IJBPAS, April, 2014, 3(4): 567-573

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIOCHEMICAL RESTORATION OF *RICINUS COMMUNIS* L LEAF EXTRACT AGAINST *VIBRIOSIS*

SRIVALLI M, DEVAKUMAR D, JAYANTHI J AND RAGUNATHAN MG*

Department of Advanced Zoology and Biotechnology, Guru Nanak College, Chennai - 600 042, Tamil Nadu

***Corresponding Author: E Mail: mgragunathan@yahoo.co.in**

ABSTRACT

The crab, *O. senex senex* was injected with *V. harveyi* (0.1 ml of 10^7 cfu/ml) . After injection the crabs were allowed to withstand for 96 hrs. Then a 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). Glucose level decreased and protein increased significantly after 96hours in the experimental group. These result suggested that the *R.communis* could combat the microbial infection by altering the biochemical mobilization in crabs.

Keywords: Protein, Glucose, *V.harveyi,O.senex senex, R.communis*

INTRODUCTION

Fish and shellfish diseases and pathologies, with a broad range of aetiologies, are increasingly being used as indicators of environmental stress since they provide a definite biological end-point of historical exposure **[1]**. The large scale settings of aquatic animal husbandry have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish and related environment **[2, 3]**. The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in theaquatic environment. Continuous use of synthetic antibiotics reveals the threats to consumers and non target organisms in the environment **[4]**. Treatments of bacterial diseases with various herbs have been safely used widely in organic agriculture, veterinary

and human medicine **[5]**. Since ancient times, medical plants have been used for the treatment of common infectious diseases **[6]** and treatments with plants having antibacterial activity are a potentially beneficial alternative in aquaculture **[4]**. Medical plants as the alternative agents are effective to treat the infectious diseases and mitigate many of side effects that are associated with synthetic antimicrobials **[7]**. In addition, plant derived phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents in this field **[7]**.

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 libitium* and the water was changed daily and was acclimatized for 15days in the prevailing room temperature. The crabs were divided into four groups of ten each – Control (Group-A), Ethanol injected crabs (Group-B), *V. harveyi* injected crabs (Group-C), *R.comunis* leaves ethanol extract treated (Group-D) 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 96hrs. after 96hrs haemolymph, hepatopancrease, ovary, spermatheca, muscle and gills was taken from ten crabs for biochemaical assays. Bacteria injected crabs were treated with 0.05ml of 80% *R. communis* leaves ethanol plant extract (1000ppm) and after 96hrs biochemical assays were repeated.

Glucose Assay

The determination of hemolymph and tissues glucose levels was carried out manually by the Enzymatic, Colorimetric Method (God-Pap Zistshimy Glucose Diagnostic Kits; **[8]**.

Protein Assay

The total protein assay of the hemolymph and tissues were performed manually by the Biuret method **[9]**.

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

Due to *R.communis* leaves ethanol extract treatment the Total glucose levels hemolymph groups D significantly decreased in group D crabs after 96hrs in hemolymph, hepatopancreas, ovary, body muscle and gills

(Table 1). Total protein levels in the hemolymph, hepatopancreas, ovary, body muscle and gills of the groups D crabs has significantly increased after 96 hours of *R.communis* leaf extract treatment **(Table 1)**.

Table 1: Levels of Glucose and Total Protein

Parameters	Tissues	Control Group A	*V. harveyi* Injected (After 96h-Group B)	*R.communis b* Injected (After 96 hr-Group C)
Glucose mg/dl	Haemolymph	24.6±0.501	*32.5±0.623	*8.95±0.293
	Hepatopancreas	32.4±0.678	*52±0.490	*28.1±0.451
	Ovary	21.3±0.534	*23±0.490	*14.1±0.707
	Spermatheca	9.3±0.269	*35±0.250	*5.26±1.089
	Muscle	11.2±0.699	*42±0.471	*4.18±0.462
	Gills	18.95±0.702	*16.3±0.544	*7.7±0.478
Total protein gms/dl	Haemolymph	6.18±0.143	*2.41±0.140	*10±0.471
	Hepatopancreas	3.13±0.150	*1.65±0.120	*8.1±0.250
	Ovary	3.13±0.150	*1.28±0.085	*7.1±0.262
	Spermatheca	4.83±0.327	*0.816±0.173	*7.1±0.262
	Muscle	5.33±0.347	*0.866±0.106	*6.25±0.383
	Gills	4.83±0.327	*1.05±0.072	*6.25±0.383

