensis* (BLOCH), which is an excellent delicious fish extensively cultured. It is very common in the brackish waters of the coastal regions of Kerala, Tamil Nadu, Pondicherry and Orissa (Talwar and Jingran, 1991). The pearl spot is suitable for culture in confined fresh and brackish water (Hora and Pillay, 1962) and has been transplanted into fresh water areas of India (Chacko *et al.*, 1953; Hora and Pillay, 1962).

Immune responses are important defense mechanisms. Their impairment will allow an increased incidence of infection and thus may indirectly influence the survival of the individual or the species. If toxic chemicals inadvertently interfere with the immune system, such interference may have serious consequences. In many cases the individual may not be aware of the exposure to environmental chemicals that may be immunosuppressant. Investigation of the immunosuppressive potential of chemical is therefore desirable.

Inflammatory response is an important component of non-specific immune system in fish. Apparently no researcher has examined the effect of pesticides upon fish inflammation. Hence an attempt was made through the present study to investigate the effect of pesticide on the inflammatory response in *Etroplus suratensis*.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Organophosphorus (OP) insecticides have largely replaced the use of organochlorine pesticides in recent years because of their rapid breakdown in water and their low environmental persistence. Organophosphate pesticides are the most widely used agricultural chemicals: an estimated 40% of all crops are treated with pesticides of this class (Wagner, 1981). However their extensive use has resulted in a high contamination risk to aquatic environment.

Acute toxicity is rare in natural environment. Sometimes it occurs due to accidents or direct application of pesticides. Voluminous literature is available on the acute toxicity of organophosphates to fishes (Konar, 1977; Qureshi *et al.*, 1983; Shaffi, 1980; Mohapatra and Noble, 1992a; Hecht *et al.*, 1994; Trivedi and Saksena, 1999).

The sub-lethal concentration may prove more deleterious than the lethal concentration because small and subtle effect on the fish may alter their behaviour, feeding habits, position in the school, reproductive success etc. Subtle effects at the organ or cellular level may alter the metabolism of the fish and hence its

ability to withstand stress. The effect of sub-lethal exposure to organophosphorus compounds in relation to growth, behaviour, biochemical, histological and physiological alterations in the body have been studied on fish. (Mukhopadhyay and Dehadri, 1980; Ramalingam and Ramalingam, 1982; Awasthi *et al.*, 1984; Kumar and Alam Ansari, 1986; Bashamohideen *et al.*, 1987; Ghosh and Chatterjee, 1989; Khillare and Wagh, 1988; Dutta *et al.*, 1994; Kumar *et al.*, 1995; Sancho *et al.*, 1997). Sublethal effects on the *haematological parameters were also reported* (Singh and Srivastava, 1994; Gupta *et al.*, 1995).

The water-soluble organophosphorus insecticide " Nuvan " is widely used in the Kolleru region of AndhraPradesh for controlling the ectoparasites such as Lernea, Argulus etc (Muthu *et al.*, 1988; Gopal Rao, 1993). But the long-range effects of this practice are not known. The chemical " Nuvan Fish 500 EC " has been granted a product licence by the Government of U.K, for use as *medicine in Salmon farming to treat sealice* (Anon, 1989). The use of these chemicals in salmon farming appears to have deleterious effect on marine invertebrate species (Egidius and Moester, 1987). Stephanie Pain (1989) links the epidemic of eye disease in salmon of the wild to the use of " Nuvan 500 EC " in farms.

There are many reports on the sub-lethal toxicity of Nuvan to fishes (Ghosh and Chatterjee, 1989; Mohapatra and Noble, 1992b; Medda, 1993; Arastra *et al.*, 1996; Venugopal *et al.*, 1996).

Sub-lethal doses of toxic agents can have effects upon immune structures and functions that may ultimately be almost as harmful as direct toxic doses. Holladay *et al.* (1996) conducted investigations on the influence of Chlorpyrifos, an organophosphate insecticide on the immune system of Nile Tilapia. Results indicated lowering of total pronephros cell counts and phagocytic function. Rainbow trout *Oncorhynchus mykiss* exposed to TTDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) displayed a variety of lymphomyeloid effects, including thymic involution, splenic lymphocyte depletion, hypocellularity of the pronephros, and peripheral leucopenia (Spitsbergen *et al.*, 1988). Similar lesions were found in rainbow trout injected with Clophen A50 (a mixture of polychlorinated biphenyls) ; these included edema and partial degeneration of lymphocytic tissues in the thymus and spleen (Thuvander *et al* ., 1993). Spot *Leiostomus xanthurus* and hogchokers *Trinectes maculates* collected in waters heavily contaminated with polycylic aromatic hydrocarbons exhibited markedly reduced phagocytic and

chemotactic activity of macrophages isolated from the pronephros, as well as defificient macrophage chemiluminescent responses (Weeks *et al.*, 1990). Jeney and Jeney (1986) observed reduced hematocrit values in common carp *Cyprinus carpio* treated with organophosphate, trichlorphon. Channel catfish *Ictalurus punctatus* exposed to malathion have displayed significantly depressed peripheral leucocyte counts (Areechon and Plumb, 1990) as well as inhibited humoral immune response (Plumb and Areechon, 1990).

Non-specific immune capacities of fish are general reactions to injury or invasion by foreign organisms. Phagocytosis and inflammation are two non-specific responses that are probably universal in fish (Finn, 1970; Corbel, 1975; Ellis, 1977). Inflammation is a protective reaction of the host in response to injury, which results in specific morphological and chemical changes in tissues and cells. The process of inflammation for any disease is essentially the same, however the features vary widely according to type of injurious agent. The general steps in inflammation are

- Vasodilation and increased vascular Permeability
- Leucocyte migration and removal of debris
- Resolution

The inflammatory response in teleost fish is poorly documented compared with that of higher vertebrates. The response has been claimed to be basically similar to that of mammals although less intense and slower to appear and resolve (Finn and Nielsen, 1971a).

Fishes are poikilotherms and it is expected that their inflammatory response would vary with temperature. The relationship between environmental temperature and the rate of development of the acute and chronic inflammatory response has been the subject of several studies (Finn and Nielsen, 1971b; Roberts *et al.*, 1973; Anderson and Roberts, 1975; Timur *et al.*, 1985; Suzuki and Iida, 1992) and the results indicate that inflammatory response is delayed at lower temperature. This delay is variable and appears to depend on factors such as type of injurious agent and the optimal temperature range of an individual species. Fibroblastic activity took twice as long to develop to the same stage at 5°C than at 15°C. Low temperature also delayed the appearance of tissue necrosis and clearance of bacteria and necrotic muscle tissue (Finn and Nielsen, 1971b). Mawdesley and Bucki (1973) studied the tissue repair in *Carassius auratus*. Epithelialisation occurred within first month in the epidermis

following the injury and dermis showed increased cellular infiltration.

Acute inflammation is an exudative reaction in which fluid, plasma protein and leucocytes exit the blood stream and infiltrate the injured area. Acute inflammation in teleost has been studied ultra structurally after injection of killed bacteria in winter flounder *Pleuronectes americanus* (Bodammer and Robohm, 1996), injection of live bacteria in striped snakehead *Channa striatus* (Chinabut, 1990), injection of live bacteria and oyster glycogen in plaice *Pleuronectes platessa* (Mac Arthur *et al.*, 1984), and intragastric intubation of live bacteria in channel catfish (Baldwin and Newton, 1993). Examination of the host response to parasites such as monogenetic trematodes on the gills (Roubal, 1986), coccidian in the liver (Hawkins *et al.*, 1981) and trematodes in the spleen and head kidney (Richards *et al.*, 1994) has also been performed.

