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AZADIRACHTIN INDUCED ULTRASTRUCTURAL CHANGES ON HAEMOCYTE IN *LAMELLIDENS MARGINALIS*

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ABSTRACT

Indian wetland supports a wide range of aquatic biodiversity of ecological and economical importance. *Lamellidens marginalis* is a freshwater edible mollusc widely distributed in the wetland of West Bengal. In India, a large section of people is involved in agricultural process and use of agrotoxin in the form of pesticide is considered as routine procedure. Freshwater ecosystem bears the risk of contamination by pesticide residues during monsoon and other seasons. Haemocytes, the chief circulatory cells of *Lamellidens* play an important function in elicitation of immune response under the exposure of xenobiotics. In the present investigation, toxicity of azadirachtin was screened in *L. marginalis* in relation to ultrastructural changes of haemocyte subpopulation. Azadirachtin exposure resulted in morphological impairment of haemocytes that may lead to possible disruption of normal functioning of these cells. The present study would provide a database for determination of the magnitude of toxicity of azadirachtin in the freshwater edible mollusc.

Key words: Mollusc, azadirachtin, TEM

INTRODUCTION

Lamellidens marginalis is an important member of freshwater benthic community. Species is a filter feeder and reduces sedimentation load of water column. *L. marginalis* thrives on plankton and aquatic plants distributed in biosafe and biounsafe environment. Currently a decline in population of *L. marginalis* is noticed in several wetlands of urban and rural West Bengal. Habitat of *L. marginalis* receives diverse toxins of known and unknown chemistry (Isman 1997). In recent years, Multineem, an azadirachtin based biopesticide is introduced in agriculture for protection of crop against pest. Highly toxic azadirachtin residues are potent neurotoxin (Schmutterer 1990) and produces adverse effects at various levels of physiological and immunological parameters of aquatic animals. Agricultural runoff loaded with azadirachtin adversely affects the non target invertebrates of aquatic ecosystem including *L. marginalis*. Here adult *L. marginalis* were exposed to sublethal concentrations of azadirachtin in the controlled laboratory condition for morphological analysis of haemocytes in different time spans.

MATERIALS AND METHODS

Collection and treatment of animal

The adult healthy *L. marginalis* with shell size of 7-8 cm were manually collected from the selected wetlands of the district of South 24 Parganas of West Bengal. Animals were transported to the laboratory in rectangular plastic containers with a dimension of 12'x18'x 6' at a density of 4-6 individuals per box in the moist condition. Prior to experimentation, animals were acclimatized for 15 days in the laboratory. During acclimatization, *L. marginalis* were maintained in aquaria with fresh supply of pond water with temperature of 29°C±3°C and the animals received uniform ration of illumination. During the course of acclimatization and experiment, the animals were fed with chopped *Hydrilla* sp. and common aquatic weeds (Raut 1991). Routine replenishment of water was carried out in every 12 hours to avoid residual toxicity. Aqueous solutions of Multuneem (Multiplex, India Private Limited, Azadirachtin E.C. 0.03%) formulations were prepared in Borosilicate glass containers with azadirachtin concentrations of 0.006, 0.03, 0.06, and 0.09 ppm. The pH of the solution was maintained at 7.2. Each experimental set consisted of 10 animals of same shell length. Animals were exposed to a volume of 5 litre of pesticide solution for varied span of exposure i.e. 1,2,3,4,7,15 and 30 days. For control, a set of animals were kept in identical volume of pesticide free analytical grade water. The experiments were carried out in static water environment and

fresh solutions of pesticide were replenished in every 12 hr.

Transmission Electron Microscopy

For TEM analysis, haemolymph was placed immediately in a microfuge tube containing a glutar aldehyde (E.M. grade, Sigma, USA) solution (0.35%) buffered with 0.1M sodium cacodylate (E.M. grade, Sigma, USA, pH 7.4) and fixed for 15-20 minutes. The fixed cells were centrifuged at 1500 rpm for 10 minutes and the resulting pellet was further fixed for 2 hr at 4°C in the cacodylate buffer. After washing, the cells were subjected to osmification for 1-2 hours by treatment with 1% osmium tetroxide (E.M. grade, Sigma, USA) with cacodylate buffer at room temperature, dehydrated through graded ethanol (50-100%), transferred to propylene oxide (E.M. grade, Sigma, USA) and embedded in Epon. Semi-thin sectioning (200-300 nm) was carried out with a glass knife for light microscopy. Ultrathin sections (50- 70 nm) were double stained with aqueous uranyl acetate (E.M. grade, Sigma, USA) followed by lead citrate (E.M. grade, Sigma, USA) treatment. Micrographs were obtained under Transmission Electron Microscope operating at 60 kV (Gagnaire 2004).

