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HERBAL ALTERNATE TO *PSEUDOMONAS AERUGINOSA* INFECTION IN A FRESHWATER CRAB, *OZIOTELPHUSA SENEX SENEX*

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ABSTRACT

The crabs, *Oziotelphusa senex senex* was injected with *Pseudomonas aeruginosa* (0.1 ml of 10^7 cfu/ml). After injection of bacteria, 30 crabs were allowed to withstand for 96 hrs. After 96 hrs ten crabs were dissected and the tissues (brain, ovary, gills) were used for histological study. The brain neurosecretory cells (NSC) were clumped; ovaries showed shrunken oocytes, germinal cells are missing. In gills, epithelial lifting (EL), necrosis (NCR), Disrupted pillar cells (DPC) were observed. Remaining twenty bacterial injected crabs were treated with 0.05 ml of 80% *Psidium guajava* leaf ethanol extract (1000 ppm), after 96 hrs, histological alterations in the tissues (brain, ovary, gills) of the experimental group crabs were restored. These results suggests that the *Psidium guajava* could combat the microbial infection by stimulating the immune response in crabs.

Keywords: Neurosecretory Cells, Necrosis, Disrupted Pillar Cells, *P.guajava*, *O. senex senex*

INTRODUCTION

Disease outbreaks are being increasingly recognized as a significant constraint on aquaculture production and trade, affecting the economic development of the sector in many countries. Shell disease syndrome is an economically important disease of crustaceans that is characterized by the degradation of the exoskeleton by chitinolytic bacteria. *Pseudomonas sps* causes hemorrhagic

septicemia resulting in hemorrhage and ulceration of the skin.

The large scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment **[1, 2]**. The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in the aquatic environment. Continuous use of synthetic antibiotics reveals the threats to consumers and non target organisms in the environment **[3]**. Safe treatments of bacterial diseases with various herbs have been used widely in organic agriculture, veterinary and human medicine **[4]**. Since ancient times, medical plants have been used for the treatment of common infectious diseases **[5]** and treatments with plants having antibacterial activity are a potentially beneficial alternative in aquaculture **[3, 6]**.

Medical plants as the alternative agents are effective to treat the infectious diseases and mitigate many side effects that are associated with synthetic antimicrobials. In addition, plant derived phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents in this field **[7, 8]**.

MATERIALS AND METHODS

Experimental Animal and Treatment

The female crabs, *Oziotelphusa senex senex* were collected from Vandalur Lake, 40 kms away from Chennai city and were acclimatized in the lab and were divided into four groups of ten each. Group -A is the initial control and was sacrificed on the 1st day of the experiment and the tissues such as brain, ovary and gills were taken for histological study. Group-B was injected with ethanol and the group- C was injected with *P.aeruginosa* 0.1 ml of 10^7 cfu/ml /crab. Group

– D experimental crabs were injected with sub lethal dose of *P.aeruginosa* 0.1 ml of 10^7 cfu/ml. After injection the crabs were allowed to withstand for 96 hrs. After 96hrs ten crabs were dissected out and the tissues such as brain, ovary and gills were taken. Remaining bacterial injected crabs were treated with 0.05 ml of 80% *P.guajava* leaves ethanol plant extract (1000 ppm) after 96 hours target organs (brain, ovary and gills) were processed for histological studies **[9]**.

RESULTS

Histological studies on the brain of uninfected crabs exhibited a normal histological structure. Group-A show more number of neurosecretory cells (NSCs) distributed along the peripheral region. The NSCs are clear with moderate staining intensity. The NSCs are with the nucleus and nucleolus. The

bacterial infection in the crabs leads to an extensive damage of the brain. The group-C show clustered NSCs pushed to one corner and have no neurosecretory materials (NSM). The cytoplasm is unclear. The nucleus and the nucleolus are invisible. The shape and size of the NSCs are indistinguishable and are found scattered on the periphery Group-D show more number of NSCs distributed along the peripheral region. The NSCs are clear with moderate staining intensity. The NSCs are with nucleus and nucleolus **(Figure 1)**.