Suzuki and Hibiya (1983) measured increased vascular permeability during inflammation in carp *Cyprinus carpio*. The process of leucocyte migration in various types of inflammation has specific features, which are controlled by a variety of chemical mediators such as complement factors, leucotrine B_4 and a

lymphokine (Suzuki and Iida, 1992). Nomenclature concerning teleost leucocytes is confusing (Ellis, 1977; Anisworth, 1992). Acute inflammatory leucocytes present in the skin of teleost have been examined after mechanical injury (Phromsuthirak, 1977; Iger and Abraham, 1990), during infection of *Ichthyophthirius multifilis* (Cross and Matthews, 1993) and in response to sudden temperature increase (Iger *et al.*, 1994). No differences were observed in the type of leucocytes, their ultrastructure, nor their relative abundance in acutely inflamed dermis and underlying muscle of channel catfish held at temperatures of 9°C, 15°C and 21°C after injection of turpentine (Croll and Grizzle, 1998).

In teleosts the type of leucocyte that respond first during inflammation varies. Neutrophils are typically the first inflammatory cells to respond during inflammation in teleost (Finn and Nielsen, 1971*a,b*; Phromsuthirak, 1977; Sohnle and Chusid, 1983; Mac Arther *et al.*, 1984; Suzuki and Hibiya, 1986; Iger and Abraham, 1990; Cross and Matthews, 1993; Iger *et al.*, 1994; Suzuki and Iida, 1992; Suzuki, 1992; Afonso *et al.*, 1998) but in some circumstances macrophages are the first cells responding to an injurious agent (Anderson and Roberts, 1975; Chinabut, 1990; Baldwin and Newton, 1993). Neutrophils have been designated with various synonyms such as heterophils (Hawkins *et al.*, 1981) and

polymorphonuclear leucocytes (Finn and Nielsen, 1971a; Roberts *et al.*, 1973; Anderson and Roberts, 1975). Monocytes are relatively immature cells present in the hematopoietic tissue and circulating blood. Once monocytes exit the circulatory system they change into macrophage. Wolke (1992) revealed that piscine macrophage aggregates or melanomacrophage centers are most commonly present in the spleen, mesonephros and liver but found in other organs especially during inflammation. Inflammatory cell response of Atlantic salmon to intraperitoneal injection of yeast glucan, glycogen or FIA is characterised by the accumulation of neutrophils, macrophages and thrombocytes in the peritoneum. However the onset and duration of response and the number of cells elicited differed with each agent (Jorgensen *et al.*, 1993).

The slower response and later predominance of macrophages were demonstrated in the skin of common carp *Cyprinus carpio* (Iger and Abraham, 1990) and three-spine stickleback *Gastrosteus aculeatus* (Phromsuthirak, 1977) after being wounded. Migration of basophils during the process of wound healing in carp was observed by Iger and Abraham (1990). Suzuki (1992) observed cellular responses in the puffer, *Takifugu niphobles* in inflammation caused by subcutaneous injection of carrageenan. Migration of basophils was clearly recognised and

maximum cell numbers were attained one day after injection. Suzuki (1986) observed inflammatory exudation of eosinophils in the peritoneal cavity of tilapia, *Oreochromis niloticus*. Inflammatory exudation of eosinophils during the process of wound healing in the skin of stickleback *Gasterosteus aculeatus* (Phromsuthirak, 1977) and to abdominal implants (Marty and Summerfelt, 1988) is also reported.

MATERIALS AND METHODS

MATERIALS AND METHODS

Finfish Pearl spot, *Etroplus suratensis* of average length 12± 1 cm and average weight 22± 2 g were selected for the experiment and were collected from Matsysfed Farm, Narakkal. The animals were acclimatised to laboratory condition by maintaining them in aquaria containing water of salinity 5± 1 ‰ and temperature 30±2°C. fishes were fed *ad libtium* with pellet feed once in a day. The faecal matter and waste material were siphoned out daily from the tank. This maintenance procedure was strictly followed during both acclimatisation and experimental phase.

Exposure to Nuvan

The commercial grade Nuvan of Hindustan CIBA-GEIGY Ltd, having the composition Dichlorovos 76% m/m, Emulsifier 19.6% m/m and Solvent 13.4% m/m was used for the experiment. 1 ml of Nuvan was dissolved in 100 ml distilled water to make a stock solution of 10 mg insecticide /ml. The desired concentration of the test media was obtained by diluting the stock solution in distilled water.

The fishes were divided into two groups. The first one served as control while the second were subjected to 0.02 ppm

Nuvan treatment for one week before the start of the experiment and throughout the experimental period. In order to maintain the concentration of Nuvan, water was exchanged completely in every 24 hrs. Each group contained 7 fishes.

Injection

Fishes were anesthetized in a solution of Benzocaine (10 ppm). Then they were injected intramuscularly (IM) with 0.1 ml Freund's Complete Adjuvant (FCA). The injection was on the left side of the fish immediately below the dorsal fin.

One fish from each group was killed at 3 hrs and 1,2,3,9,13,15 days after injection. Samples from each fish were excised from the injection site and fixed in 10% buffered neutral formalin. After proper fixation, tissues were processed by routine histological technique (Bullouck, 1978).Paraffin embedded sections was cut at 6 μ thickness in rotary microtome. The deparaffinised sections were stained with three different stains.

The stains used were:

a) Haematoxylin-Eosin (Culling et al., 1985)

b) Van Gieson's stain (Melby and Altman, 1984)

c) Hematoxylin-Phloxine Saffron Masson, Modified (Melby and Altman, 1984)

All sections were examined under light microscope and photographs were taken wherever necessary.

RESULTS

RESULTS

Skeletal muscle tissues of both control and treatment group were collected at 3 hrs and 1,2,3,9,13 and 15 days post injection of Freund's Complete Adjuvant (FCA) intramuscularly (IM). They were processed for histological studies.

At 3 hrs the subcutaneous tissue and muscles showed different changes. The muscle tissue underwent necrosis with fragmentation of muscle fibres and separation of sacrolemal cells. Haemorrhage was seen in subcutaneous tissue. Mild neutrophilic infiltration was also seen (Fig 1). Treated group showed same type of changes in subcutaneous tissue. There was not much significant difference between control and treatment.

On the first day, neutrophils along with lymphocytes were seen in the interstitial tissue in control group but the prominent cells were neutrophils. A few numbers of macrophages were also found in the tissue. In subcutaneous tissue the venules and capillaries were engorged with blood (Fig 2&3). In dermis and epidermis large number of leucocytes were seen. Haemorrhage was evident. Exudates contained considerable amount of fibrin. Treatment group also showed infiltration of neutrophils along with

dilatation of lymph channels. However the changes were comparatively less intensive.

On the second day control group revealed extensive area of haemorrhage along with infiltration of neutrophils, lymphocytes and macrophages (Fig 4). Some of the macrophages appeared to acquire more cytoplasm and converts into epithelioid cells. Vacoulation of muscle fibres were seen and active myophagia was evident. Dilation of blood vessels were also seen. Collagen appeared in the inflammatory area and macrophages tended to accumulate in focal areas. Fibrin was also present. In treatment group the inflammation proceeded in a much slower pace. Though myophagia, number of macrophages, lymphocytes and epithelioid cells were present,they were much reduced compared to control group of animals. Fibroblast proliferation was also noticed. Areas of haemorrhage were still present but neutrophils were the prominent cells. (Fig 5).