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

DISCUSSION

The herbal plants may be used as a potential and promising source of pharmaceutical agents against fish pathogens in organic aquaculture **[10]**. The screening results of our study confirm the possible use of medicinal herbs as a source of antimicrobial agent for this purpose. In the present investigation herbal plant *R.communis* leaf methanol extract was tested against *V. harveyi.*

The present study shows a significant increase in glucose levels in the haemolymph, hepatopancreas, ovary, muscle and gills of the bacteria treated group. During stressful situation higher blood glucose is maintained in fish normally through breakdown of glycogen from liver, mainly through glycogenolysis **[11]**. The glucose level increased in the infected or stressed animals **[12]**. It was also reported that the glucose level elevated in stress condition **[13]**. Injection of *A. hydrophila* was a stress indicator, which was indirectly confirmed by the glucose level during that period and the entire metabolic pathway produces a burst of energy to prepare the fish for an emergency situation **[14]**.

This shows that the decrease in the carbohydrate level was due to glycogenolysis, possibly by increasing the activity of glycogen phosphorylase to meet the energy demand under stress condition or the toxicant has an effect on glycogenesis by inhibiting the activity of carbohydrate metabolism **[15]** or also due to hypoxic condition in the tissues as a result of pollutant stress **[16]**.

In the *R.communis* ethanol extract treated group the glucose has significantly decreased in all tissues. Generally, the glucose level increases in infected or stressed animals to ward off the infection or stress. The possibility of high levels of glucose and total carbohydrate in hemolymph might be due to the transport of glucose and carbohydrate from hepatopancreas and muscle to hemolymph. **[17]** showed a significant elevation of blood glucose in *P. monodon* in stress condition.

The *V. harveyi* treated group shows decrease in the protein content which might be due to the existence of proteolysis. The depletion in the protein content may also be due to the blocking of protein synthesis or protein denaturation or interruption in the Amino acid synthesis **[18]** or due to the rapid utilization of tissue protein as the food utilization decrease when the animals are under stress conditions. A similar report on the decrease in the protein content in the tissues of the prawn, *Palaemon serratus* and the crab, *Uca annulipes* during Cd exposure **[19, 20]** and Naphthalene exposure in the crab, *S.serrata* **[21]** was reported.

In the *R.communis* ethanol extract treated group the protein has significantly increased in all tissues. The protein is the major intake of energy source. It has been observed that the proximate composition of aquatic animals under various feeding regimes and the energy gain or depletion from the body is due to the changes in the amount of content of the cell is considered to be an important tool for the evaluation of physiological standards. The tissue protein is metabolized to produce glucose by the process of gluconeogenesis and it is utilized for the energy production under stress condition **[22]**.

The herbal plants may be used as a potential and promising source of pharmaceutical agents against fish and Shellfish pathogens **[10, 23-26]** this present study has shown that the plant leaf extract of *R.communis*is a good antimicrobial agent against *V. harveyi* Medicinal herbs as immunostimulants increase resistance to disease by enhancing non specific and specific defense mechanisms. The results suggest that some high concentration of active principle in the extracts of the tested plants may be present which has produced potential antibacterial activity.

REFERENCES

[1] Matthiessen P, Thain JE, Law RJ and Fileman TW, Attempts to assess the environmental hazard posed by complex mixtures of organic chemicals in UK estuaries. Mar. Poll. Bull. 26, 1993, 90-95

[2] Sorum HL and Abee-Lund TM, Antibiotic resistance in food- related bacteria-a result of interfering with the global web of bacterial genetics, Int. J. Food. Microbial., 78, 2002, 43-56.

[3] Alcaide E, Blasco MD and Esteve C, Occurrence of drug resistant bacteria in two European eel farms, Appl. Environ. Microbiol., 71, 2005, 3348-3350.

[4] Abutbul S, Golcan-Goldhirsh A, Barazani O, Ofir R and Zilberg D, Screening of desert plants for use against bacterial pathogens in fish Isr, J. Aquacult- Bamid, 57 (2), 2005, 71-80.

[5] Direkbusarakom S, Application of medicinal herbs to aquaculture in Asia, Walailak J. Sci. and Technol., 1, 2004, 7-14.

[6] Rios JL and Recio MC, Medicinal plants and antimicrobial activity, J. Ethnopharmacol, 100, 2005, 80-84.

[7] Punitha SMJ, Babu MM, Sivaram V, Shankar VS, Dhas SA, Mahesh TC, Immanuel G and Citarasu T, Immuno stimulating influence of herbal biomedicines on nonspecific immunityin Grouper *Epinephelustauvina* juvenile against *Vibrio harveyi* infection, Aquaclt., Int.,16, 2008, 511-523.

[8] Ashwell G, Colorimetric analysis of sugars, Methods Enzymol., 3, 1957, 378–386.

