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Influence of *Vibrio parahaemolyticus* MTCC 451 on the levels of ascorbic acid and histamine in *Penaeus monodon* (Fabricius)

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Abstract: *Juvenile shrimps (Penaeus monodon) weighing about 15-20 gms were procured from the grow-out ponds and reared under laboratory conditions. They were challenged with Vibrio parahaemolyticus MTCC 451 and their LD_{50} value was evaluated. The ascorbic acid and the histamine activity of both the control and experimental groups were assessed. The results showed a marked decline in both the parameters at all intervals. This reveals the pathogenesis of the inoculated bacterial strain, which affects the defense mechanism by reducing the glutathione content by the decreased level of ascorbic acid and letharginess by the decreased histamine content of the experimental prawns.*

Key words: *Penaeus monodon, Vibriosis, Ascorbic acid, Histamine and pathogenesis.*

Introduction

Penaeid shrimp production is a worldwide economic activity, primarily important for inter-tropical developing countries. The intensification of shrimp farming over the last few decades has been accompanied by the development of infectious diseases from viral, bacterial and in some cases, fungal origin. The bacterial strains responsible for virbriosis in the successive stages are usually considered to be different and their virulence specificity has been reported both at the species level and at the stage levels. *Vibrio spp*. are most often considered opportunistic pathogens in shrimps, but primary disease caused by highly virulent strains has as also been reported. Diseased prawns display a wide spectrum of clinical signs, including disoriented swimming, lethargy, weakness and abnormal colouration of the body and appendages (Lightner, 1996). It is known that in animals, manifestation of the disease by pathogens involve the breakdown of homeostasis, when the latter organisms and their load reach beyond the threshold limit of tolerance of the host animals.

Ascorbic acid (Vitamin C) is a water-soluble vitamin and acts as an enzymatic chain breaking antioxidant (Mayes, 1988). It reduces the glutathione content for defense mechanism during its role in the stress and disease resistance. As an antioxidant, it protects the cellular components from free radical damage. Ascorbic acid has also been proven to protect the membranes and other hydrophobic compartments from damage by regenerating the antioxidant form of Vitamin E, and its action has been reviewed extensively by Som *et al*., (1980). Since a monohydroascorbic acid with a single electron product, it is highly stable and comparatively non-reactive and serves as good antioxidant. It has been shown to scavenge free radicals directly in the aqueous phases of cells and the circulatory system. Vitamin-C has been the most frequently studied factors relating to disease resistance in animals, including fish. There are reports of a positive effect of high ascorbic acid on disease resistance in fish.

A perusal of review of literature reveals that vitamin C undoubtedly has a positive effect on some mechanisms of the natural resistance against infections as well as on the immune response. Shrimp with ascorbic acid deficiency shows moulting incompetence, malformation of carapace, disorder of the gill and associated high mortality. Information of vitamin deficiency in shrimp is scarce. The well-documented evidence is the black death syndrome related to vitamin C deficiency in penaeid shrimps (Lightner, 1977).

Merchie *et al.,* (1997) suggested the addition of ascorbic acid feed for the enhancement of resistance to stress conditions and bacterial infections in shrimps. Levenson *et al.,* (1946) revelaed an abrupt and often sustained drop of ascorbic acid in the blood and tissues of animals after pathogenesis. Hunter *et al.,* (1979) reported black death in *Penaeus californiensis* due to deficiency of ascorbic acid, which was revealed to be essential for the hydroxylation of proline in collagen formation in shrimps. Chen and Chang (1992) revealed the necessary requirement of ascorbic acid for *Penaeus monodon* for its optimal growth. Michael *et al.,* (1998) revealed an inverse relationship between the ascorbic acid level and antibody response in *Oreochromis mossambicus*. Ciereszko *et al.* (1999) correlated the lack or low-level of ascorbic acid to the damage of male germ cells in rainbow trout. Ascorbyl 2 sulfate applied directly in the sea water (used to raise the black tiger shrimp larvae) could act as an anti-stress factor against toxic substances and some unfavourable conditions (Beyer, 1994).

Histamine, a biogenic amine present in the neuronal cells, is involved in the modulation and coordination of the nervous system. It is a neurotransmitter and neuroregulatory substance present in diverse species. It serves as the neurotransmitter of photoreceptor in insects and other arthropods. The development pattern of appearance of histamine in the crustacean nervous system has been studied by Beltz (1999). He has revealed the involvement of this biogenic amine in wide spread modulation and coordination

Table – 1: Ascorbic levels in tissues of *Penaeus monodon* (control) Vs inoculated with *Vibrio parahaemolyticus* MTCC 451 at different post inoculation intervals.