On the third day, control group showed necrosis on a large scale and active myophagia. (Fig 6). Large numbers of epitheliod cells were present. Dilation of blood vessels in the subcutaneous areas were visible. Capillary walls were adhered with leucocytes. Macrophages and monocytes were seen. Fibroblastic

proliferation was prominent. In treatment group at 3rd day the changes noticed were similar to 2nd day of control group. Necrosis was noticed on a large scale and there was moderate lymphocyte infiltration. There was accumulation of phagocytes. But granulomatous reaction failed to appear.

On 9th day the control group revealed large number of epithelioid cells surrounding oil droplets. In certain areas large number of lymphocytes were present. Fibroblastic proliferation continued (Fig 8). Treated group also showed similar changes in a reduced manner (Fig 9).

On 13th day, control group showed extensive epithelioid reaction continued with mononuclear cell infiltration. Focal areas of neutrophil infiltration were still present. Fibroblastic activity was also evident. Exudates contained *considerable amount of fibrin (Fig 12).* Myophagia was very high. In treatment group, moderate epithelioid reaction was noticed. Granuloma formation (Fig.11) and fibroblast proliferation were also evident (Fig. 10).

The granulomatous reaction was very prominent. Giant cells appeared in tissues (Fig 15). Infiltrating cells were mostly

mononuclear. Connective tissue formation was more compared with treatment group (Fig 14). Cyst formation was very prominent. Muscle started regenerating (Fig 16). In treatment group, changes were very much reduced. Giant cells were not found. Connective tissue formation was also less compared to control group.

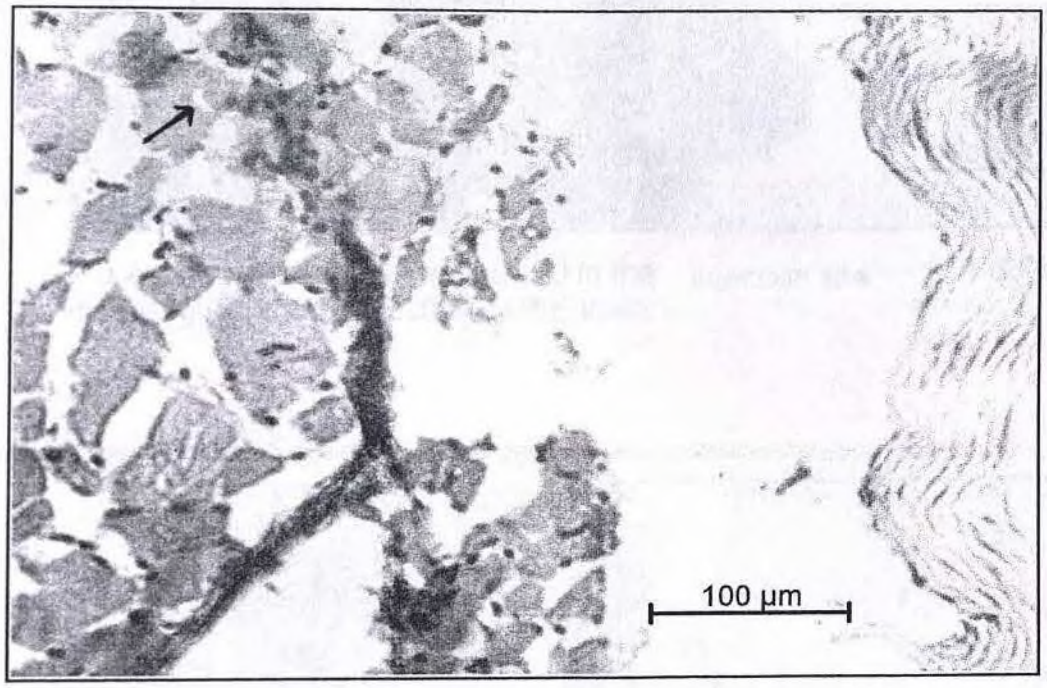

Fig.1 - Mild neutrophilic infiltration and muscle necrosis at 3 hpi of FCA in control group; H&E stain

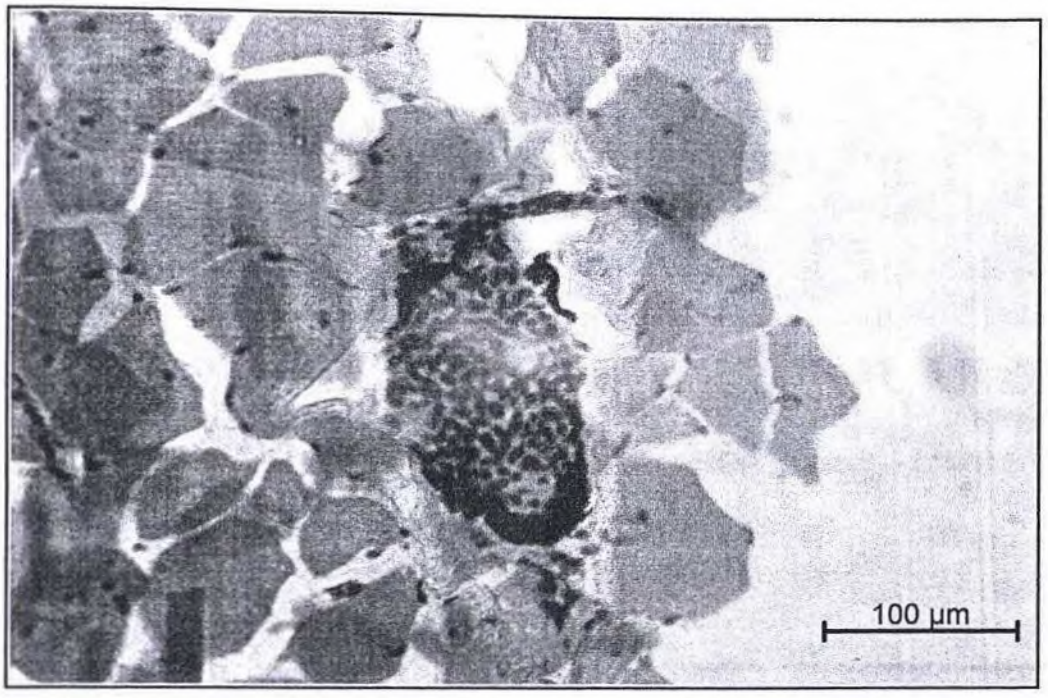

Fig.2 - Congestion of blood vessel in the injection site at 1 dpi in the control group (cross section); H&E stain

