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EFFICACY OF *RICINUS COMMUNIS* L. LEAF EXTRACT AGAINST *VIBRIO HARVEYII*INFECTION IN A FRESH WATER CRAB, *OZIOTELPHUSA SENEX SENEX*

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ABSTRACT

The crab, *O. senex senex* was injected with *V. harveyi* (0.1 ml of 10^7 cfu/ml). After injection of bacteria the crabs were allowed to withstand for 96 hrs. After 96 hrs one group of crabs were dissected, some tissues and haemolymph were used for biochemical assays. Remaining bacterial injected crabs were treated with 0.05 ml of 80% *R.communis* leaves ethanol extract (1000 ppm), after 96 hours acid phosphatase and alkaline phosphatase significantly decreased in the experimental group. These result suggested that the *R. communis* could combat the microbial infection by stimulating the immune response in crabs.

Keywords: Acid Phosphatase, Alkaline Phosphatase, *V. harveyi*, *O. senex senex*, *R. communis*

INTRODUCTION

Bacteria, the major group of pathogens, pose one of the most significant threats to successful fish and shellfish production throughout the world. Bacterial diseases are responsible for heavy mortalities in both culture and wild fishes throughout the world and most of the causative microorganisms are naturally occurring opportunist pathogens which invade the tissue of a fish host rendered susceptible to infection **[1]**. Among all other bacteria, *Aeromonad, Pseudomonad* and *Edwardsiella tarda* are the major bacterial fish and shellfish pathogens which are widely distributed in aquatic organisms in nature **[2, 3]**. In coastal regions, fish have been suffered

from *vibriosis*, a bacterial disease causing losses in the fish production **[4]**.

Several *Vibrio* spp. form part of the natural biota of fish and shellfish **[5]**. Some of the *Vibrio* species such as *V. harveyi* and *Vibrio parahaemolyticus* are also associated with bacterial infections in shrimp **[6]** and are generally considered tobe opportunistic pathogens causing disease when shrimp are stressed. Among more than 20 *Vibrio* species known to be associated with human disease, *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are most important. Depending on the species involved, the clinical manifestations are different, ranging from gastroenteritis to septicaemia and wound infection **[7]**. Many sea food associated disease outbreaks have been reported worldwide **[8]**.

The World Health Organization reported that 80% of the world's population relies chiefly on traditional medicine and a major part of the traditional therapies involve the use of plant extracts or their active constituents. Due to the indiscriminate use of antimicrobial drugs microorganisms have developed resistance to many antibiotics. This has created immense clinical problems in the treatment of infectious diseases **[9]**. In India herbal medicines have been the basis of treatment and cure for various diseases / physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Although antibacterial activities of indigenous plants have been reported from many regions, they have not been systematically conducted except in a few cases thereby leading to confusion in drawing meaningful conclusions. **[10-12]** medical plants have been used for the treatment of common infectious diseases **[13]** and treatments with plants having antibacterial activity are a potentially beneficial alternative in aquaculture **[14-16]**. The objective of the present study is to evaluate the antibacterial activity and biochemical changes of *V. harveyi* infected *O. senex senex* in ethonolic extracts of *R.communis* leaf extract.

MATERIALS AND METHODS

Experimental Animal and Treatment

The female crabs, *Oziotelphusa senex senex* collected from Vandalur Lake, Tamil Nadu were brought to the laboratory and maintained in plastic tubs. Crabs were fed with beef mutton *ad libitum* and the water was changed daily and was acclimatized for 15 days in the prevailing room temperature. The crabs were divided into four groups of ten crabs each - Control (Group-A) *V. harveyi* injected crabs (Group-B), *R. communis* leaves ethanol extract treated crabs (Group-C) experimental crabs injected with sub lethal dose of *V.*

harveyi 0.1 ml of 10^7 cfu/ml. After injection the crabs were allowed to withstand for 96 hrs. After 96 hrs haemolymph, hepatopancreas, ovary, spermatheca, muscle and gills was collected from ten crabs for biochemical assays. Bacteria injected crabs (Groups) were treated with 0.05 ml of 80% *R. communis* leaves ethanol plant extract (1000 ppm) and after 96 hrs biochemical assays were repeated.

Acid Phosphatase and Alkaline

Phosphatase Analysis

Acid phosphatase and alkaline phosphatase were estimated following **[17]**.The statistical analysis system (SPSS version 17.0) software was used to analyse all the data. The data were expressed as mean ± standard error of mean (S.E.M) and the data were analysed using the Students T-test and one-way analysis of variance (ANOVA) followed by Tukeys posthoc multiple comparison test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The acid Phosphatase and alkaline shows significant increase in haemolymph, hepatopancreas, ovary, spermatheca, muscle and gills of the group-B when compared with the group-A, The level of significance is P<0.001. The acid Phosphatase and alkaline shows significant decrease in haemolymph, hepatopancreas, ovary, spermatheca, muscle and gills of the group-C when compared with the group-B, The level of significance is P<0.001 **(Table 1)**.