RESULTS

Electron microscopic study of haemocytes exhibited diverse types of cells. The hyalinocytes were round with scarce cytoplasmic granules (Fig. 1). The nucleus was irregularly shaped. The agranulocyte possess a nucleus with clumped mass of chromatin (Fig. 3). These cells are devoid of cytoplasmic granules. The granulocytes possess round nucleus and exhibit chromatin clumps. The cytoplasm is packed with round granules (Fig. 5). The granules are membrane limited and contain homogenous electron-dense matrix. Hyalinocytes of azadirachtin exposed (0.06 ppm/15 days), *L. marginalis* developed eccentric nuclei, granular cytoplasm and membrane blebs. In TEM study, the nuclei were pushed towards the periphery and the cytoplasm appears to be more granular (Fig. 2). Treatment of 0.03 ppm/30 days of azadirachtin resulted in a marked enlargement of nuclear volume of agranuocytes in relation to that of cytoplasm and the nucleus was transformed to lobate in appearance. The nucleus exhibited sign of disintegration of chromatin clumps (Fig. 4). Exposure of azadirachtin of 0.09 ppm/ 7 days of span resulted in rupture of cell membrane and release of cytoplasmic granules from granulocytes. The nucleus is placed towards the periphery and exhibited sign of disintegration of chromatin clumps (Fig. 6).

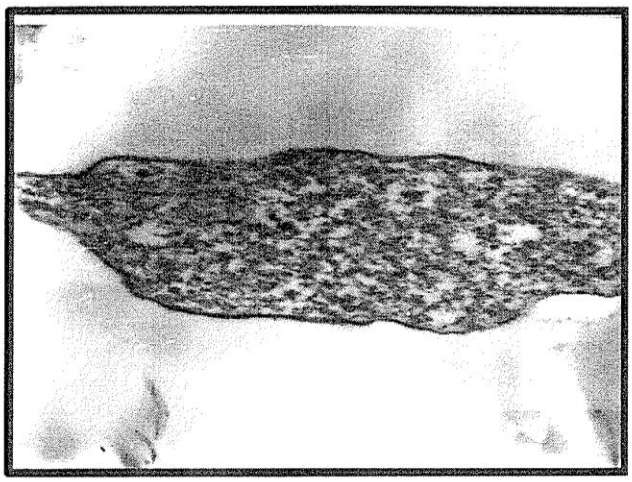

Fig. 1. TEM of hyalinocyte of *L. marginalis*: Oval cell shape with scarce cytoplasmic granules. X 8400.

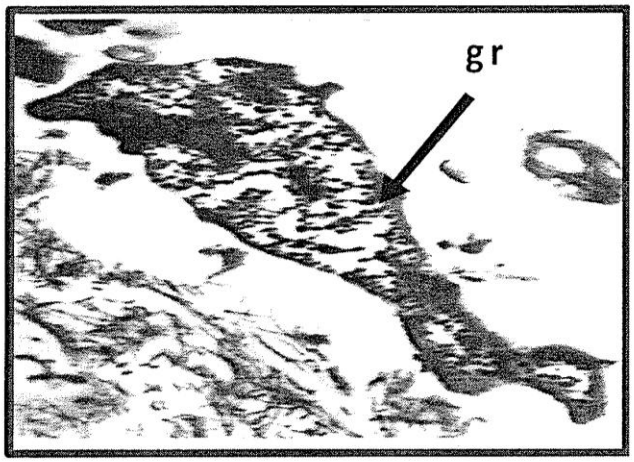

Fig.2. TEM of hyalinocyte of *L. marginalis* (0.06ppm/15days of treatment): Cell showing appearance of cytoplasmic granules (gr). X 8400.

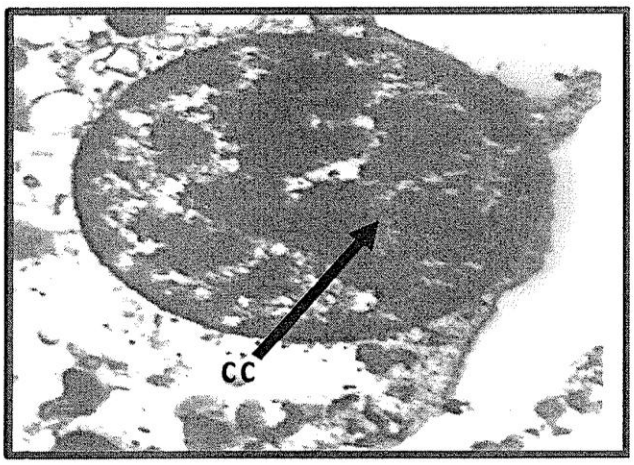

Fig.3. TEM of agranulocyte of *L. marginalis*: Cell with round nucleus exhibiting chromatin clumps (cc) .X 8400.