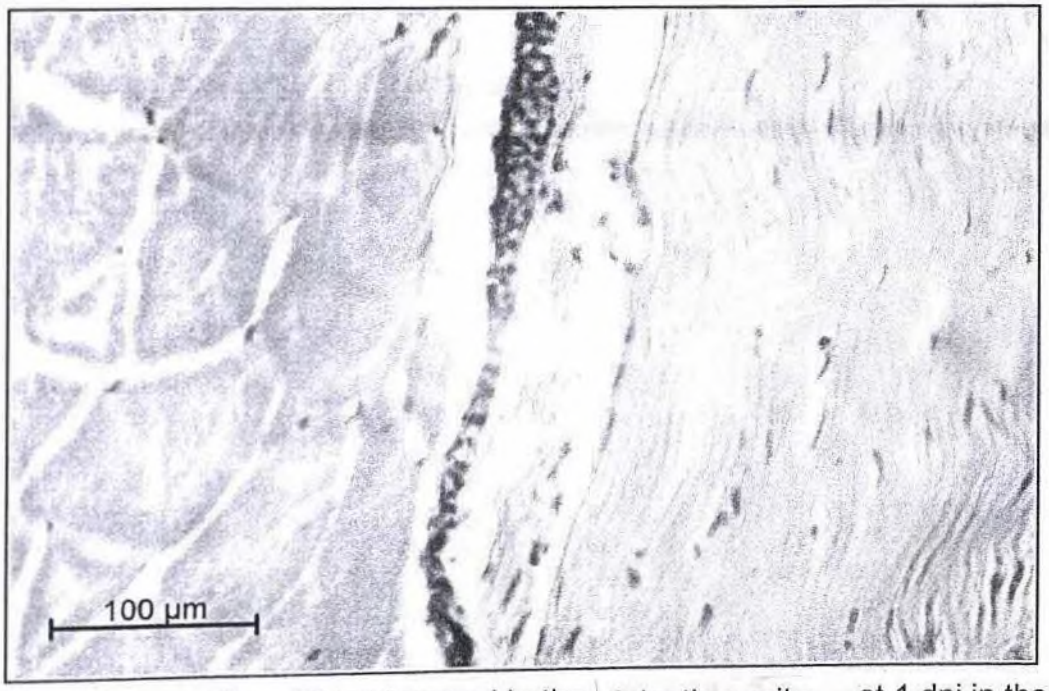

Fig.3 - Congestion of blood vessel in the injection site at 1 dpi in the control group (sagittal section); H&E stain

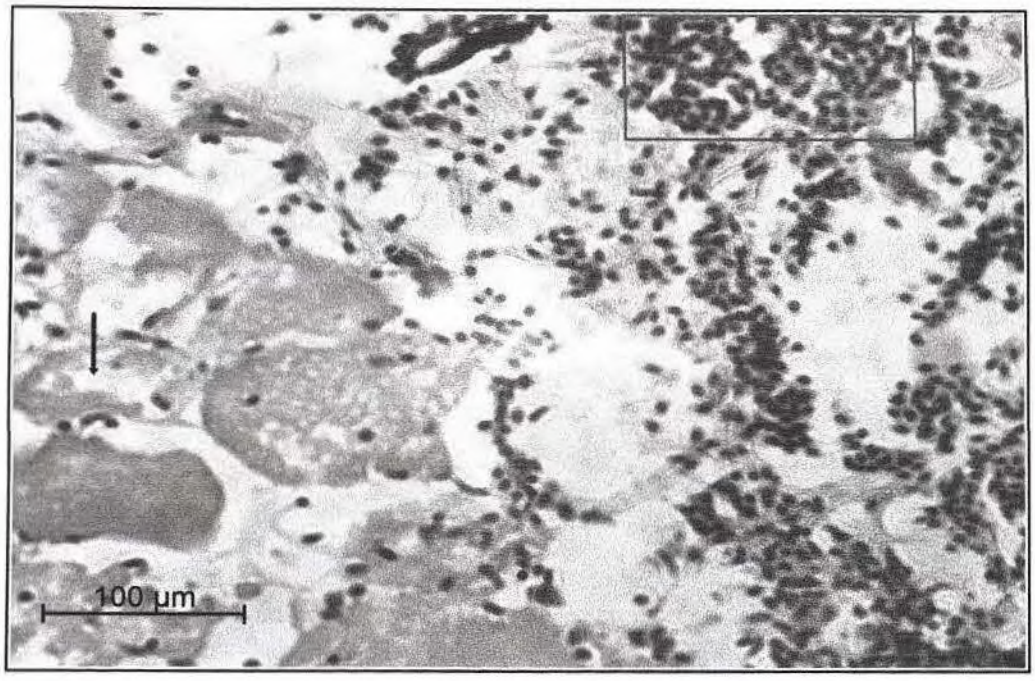

Fig.4 - Muscle necrosis (arrow) and haemorrhage (box) and infiltration of phagocytic cells in the inflammatory site at 2 dpi in the control group; H&E stain

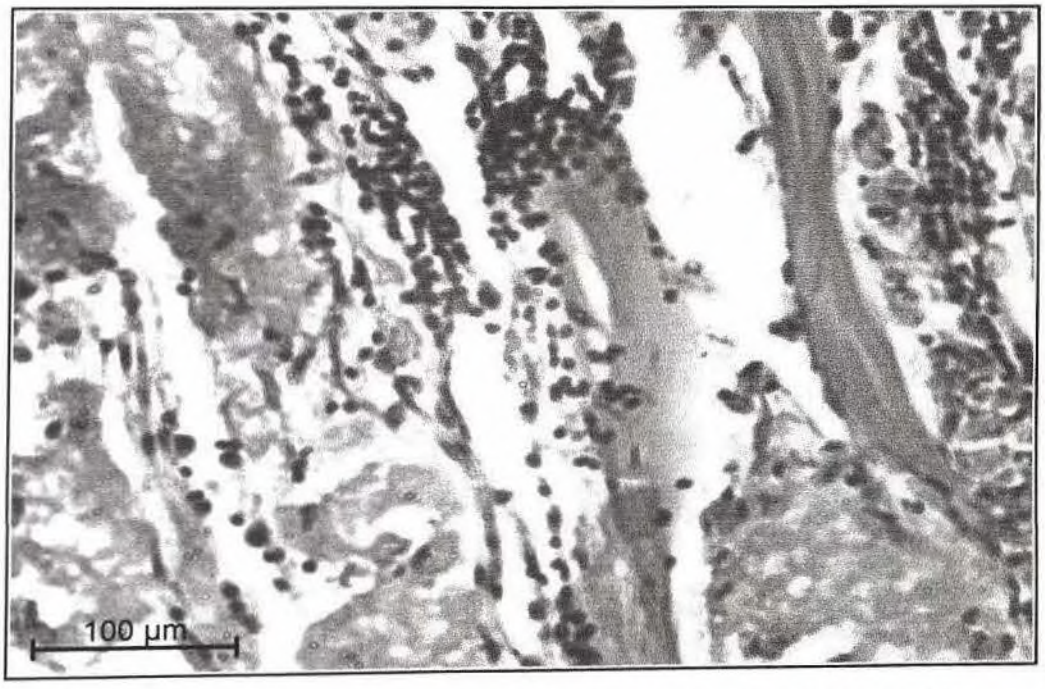

Fig.5 - Inflammatory area showing muscle necrosis and neutrophil infiltration at 3 dpi in treatment group; H&E stain