Table 1: Levels of Acid and Alkaline Phosphatases

Parameters	Tissues	Control	*V. harveyi*Injected	*R.communis*Injected
		Group A	(After 96h-Group B)	(After 96 hr-Group C)
	Haemolymph	3.23±0.207	*14.4±0.884	*2.5±0.310
	Hepatopancreas	4.45±0.123	*20.1±0.490	*3.2±0.154
Acid Phosphatase	Ovary	2.21±0.142	*12.66±0.501	*1.5±0.256
IU / L	Spermatheca	3.83±0.272	*11.0±0.596	*1.5±0.256
	Muscle	5.12±0.135	*15.0±0.471	*2.3±0.253
	Gills	4.71±0.099	*12.16±0.443	*3.0±0.178
	Haemolymph	10.5±0.308	*110.5±0.623	*9.5±0.623
	Hepatopancreas	53.9±0.53	*200.1±0.490	*49.3±0.403
Alkaline Phosphatase	Ovary	8.1±0.490	*54.3±0.544	*4.6±0.620
IU / L	Spermatheca	57.5±0.613	*121.3±0.720	*40±1.003
	Muscle	43.7±0.678	*110.5±0.623	*31±0.389
	Gills	35.7±0.444	*81.3±0.403	*25±0.298

NOTE: Mean ± SD of Ten Individual Observations; Group-A Vs B Vs C , *P<0.001

DISCUSSION

The present study was carried out to investigate whether the injection of ethanol extract of *R.communis*. Protecting *O.senex senex* against *V. harveyi* therefore, the leaves extract was used for challenging with *V. harveyi* and for studying the

specific immunity on crab. Opportunistic pathogen *V. harveyi* is an easily infectious bacterium, mostly when crabs are exposed to both physical and environmental stressors.

In the present investigation, the marker enzymes such as Acid Phosphatase, Alkaline Phosphatase, show variable results within the tissues of *O.senex senex*. Phosphatases are phosphomono-esterase having pH specificity, which hydrolyze various phosphate esters and liberate phosphate from the substrate. The phosphate also plays a major role in molting physiology of many crustaceans **[18]**. Alkaline Phosphatase hydrolyses the phosphorous esters in acidic medium and autolysis process of the cell after its death. Alkaline Phosphatase is a brush border enzyme involved in carbohydrate metabolism, growth and differentiation.

Medicinal plants are progressively being estimated as appropriate alternative sources of antibacterial and antiviral agents. The extract of plant *R.communis* was found to be highly effective in preventing the growth of *V. harveyi* infection in crabs. In the present study, the Acid Phosphatase and Alkaline Phosphatase levels increased in all the tissus after 96 hours of *V. harveyi* increase than in the control. The treatment of *R.communis* Acid Phosphatase and Alkaline Phosphatase activity significant decreased in all the tissues. Decrease or increase in the enzyme activity represents the stress in any organism that resuls in metabolic burden. Generally, the increased activity of acid phosphatase was attributed to the activation of the enzyme which was kept in a latent state inside the membrane of lysosomes, due to the disruption of the membrane **[19]**. Phosphatases play an important role in carbohydrate metabolism **[20]** and reported have that increase in acid phosphatase activity due to accumulation of mercury in the lysosome and blockage in the release of enzymes and carbohydrate forms the major reserve of many crustaceans accumulated in the hepatopancreas **[21]** and they were of the opinion that the degradation and necrosis induced by toxicants in hepatopancreascauses release of acid phosphatase. It was concluded that both induction and inhibition of phosphatase takes place depending on the concentration of metals. **[22]** Increased acid phosphatase activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphate to release energy in view of impaired ATPase system during metal stress **[22]**.

Alkaline phosphatase is a brush border enzyme that splits various phosphorus esters at an alkaline pH and mediates membrane transport **[23]** It is also involved in synthesis

of certain enzymes, **[24]** active transport, protein synthesis , glycogen metabolism and secretary activity. Any alteration in the activity of alkaline phosphatase affects the organisms in a variety of ways**. [25]** has reported the effect of pyrethroid and mortality on the fish, *Clarias bactrachus* and found that alkaline phosphatase decreased in response to the toxicant. **[26]** reported the effect of copper on oxygen consumption and phosphatase in *S.serrata* and concluded that there was a decrease in alkaline phosphatase activity in muscle, hepatopancreas and haemolymph.

The decreased activity of Acid Phosphatase and Alkaline Phosphatase indicates the disturbance in the structure and integrity of cell organelles (lysosome) and physiological process (hormonal regulation) of organism, causing deleterious consequences **[27, 28]**.

CONCLUSION

Present study has shows that the plant *R.communis*has restored in the biochemical immune parameters in *O.senex senex*. These results suggested that *R.communis* extract may provide a new therapeutic value in specific and non-specific immunity in the fresh water crabs.

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