Tissues:	Control (0hr)	24 hr	48 hr	72 hr	96 hr
Haemolymph	1127.46 ± 15.86	775.91 ± 13.44	1075.6 ± 21.31	481.88 ± 12.51	442.74 ± 22.7
Hepatopancreas	337.03 ± 18.06	54.89 ± 8.04	53.23 ± 7.12	33.55 ± 4.97	326.93 ± 26.51
Body muscle	312.29 ± 19.21	282.56 ± 36.04	224.13 ± 17.85	207.23 ± 13.82	317.75 ± 20.75

Mean ± SD (µg/ml/µg/100mg).

Table – 2: Histamine levels in tissues of *Penaeus monodon* (Control) Vs inoculated with *Vibrio parahaemolyticus* MTCC 451 at different post inoculated intervals.

Tissues	Control (0 hr)	24 hr	48 hr	72 hr	96 hr
Haemolymph	31.45 ± 6.9	58.13 ± 2.78	36.53 ± 1.40	44.20 ± 7.89	14.93 ± 2.632
Hepatopancreas	21.61 ± 2.45	21.19 ± 2.19	23.39 ± 3.85	28.35 ± 3.90	5.09 ± 1.41
Body muscle	35.41 ± 4.32	28.19 ± 2.59	32.26 ± 3.88	23.87 ± 3.82	23.94 ± 2.61

Mean ± SD (µg/ml/µg/100mg).

within the nervous system. This transmitter acquisition is co-regulated with target maturation providing its functional contributions in mature organisms. Histamine is a messenger molecule synthesized from the amino acid L- histidine, which is done by the L-histidine decarboxylase enzymes.

Histamine production in post captured fish by the contaminating bacteria is cited by many authors as the primary cause of scombroid poisining. Potent histamine producing members belong to the family of Enterobacteriaceae (eg., *Klebsiella, Morganella* and *Hagnia*). These bacteria are commonly associated with scombroid poisoning and contain the enzyme histidine decarboxylase that converts free L-histidine to histamine.

The purpose of histamine is to produce microcirculatory changes to counter pathological changes and also to maintain homeostasis. The relationship between histamine release and proteolysis in the infected tissues had prompted the formulation of a hypothesis that histamine is bound to cell protein (Ungar, 1956). Jorge *et al.* (1998) revealed the relationship between the function of histamine and the muscular contraction and relaxation in the crab, *Cancer borealis*. There is substantial evidence that histamine is the primary factor of insect photoreceptor neurotransmitter both in visual and extra ocular systems. The purpose of this study is to correlate the clinical signs with the changes of histamine and ascorbic acid levels.

Materials and Methods

Specimens of *Penaeus monodon* weighing about 12-15 gms were collected from the local shrimp farms located around the coastal regions of Chennai, India. They were acclimatized to the laboratory conditions in glass aquarium tanks containing sea water with 35 ‰ salinity. The bacterial strain *Vibrio parahaemolyticus* MTCC 451 was procured from the Institute of Microbial Type Culture and collection (IMTCC), Chandigarh, India. The bacterial strain was cultured and inoculated at the sixth segment on the ventral side of the shrimp as described by Lightner and Lewis (1975). The lethal dosage (LD_{50}) of the bacterial strain was determined as described by Reed and Muench (1938). During the experiment, the prawns were subjected to natural Light-Dark regime (12L:12D) and the mechanical disturbances were avoided by isolating the specimens in separate tanks. Both the control and the experimental groups were maintained simultaneously.

The level of ascorbic acid and histamine in the haemolymph, body muscle and hepatopancreas were estimated using high performance liquid chromatography by the method followed by Ashman and Bosserhoff (1983).

Ascorbic acid and histamine were separated on a C-18 reversed phase HPLC at 210 nm (supelco). The ortho-phthaldialdehyde/mercaptoethanol (OPA) mixture consisting the stock solution stored at 4ºC was diluted to 1:10 with the reaction buffer (CAN) and stored. The automatic derivatisation was achieved by the sample processor made by two injections, one was OPA and the other one was the test sample with solvent NaH_2PO_4 (25mM) and sulfonate (5mM) flow set to zero. The flow rate was then increased from zero to $0.1ml^{-1}$ over the next two minutes, and further increased to $0.1ml^{-1}$ over a period of 30 seconds. Finally, the flow rate was held constant for rest of the run. The ascorbic acid derivative was eluted from the column with the gradient or buffers a and b. Both the experiments were carried out at regular intervals of 24, 48, 72 and 96 hrs respectively. The results obtained for ascorbic acid and histamine contents, in the time course study on the inoculation of *Vibrio parahaemolyticus* MTCC 451, in the various tissues were subjected to standard deviation and the significance of the difference obtained was assessed by two-way classification with replication type analysis of variance (ANOVA) for the comparison of control and experiment (Zar, 1974).