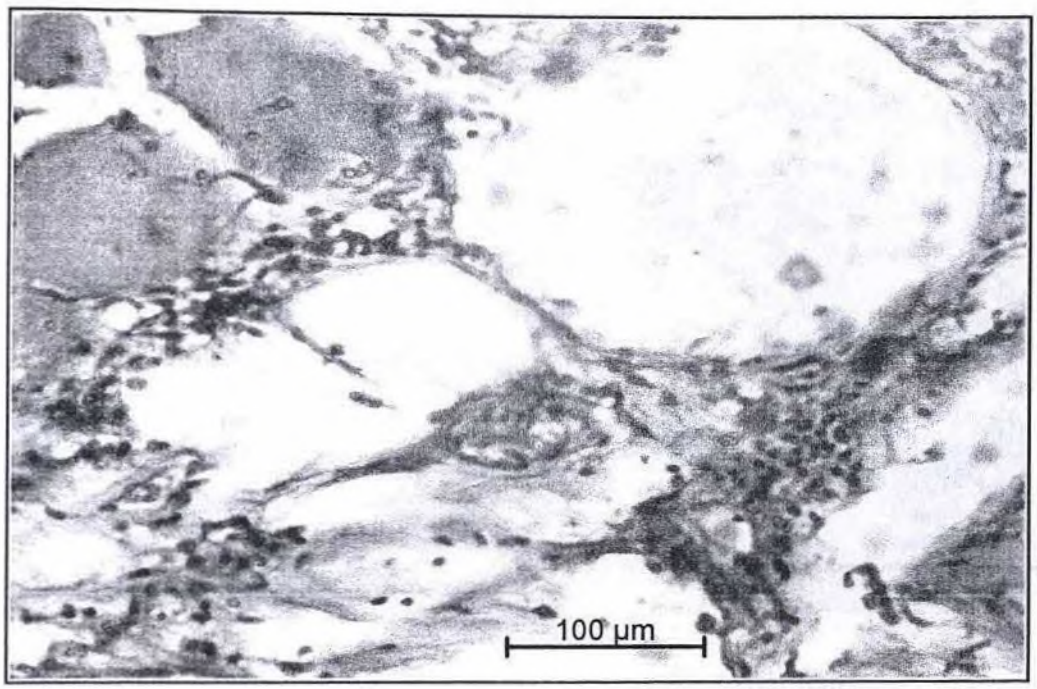

Fig.6 - Inflammatory area showing the adjuvent droplets surrounded by phagocytic cells at 3 dpi in control group; H&E stain

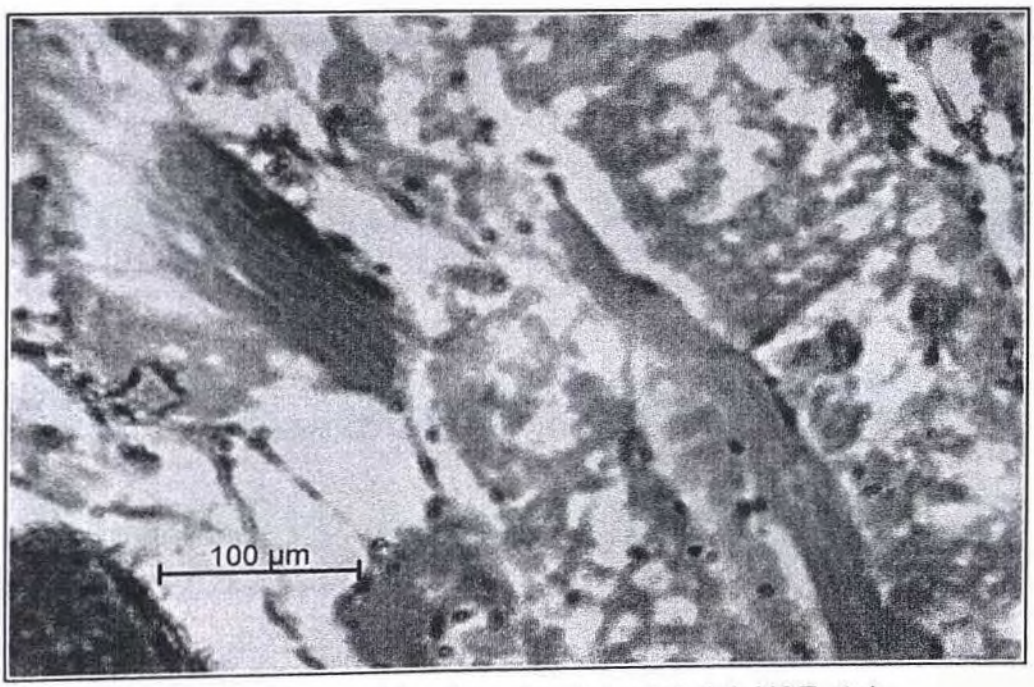

Fig.7 - Inflammatory area of treatment group at 3 dpi; H&E stain

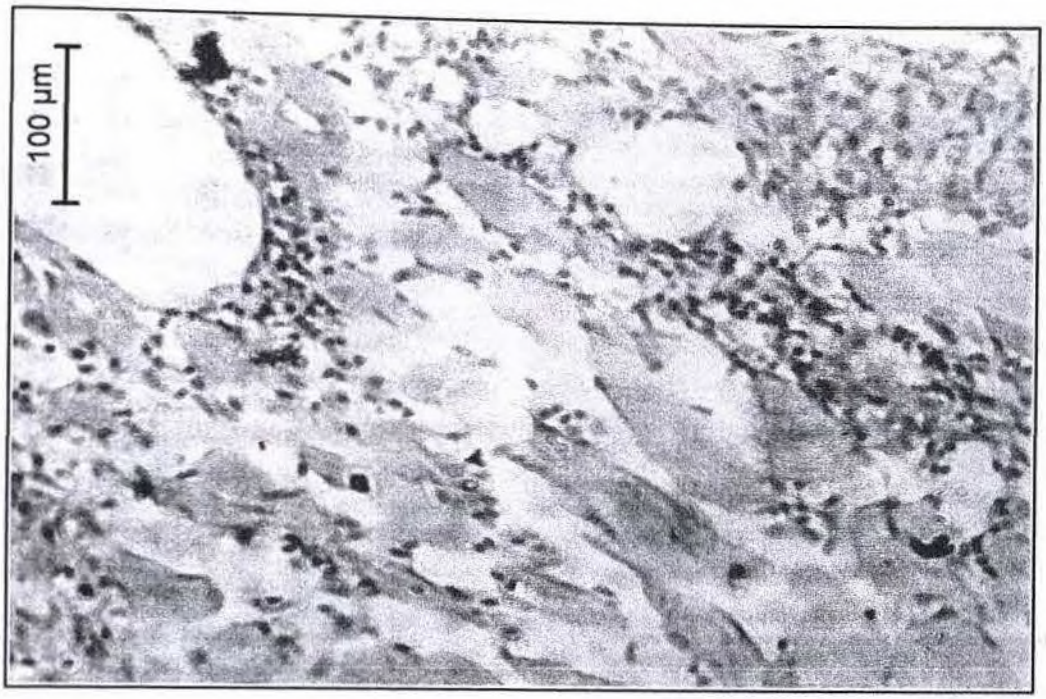

Fig.8 - Inflammatory lesion at 9 dpi in the control; H&E stain

Fig.9 - Inflammatory lesion at 9 dpi in the treatment group; Phloxine-Saffron Masson (modified) stain