[9] Reinhold JG, Manual determination of serum total protein, albumin and globulin fractions by biuret method, In: Reiner, M. (Ed.), Standard Method of Clinical Chemistry. Academic Press, New York, 1953, 88.

[10] Bahaduria S and Kumar P, (2004). Antibacterial activity of some extracts of *Lawsonia alba Solanum dulcamara and Allium sativum* against four fish pathogenic bacteria, J. Phyto. Res., 17, 2004, 191-193.

[11] Vijayan MM, Pereira C, Grau EG and Iwama GK, Metabolic responses associated with confinement stress in tilapia: the role of cortisol, Comp. Biochem. Physiol., 116, 1997, 89-95.

[12] Yoganandhan K, Thirupathi S and Sahul Hameed AS, Biochemical physiological and hematological changes in white spot syndrome virus-infected shrimp, *Penaeus indicus*, Aquacul., 221, 2003, 1-11.

[13] David M, Shivakumar R, Mushigeri SB and Kuri RC, Blood glucose and glycogen levels as indicators of stress in the freshwater fish, *Labeo rohita* under fenvalerate intoxication, J. Ecotoxicol. Environ. Monit., 15, 2005, 1-5.

[14] Rottmann RW, Francis-Floyd R and Durborow R, The Role of Stress in Fish Disease, SRAC Publication No. 474, 1992, 4.

[15] Ghosh R and Shrotr RV, Blood glucose and tissue glycogen interrelationship in *Scylla serrata* (Forskal) chronically exposed to thiodan, Environ. Biol., 120, 1992, 379-384.

[16] Dhavale DM and Masurekar VB, Variation in the glucose and glycogen content in the tissue of *Scylla serrata* (Forskal) under the influence of cadmium toxicity, Geobios., **13,** 1986, 139-142.

[17] Hall MR and Van Ham EH, The effect of different types of stress on blood glucose in the giant tiger prawn *Penaeus monodon*. J. World Aquacult. Soc., 29, 1998, 290-299.

[18] Jha BS, Alterations in the protein and lipid contents of intestine, liver and gonads in the lead exposed freshwater Murrel, *Channa Punctatus* (Bloch), J. Ecobiol., 3, 1991, 29-34.

[19] Papathanassiou E and King PE, Ultrastructural changes in hepatopancreatic cells of the prawn, *Palaemonserratus* induced by exposure to acutely toxic cadmium concentration, Dis. Aquat. Org., 2, 1986, 39-47.

[20] Prasad DVLN, Aspect of physiology of marine Fiddler crab, *Uca* (*celuca*) *laceta annulipes* in relation to exposure to cadmium chloride. Ph.D. thesis. S.V. University, Tirupati, India, 1990.

[21] Vijayavel K and Balasubramanian MP, Fluctuations of biochemical constituent and marker enzymes as a consequence of naphthalene toxicity in the edible estuarine crab, *Scylla serrata*, Ecotoxicol. Environ. Saf., 63, 2006, 141-147.

[22] Elumalai M and Balasubramanian MP, Influence of naphthalene on esterase activity during vitellogenesis of marine edible crab, *Scylla serrata*. Bull. Environ. Contam. Toxicol., 62 (6), 1999, 743-748.

[23] Devakumar D, Mahesh TC, Priyadarshini R, Jayanthi J and Ragunathan MG, Restoration ofPhosphatases enzyme activity by *Psidium Guajava* Leaf Extract in *Pseudomonas aeruginosa* infected fresh water crab, *Oziotelphusa senex senex*, Life Sci. Bulletin, 8 (2), 2011a, 181-184.

[24] Devakumar D, Mahesh TC, Priyadarshini R, Jayanthi J and Ragunathan MG, Ameliorative effects of *Psidium Gaujava* Leaf Extract on the Transaminase enzyme activity affected by*Pseudomonas*

aeruginosa infection in a fresh water crab, *Oziotelphusa senex senex*, National J. Life Sci., 8 (2), 2011b, 43-46.

[25] Devakumar D, Kumaran T, Jayanthi J and Ragunathan MG, Efficiency of *Psidium guajava* leaf extract on the nitrogenous wastes in *Aeromonas hydrophila* infected fresh water crab, *Oziotelphusa senex senex*, J. Recent Trends Biosci., 2 (1), 2012, 47-52.

[26] Devakumar D, Jayanthi J and Ragunathan MG, Herbal alternate to *Pseudomonas aeruginosa* infection in a freshwater crab,*Oziotelphusa senex senex*, IJBPAS, 2 (11), 2013, 2142-2147.