Immunological Activity of Snail Hemocytes as Biomarkers of Environmental Quality

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List of abbreviations

°C	Degrees Celsius
AAS	Atomic Absorption Spectroscopy
Ab	Antibody
BSA	Bovine serum albumin
D	Degeneration
DC	Digestive Cells
DHC	Different Hemocyte Counts
DNA	Deoxyribonucleic acid
DT	Digestive gland Tubules
E.M	Electron Microscope
EC	Epithelial Cells
ELISA	Enzyme Linked Immunosorbent Assays
F	Filaments
G	Granulocytes
Hsp 70	Heat shock protein-70
Ig	Immunoglobulin
IL-2	Interleukin-2
IL-6	Interleukin-6
INF-γ	Interferon gama
m	Mitochondria
M	Mucous
M. edentula	*Macoma edentula*
MF	Muscle Fibers
MFO	Mixed Function Oxygenases
n	Nucleus
PCBs	polychlorinated Biphenyls

PHAs	Polycyclic Aromatic Hydrocarbons
R.decussatus	*Ruditapes decussatus*
RER	Rough Endoplasmic Reticulum
S	Secretion
SC	Secretory gland Cells
SER	Smooth Endoplasmic Reticulum
THC	Total Hemocyte Counts
TNF-α	Tumor necrosis factor alpha
UV	Ultra violet
V	Vacuolation

Abstract

ABSTRACT

The aim of the present study was to investigate some hematological, histopathological and immunological parameters in the clams, *Ruditapes decussatus* (*Veneridae*) and *Macoma edentula* (*Tellinidae*) to serve as a reference for studies in the monitoring of different environmental pollutants through the potential change in the clam defence activities. Two localities of the Lake Timsah exhibiting a similar pattern of seasonal variation in hemocyte abundance and a distinct degree of environmental contamination were selected for a comparative study of *in vitro* hemocyte activities. The results of DNA strand breakage,were expressed as the mean tail DNA content and the mean tail length, The two measured comet parameters in both species,showed a significant differences ($P>0.05$) between the unpolluted and polluted groups. Hsp 70 activity of *R. decussatus* and *M. edentula* was studied for natural variability related to water pollution, Hsp 70 activity was significantly higher in normal unpolluted than polluted samples. Water pollution was observed to cause significant histopathological changes in the mantle of both snails species by using electron and light microscopic studies. Histopahtological changes of mantle from both *R. decussatus* and *M. edentula* are represented in the arrangement or regularity of different cells. Vacuolation and swelling of the gill filaments occurred and distortion and damage of gill filament also occurred. The amount of secretion of glandular cells increased in polluted *R. decussatus*. The secretion was significantly decreased in *M. edentula* and the cells became completely necrotic and vacuolation occurred in most parts of digestive tubules. Later on, digestive gland tubules completely damaged. Polluted *R. decussatus* and *M.edentula* showed a statistically significantly, higher levels of IL-2 than non-polluted samples. The levels of IL-6 in both the polluted *R. decussatus* and *M.edentula* were significantly lower than the non-polluted control. The INF-γ levels in the polluted *R. decussatus* exhibited a non-significant increase as compared with non-polluted animals. However, the levels of INF-γ in the polluted *M. edentula* exhibited significant elevation as compared with non-polluted animals. The polluted *R. decussatus* showed low levels of TNF-α than non-polluted control. Also, the levels of TNF-α in the polluted *M. edentula* were significantly decreased as compared to non-polluted animals.These results suggest that the parallel use of polluted and native bivalve in environmental biomonitoring can improve the characterization of the study area.

Key words: Mollusc bivalve; *Ruditapes decussatus*; *Macoma edentula*; DNAdamage; Comet assay; Hemocytes; Mantle; Gills; Digestive gland; Immune function; Biomarkers; Ecotoxicology.

Introduction

INTRODUCTION

The immune system of invertebrates is relatively simple in contrast to that of vertebrates, providing accessible means for monitoring immune function. Unlike vertebrates, invertebrates lack immune specificity and memory, characteristic of the adaptive immunity observed in mammalian subjects. Invertebrates have evolved innate defence mechanisms that can identify and protect against non-self material, with invertebrate immune response centered largely on the multifunctional hemocytes **(Cheng, 1981)**. The various parameters that contribute to invertebrate host defence act to provide a multifaceted immune response. It has been suggested that an integrated suite of biomarkers can be used for assessing immunocompetence of invertebrates **(Pipe and Coles, 1995)**.

Advances in environmental risk assessment have highlighted the value of biomarkers to allow rapid evaluation of contaminant exposure and effect **(Galloway *et al*., 2002)**. These biomarkers may be molecular, cellular or physiological endpoints that indicate exposure to and/or damage incurred by environmental pollutants providing early-warning indicators of contaminant-induced stress. The use of biomarkers has been recognized as a cost-effective technique in identifying the effects of pollutants on biota **(Galloway *et al*., 2002)**.

The majority of biomarker studies to date have been based on the mussel *Mytilus edulis*, and the American oyster *Crassostrea virginica* **(Wootton *et al*., 2003)**. Bivalve exhibit worldwide distribution and sedentary, filter feeding behavior. They have an open circulatory system that is continually exposed to fluctuating environmental conditions and