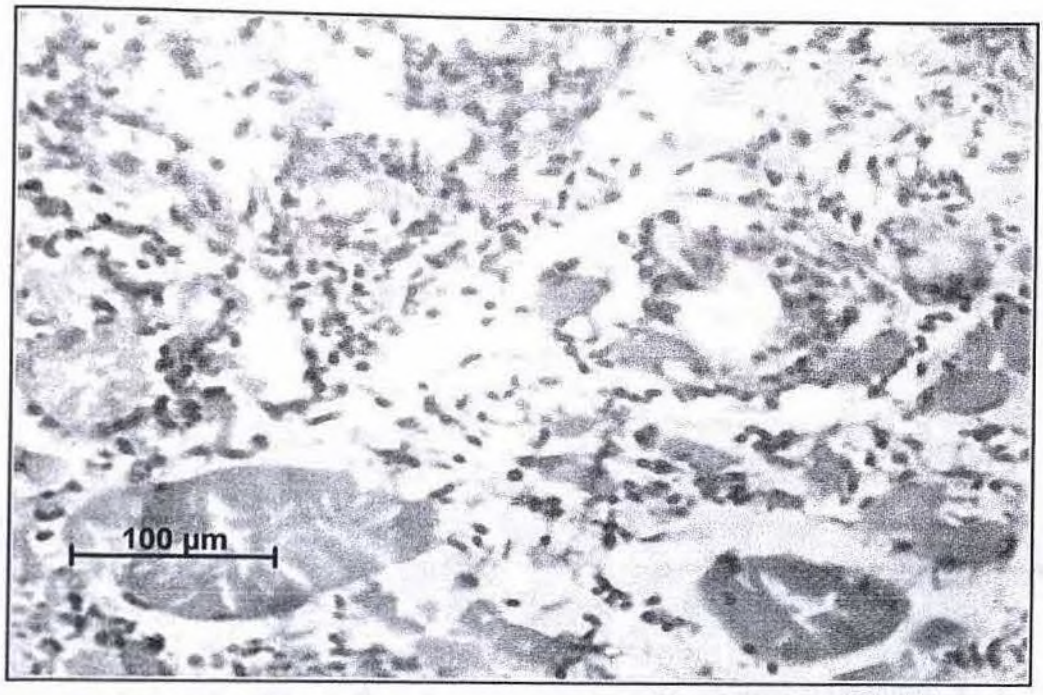

Fig.10 - Fibroblast proliferation in the inflammatory lesion at 13 dpi in tratment group; H&E stain

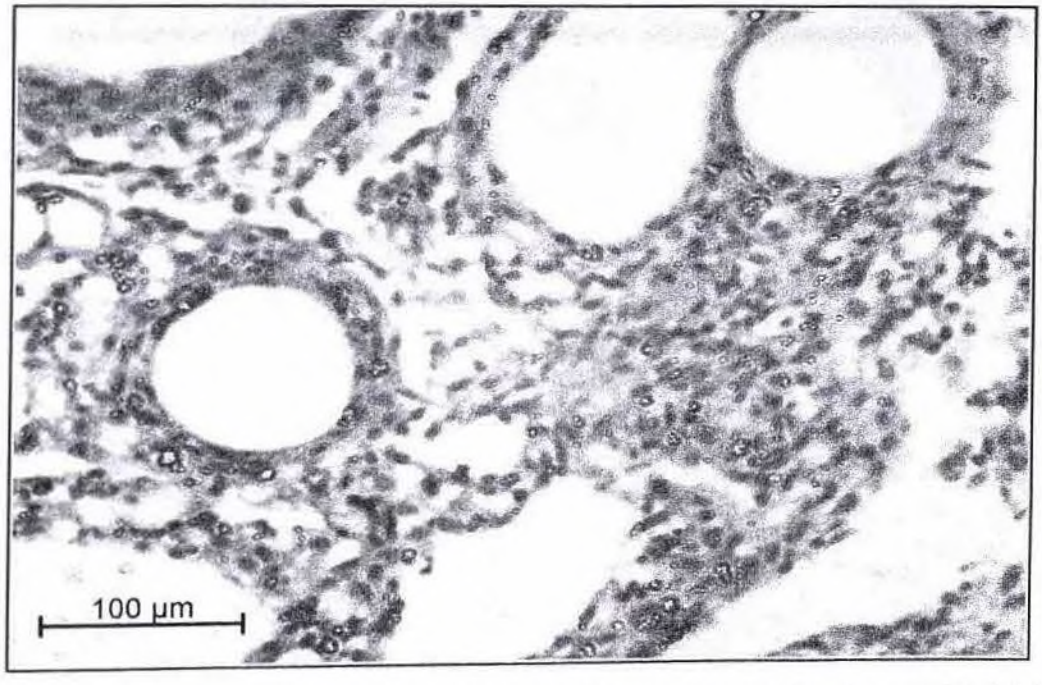

Fig.11 - Encapsulation response around the adjuvent droplets at 13 dpi in the treatment group; H&E stain

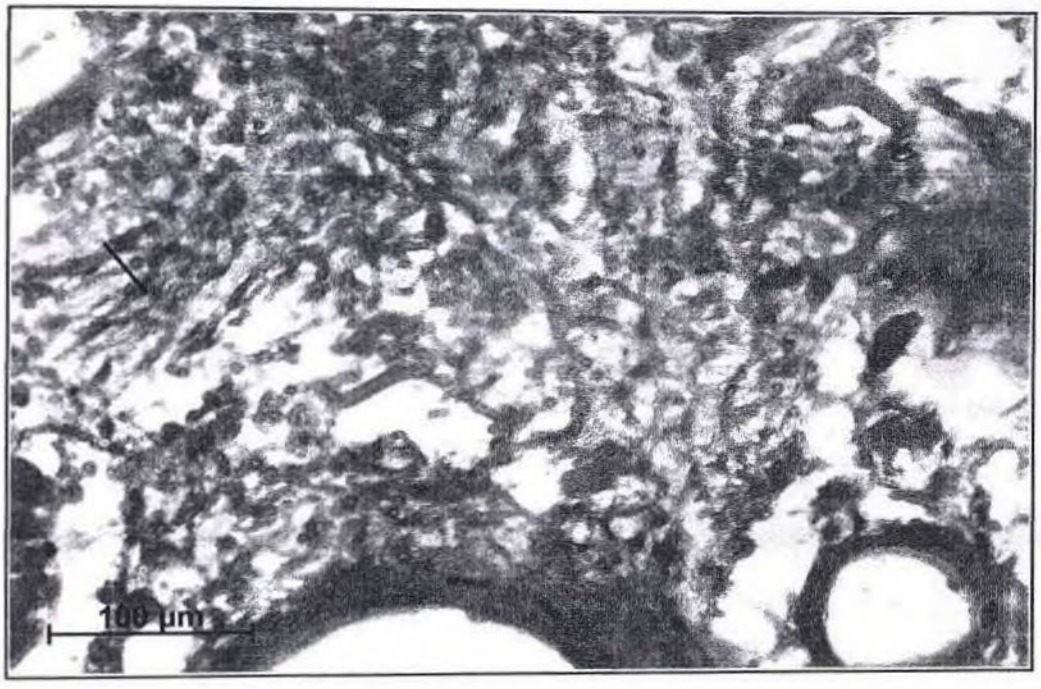

Fig.12 -Fibrinous exudate at 13 dpi in control group; Phloxine-Saffron Masson (Modified) stain