Ovaries of uninfected crabs showed normal histological architecture with many oocytes showing various stages of maturation. Histopathological studies of infected ceab's ovary showed thin ovarian wall and ruptured oocytes, and some oocytes shows disintegration. The oocytes were poorly developed and very less in number. All the oocytes lost their ooplasmic contents. *Psidium guajava* treated crabs show intact oocytes. The size of the oocytes were enlarged. In all the oocyte the nucleus and nucleolus is invisible and are laden with homogenous yolk substances **(Figure 2)**.

Uninfected crabs gill rachis is normal with less haemocytes and the pillar cells are intact. Necrosis is not seen. The gill filaments are intact with even interlamellar space. *P.aeruginosa* infected crabs gills haemocytes (HE) are accumulated in the haemocoelic space. Necrosis (NCR) is seen in most of the gill rachis. Epithelial lifting (EL) and Disrupted Pillar cells (DPC) were observed. After 96 hrs *P.guajava* treated crabs show intense staining reaction with intact gill rachis and gill filaments. The pillar cells (PC) are intact. The gill filaments are with moderate haemocytes (HE).The gill tips are intact **(Figure 3)**.

DISCUSSION

The present study was carried out to investigate whether the injection of ethanol extract of *P.guajava* protecting *O.senex senex* against *P.aeruginosa* infection Therefore, the leaves extract was used to challenge with *P.aeruginosa* to study the specific immunity on crab. Opportunistic pathogen *P. aeruginosa* is an easily infectious bacterium, mostly when crabs are exposed to both physical and environmental stressors. The histological studies of brain, ovary and gills show interesting results.

The present investigation not only reveals that the crabs are in stress due to *P.aeruginosa* infection it also reveals that there occurred a tissue damage in all the tissues (brain, ovary and gills) studied. In brain the NSM is very little and the sizes of the NSC are very small and are indistinguishable into different types as reported by **[10]**. The ovarian histology of

the crabs studied in this present investigation is entirely different from the normal non contaminated crabs reported earlier from our laboratory. The oocytes are very much shrunken, reduced in size, germinal cells are missing and disintegration of ooplasmic substances are seen. Further the results of the gill histology also clearly suggest the effect of *P.aeruginosa.*

In aquatic organisms, the gills represent a vital organ, since they play an important role in the transport of respiratory gases and regulate the osmotic and ionic balance. Toxic substances may cause damage to gill tissues, thereby reducing the oxygen consumption and disrupting the osmoregulatory function of aquatic organisms **[11]**. In the present study, bacterial treatment has resulted in notable structural alterations of the gill lamellae including accumulation of haemocytes (HE) in the haemocoelic space, epithelium lifting (EL), necrosis (NCR), disrupted pillar cells (DPC). Similar lesions have been reported to occur in the prawns, *M. kistensis* exposed to copper sulphate **[11]**. The results suggest that *P. guajava* extract may provide a new therapeutic value in specific and nonspecific immunity in the fresh water crabs.

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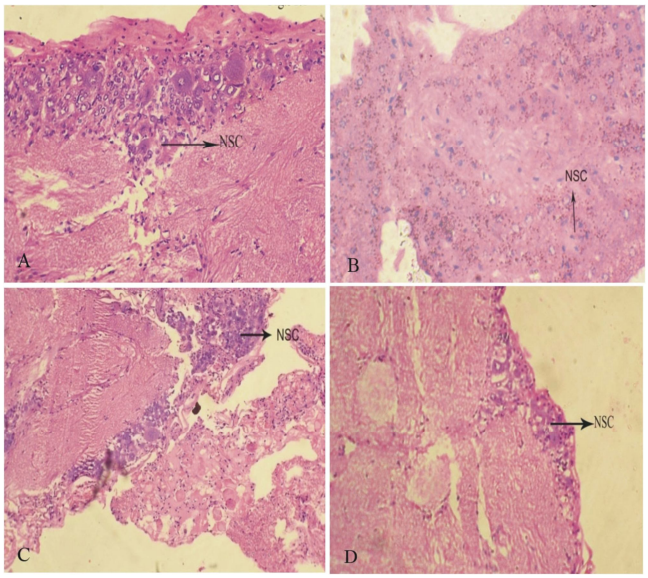