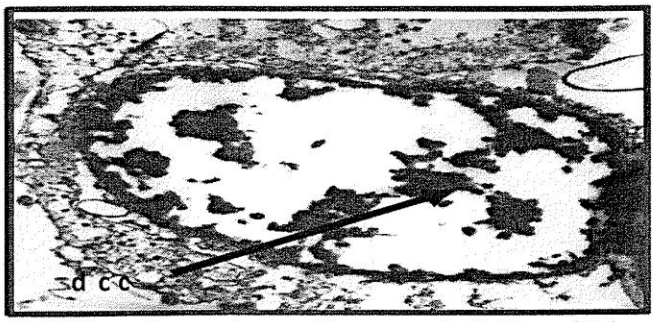

Fig.4. TEM of agranulocyte of *L. marginalis* (0.03ppm/30days of treatment): Cell exhibiting disintegrates chromatin clumps (dcc). X 8400.

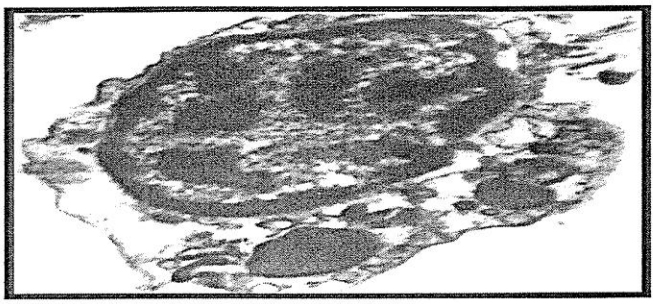

Fig.5. TEM of granulocyte of *L. marginalis*: Cell with round nucleus and cytoplasm granules. X 6300.

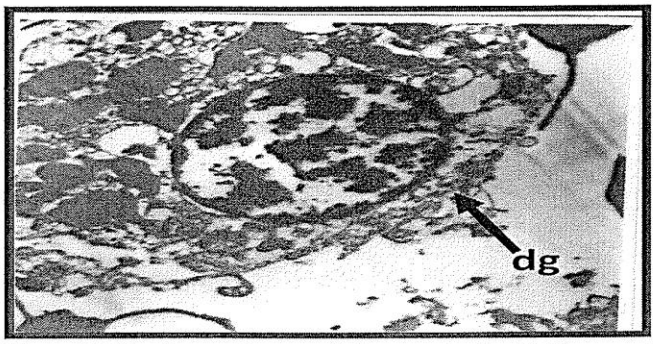

Fig.6. TEM of granulocyte of *L. marginalis* (0.09ppm of azadirachtin/7days of treatment): Cell showing degranulation (dg) tendency. X 6300.

DISCUSSION

Haemolymph, the blood of *L. marginalis*, is composed of haemocytes and fluid components. In molluscs, haemocytes are considered as blood cells which circulate in the haemolymph following a definite route and are capable of eliciting toxicological response upon exposure to xenobiotics (Sauve et al. 2002). In the haemolymph, specific cells such as hyalinocyte (Fig. 1), agranulocyte (Fig. 3) and granulocyte (Fig. 5) were reported using electron microscopic technology. In TEM study, it was revealed that the nuclei were pushed towards periphery and cytoplasm appeared to be more granular in hyalinocytes (Fig. 2). TEM study of the agranulocyte revealed that azadirachtin treatment resulted in a marked enlargement of nuclear volume in relation to cytoplasm and nucleus is transformed to lobate in appearance (Fig. 4). Exposure of azadirachtin resulted in disintegration of cell membrane in granulocytes as evident from TEM analyses (Fig. 6). Morphological analyses of haemocytes were indicative to a potential toxicity of azadirachtin in the hyalinocyte, agranulocyte and granulocyte. Morphological impairment of haemocytes is indicative of possible disruption of normal functioning of these cells (Chakraborty et al. 2008). Xenobiotics induced alteration of morphological structure of haemocytes may lead to decline of the population of *L. marginalis* in its natural habitat resulting in a gradual depletion of existing biodiversity of freshwater environment. Moreover, *L. marginalis* is an important biological component of freshwater ecosystem and an efficient bio accumulator of toxin residues. Being a filter feeder, this species performs biological filtration of ambient water and keeps the water body biologically safe for other species (Das and Jana 1999). Present investigation would help to establish a suitable biomonitor species of aquatic pollution with reference to neem based pesticide and allied xenobiotics.

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