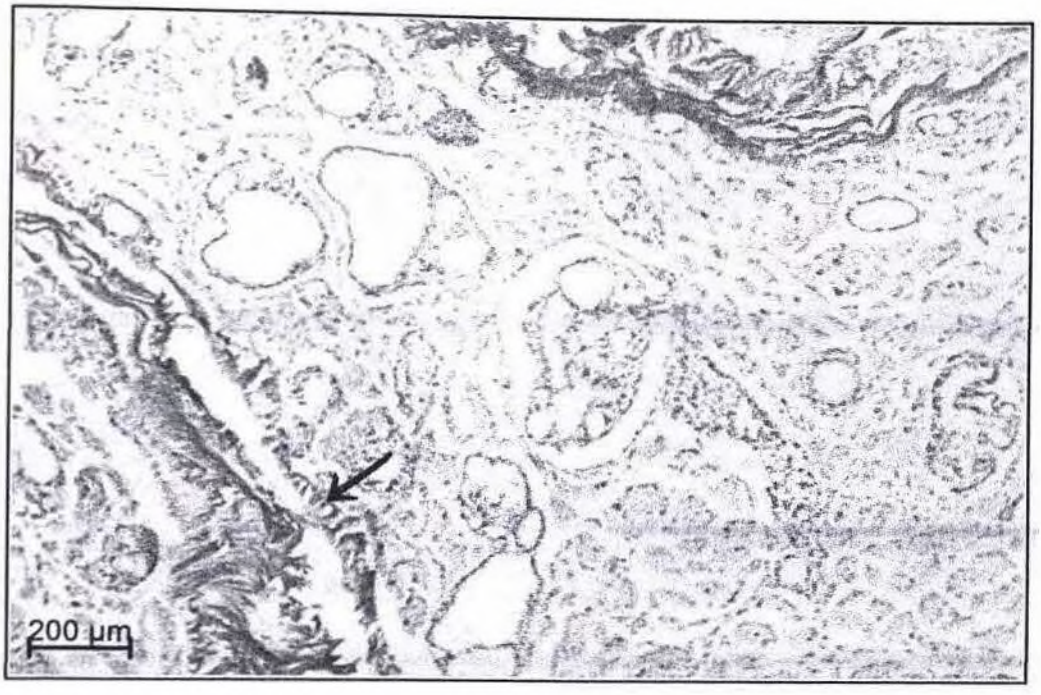

Fig.13 - Connective tissue formation at 15 dpi in the control group; Van Gieson's stain

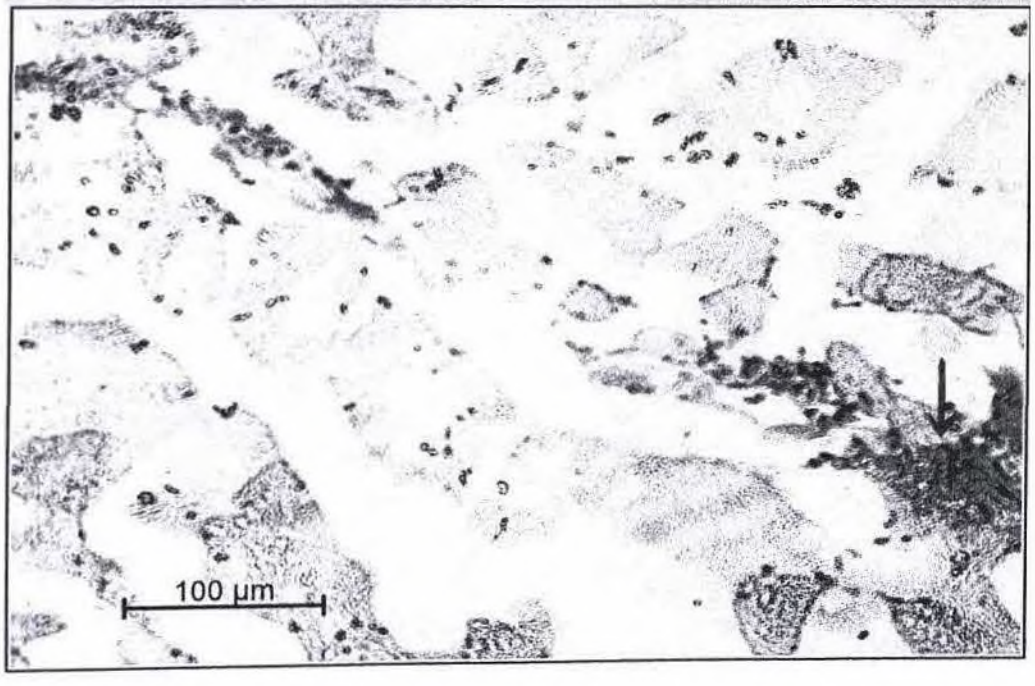

Fig.14 - Connective tissue formation at 15 dpi in the treatment group; Van Gieson's stain

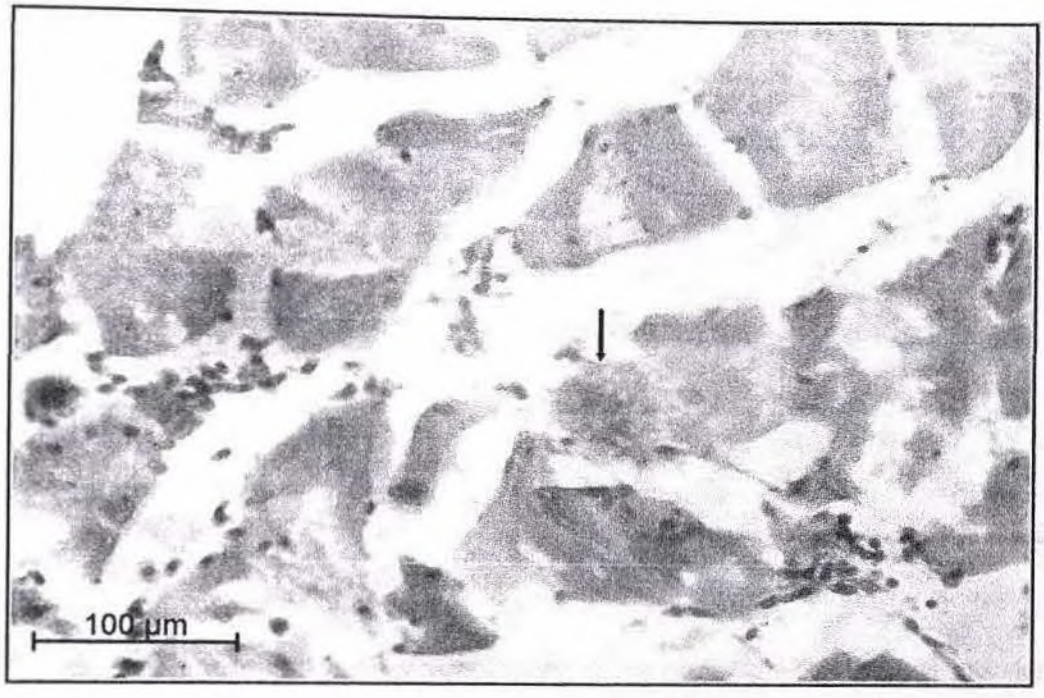

Fig.15 - Formation of multinucleated giant cell (arrow) at 15 dpi in the control group; H&E stain