Figure 1: Photomicrograph of Cross Section of Brain Showing Neurosecretory Cells
NOTE: NSC – Neurosecretory cells, Stained in HE X 425

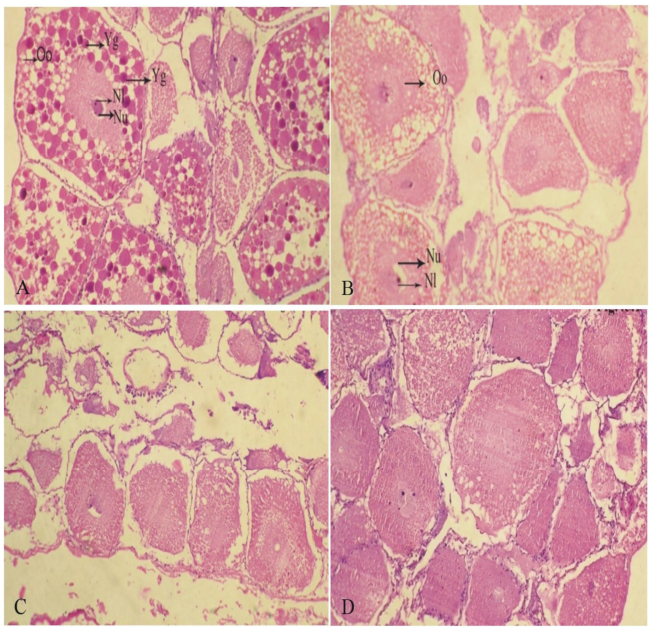

Figure 2: Photomicrograph of Cross Section of Ovary Showing Oocytes
NOTE: Oo – Oocytes Yg – Yolk Globules, Nu – Nucleus, Nl – Nucleolus Stained in HE X 425

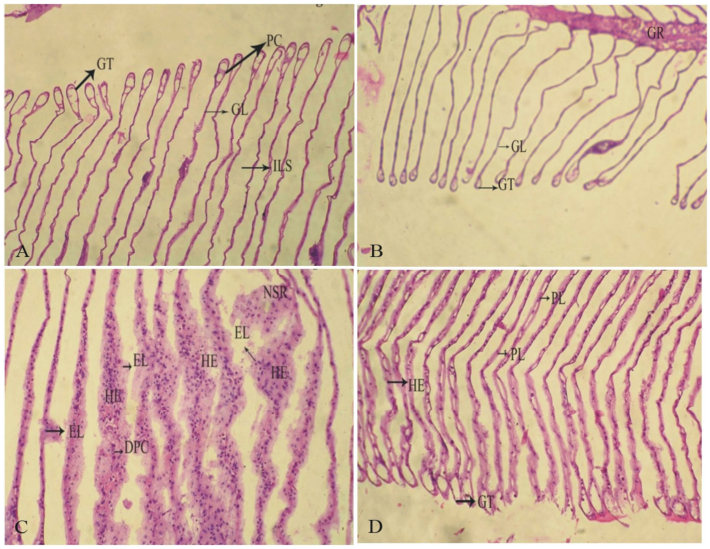

Figure 3: Photomicrograph of Cross Section of Gills Showing Gill Lamellae; GL – Gill lamellae, ILS – Interlamellar space; GT – Gill Tips; PC – Pillar cells; GR – Gill rachis; HE – Haemocytes; NCR– Necrosis; DPC – Disrupted Pillar Cells; EL – Epithelium lifting, Stained in HE X 425