Results and Discussion

The LD_{50} value of *Penaeus monodon* inoculated with *Vibrio parahaemolyticus* MTCC 451 for 96 hrs was determined to be 2.46×10^7 cfu/0.05ml.

The levels of ascorbic acid and histamine in all the three tissues declined significantly in the experimental prawns

at all intervals with respect to the control group (Table 1 and 2).

The ascorbic acid level significantly decreased in all the three tissues viz., haemolymph ($p<0.05$), hepatopancreas ($p<0.05$) and body muscle ($p<0.05$) of the inoculated prawns at all intervals with respect to the control group.

In the present study, the ascorbic acid level decreased significantly in all the tissues and the decrease in ascorbic acid level was established as a symptom in prawns subjected to pathogenesis in earlier studies. Catacutan *et al.* (1993) reported the outbreak of several pathogenic diseases in commercial penaeid farms due to deficiency of ascorbic acid. The decreased level of ascorbic acid might be due to the low intake of ascorbic acid, as the experimental prawns did not feed due to loss of appetite and hence the less incorporation of dietary ascorbic acid. This reflected in ascorbic acid composition of tissues.

The decreased level of ascorbic acid might lead to the inhibition of moulting, poor growth and survival rate, which are the clinical signs of vibriosis. In this context, Sreevatsava and Neelkamal (1988) revealed the effect of ascorbic acid on the survival and moulting of *Macrobrachium rosenbergii* and reported highest survival rate with an increase in the ascorbic acid level. This condition might also imply poor defense mechanism in the experimental shrimps and in turn, might reduce the glutothione content, which is utilized in the defense mechanism (Mayes, 1988).

Ascorbic acid being an antioxidant plays an important role in preventing the biomembranes being attacked by the inoculated Vibrios. Since the ascorbic acid level decreased due to pathogenesis, the inoculated Vibrio act as a biological scavenger to reduce or control the lipid peroxidation of the biomembranes (Davis, 1987), thus affecting the integrity and configuration of the biomembranes.

The decrease in the ascorbic level in the present investigation also reveals the inhibition of collagen synthesis in the Vibrio-inoculated prawns. In this context, Cahu *et al.,* (1991) revealed the role of ascorbic acid in collagen synthesis during embryogenesis in *Penaeus indicus* and Hunter *et al.,* (1979) also revealed the same in *Penaeus californiensis*.

The decreased level of histamine content might be attributed to the bacteriolytic activity of the incoluated vibrio strain. The level of the enzyme L-histidine decarboxylase responsible for the conversion of the amino acid histidine to its amine histamine might be reduced which in turn could have resulted in the decreased state. This also gives a clue that *Vibrio parahaemolyticus* unlike other mesophilic bacteria, failed to convert histidine to histamine and not elevated the histamine level ie., scombroid poisoning in prawns and fishes.

There are more evidences proving the function of histamine as a neurotransmitter in the modulation and cooridination of the nervous system (Beltz, 1999) and their role in the muscular contraction and relaxation in crustaceans.

Though there are very meager reports to support the present study and results Lenistea (1971) has adduced evidence to the present study stating that histaminases from bacterial strains could limit the histamine production by autoregulation and may be considered as the factor for the decreased histamine content in the tissues as observed in the present study.

Behavioural studies on the infected fishes and prawns reveal uncoordination movements and letharginess of morbidity. Considering the above responses in fishes and prawns, microbial infection results in the inhibition of histamine synthesis in the tissues of the host and lead to disturbances in the neuromuscular activity stands justified.

In the experimental prawns, there appeared letharginess, which may be due to the decreased level of histamine in the tissues, particularly in muscles, wherein it might have caused an increase in the muscle relaxation period, attributing to lethargy. Lethargy of penaeid shrimps which represents one of the clinical signs of vibriosis (Lightner, 1983), corroborates the present results with histamine. The present study also emphasizes that low histamine levels in tissues may be taken as a biochemical indicator to the signs of vibriosis and the enzymes histidine decarboxylase may also be considered as the marker enzyme profile in the diagnosis, similar to cholinesterase in the organophosphate poisoned animals.

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