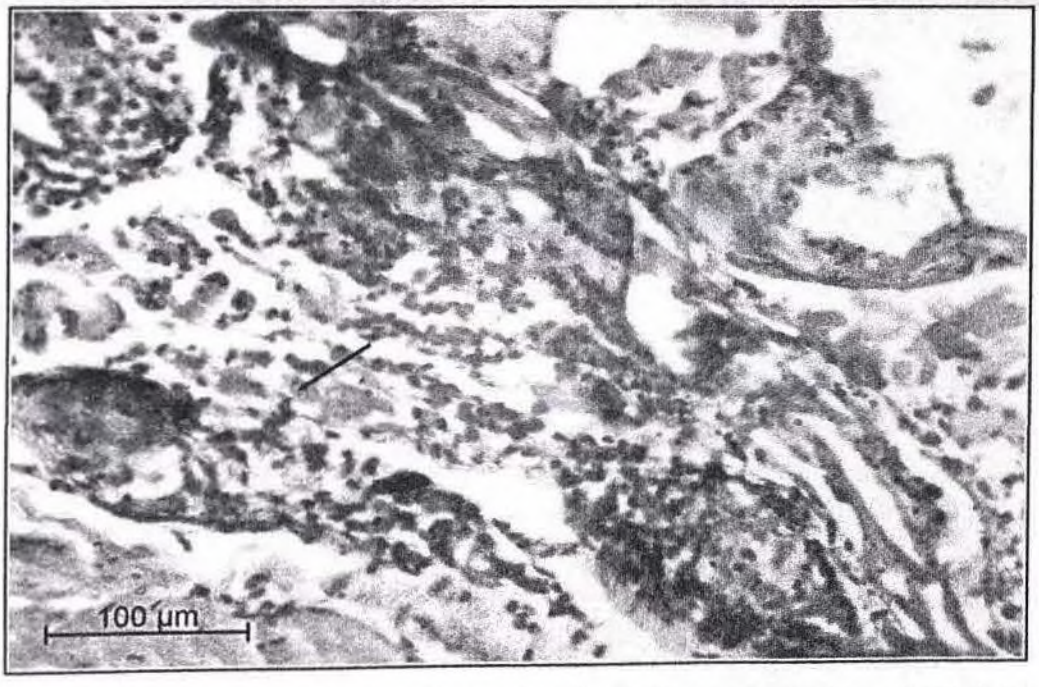

Fig.16 - Muscle regeneration (arrow) in the inflammatory area at 15 dpi; H&E stain

DISCUSSION

DISCUSSION

Present study consisted of induction of inflammation in *Etroplus suratensis* by intramuscular injection of Freund's Complete Adjuvant (FCA). Inflammation was induced in two groups of animals. The first group served as control, whereas the second groups of animals were exposed to 0.02 ppm Nuvan, an organophosphorus pesticide one week before the start of the experiment and throughout the experimental period. The inflammatory response at 3hrs and 1,2,3,9,13,15 days post injection were assessed by histological method.

The initial response in two groups was characterised by vascular congestion, increased vascular permeability and mild neutrophilic infiltration. There was not much difference between the two groups. In vertebrates the initial inflammatory changes are vascular congestion, increased vascular permeability and neutrophilic infiltration. The studies conducted in fish also revealed the same. Weinreb (1959) in experimental turpentine inflammation of rainbow trout observed edema, dilation and congestion of blood vessels with endothelial swelling continuing throughout the first 6 hrs.

Rapid migration of neutrophils to the inflammatory site observed in the present study was also reported earlier by a number of workers. Finn and Nielsen (1971a,b) observed that in rainbow trout inflammation neutrophils were the first migrating cells followed by macrophages. Phromsuthirak (1977) stated that in stickle back neutrophils increased within the first 24 hrs followed by macrophages for the next 3 days. Mac Arthur *et al.* (1984) and Croll and Grizzle (1998) reported that neutrophils reached peak within 2 days of the start of the inflammation, while macrophages appeared more gradually and reached maximal numbers on the 7^{th} day after injection.

Suzuki and Hibiya (1983) reported detailed vascular changes in fish. Increased permeability of blood vessels was detected by intravascular injection of colloidal carbon (Manjo and Palade, 1961). Increased vascular permeability also resulted in the exudation of plasma protein (Suzuki and Hibiya, 1981). In our study the neutrophil infiltration was present from 3 hrs and continued upto 13 day. Macrophages started appearing from 24 hr onwards. The present observations were in agreement with the above described studies.

Though our study is in general agreement with the works done on inflammation by other workers, there are some differences. The neutrophils appeared during early stages in the inflammation but the peak appeared much later. Most of the works on inflammation has been done in temperate species of fishes. But the present study was conducted on a tropical species, which may explain the differences observed in the responses.

Lymphocytes were the cells appeared second to neutrophils. They started appearing since 24 hr post injection. Finn and Nielsen (1971a,b) and Mac Arthur et al. (1983) also observed the same type of cells in their study.

Muscle necrosis was observed in the present study at 3rd hr onwards and necrotic muscles were attacked by phagocytic cells (myophagia). Myophagia commenced on the 2nd day. Anderson and Roberts (1975) reported early myophagia at 24 hrs. Finn and Nielsen (1971a,b) reported myophagia at 48hrs. Fibroblastic proliferation started 2nd day onwards and continued beyond 15 days. This is due to the healing process and in response to the products released at the site. Finn and Nielsen (1971a) reported fibroblast to be initially present within 4-8 days.

Finn and Nielsen (1971a) observed no sarcoplasmic budding or muscle fibre regeneration over the 16-day period. Similarly Roberts *et al.* (1973) working with salmon, and Mawdesley-Thomas & Bucke (1973) working with gold fish found myofibrilar regeneration to be far outweighed by replacement fibrosis. However in the present study myofibrillar regeneration was observed on the 15th day following adjuvant administration. In the above cited studies severe fibrosis was observed because the muscle damage was extensive. Hence replacement by connective tissue might have taken place. However the present results were similar to the results obtained by Finn and Nielsen (1971a).

In our study the neutrophil infiltration was less intensive in Nuvan treated group compared to control group. Similarly in control group granulomatous inflammation appeared on 3rd day which progressed beyond 15th day and presence of giant cells were noticed on 15th day. Whereas in treatment group granulation reaction did not appear on 3rd day. It was evident on 9th day however the 9th day reaction were less intensive compared to the control group .On 15th day though fibrosis appeared the fibroblastic changes were less intensive and muscle regeneration was also comparatively less indicating much suppressed reaction.

In mammals chronic stress causes hypersecretion of corticosteriods. This will reduce the inflammatory response as well as immune reactions (Wedemeyer, 1970). In fishes administration of corticosteroids induces susceptibility to diseases and retard wound healing, immune response, inflammatory response and migration of leucocytes (Pickering and Duston, 1983; Mac Arthur *et al.*, 1984; Roubal and Mullock, 1988; Carlson *et al.*, 1993)

The work by Holladay *et al.* (1996) established that organophosphorus compounds lower the leucocyte precursors in pronephros. The neutrophils stored in the pronephros is the source of cells in the peripheral blood and a reduction in the pronephros cell would affect the migration of leucocyte into the inflammatory site.

The much suppressed reaction in the treated group could be explained either due to stress acting through the release of corticosteroids from the adrenal or due to the reduction in the number of cells in the hematopoietic tissue. In the present study we have not measured the corticosteriod level in the serum and also not examined the pronephros. Further studies in this direction are necessary to point out the exact cause. However from the present study it is evident that the pesticide can modulate the inflammatory response.

SUMMARY

SUMMARY

- A study was conducted to evaluate the effect of Nuvan (organophosphorus pesticide) on the inflammatory response in *Etroplus suratensis*.
- Fishes were exposed to 0.02ppm Nuvan one week before inducing inflammation and throughout the experimental period. Control group of animals was also maintained.
- Tissue samples from inflammatory site were taken at intervals of 3hrs and 1,2,3,9,13 and 15 days post injection and subjected to histological analysis.
- The initial inflammatory response in both groups consisted of vascular congestion, neutrophilic infiltration and muscle necrosis.
- Macrophage started appearing on 1st day onwards.
- Granulomatous reaction appeared on the 3rd day in control group while the treated group failed to show this reaction on 3rd day.
- Myophagia was observed in both groups; however in the treatment group the response was less.
- Giant cells appeared on 15th day in control group; whereas giant cells were not seen in the treatment group on 15th day.
- Connective tissue formation was less in treated group compared to control group.
- Muscle regeneration was observed on 15th day in control group.

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