L-Ascorbate mediated detoxification of Lambda Cyhalothrin induced histopathological changes in the gill epithelium of an experimental model, fresh water bivalve, *Lamellidens Marginallis*

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**Abstract**

The present study was conducted to evaluate the effectiveness of L-ascorbic acid in Lambda Cyhalothrin-induced toxicity in an experimental model, the fresh water bivalve, *Lamellidens marginallis*. Histopathological changes were recorded in the gill of fresh water bivalve, *Lamellidens marginallis* after acute exposure to Lambda Cyhalothrin alone & in combination with 50mg/L of L-ascorbic acid. Due to Lambda Cyhalothrin intoxication damage to the gill was extensive resulting epithelial shrinkage, necrosis, hypertrophy in secondary gill lamellae & connective tissue core at 24 hours of exposure. The severity of gill damage was progressed with increase in exposure period. After 96 hours of exposure to Lambda Cyhalothrin histopathological changes like shrinkage, necrosis, degeneration of the epithelial cells along with sub epithelial spaces in the secondary gill lamellae were noted.

Exposure to Lambda Cyhalothrin in combination with 50mg/L of L-ascorbic acid showed considerable reduction in nature of damage. The pre-exposed bivalve to Lambda Cyhalothrin alone showed fast recovery in presence of L-ascorbic acid than the recovery in the untreated fresh water. The probable cause of protection by the L-ascorbic acid in Lambda Cyhalothrin induced toxicity will be discussed in the paper.

**Keywords:** *Lamellidens marginallis*  |  Lambda Cyhalothrin  |  gill  |  histopathology  |  L-ascorbic acid

**Introduction**

The pollution of rivers and streams with chemical contaminants has become one of the most critical environmental problems of the century. Some of these chemicals are biodegradable and quickly decay into harmless or less harmful forms, while others are non-biodegradable and remain dangerous for a long time. The use of various classes of pesticides
as organophosphate, organochlorine, carbamate and pyrethroids have been increased many fold for the last 10 years (Wolansky et al., 2006). Lambda Cyhalothrin is one of the class of chemicals called Pyrethroids and its trade name includes Karate. It is classified as Restricted Use Pesticide (by Environmental Protection Agency) because of its acute toxicity to humans. It is used for foliar treatment of vegetable, fruit and field crop, cotton, commercial, ornamentals and in and around poultry houses and dairies. Lambda Cyhalothrin is highly toxic to aquatic invertebrates, when absorbed through the mucous membrane of the respiratory tract, resulting in systemic intoxication. The bivalves are considered as the suspension filter feeders and influenced by the organization and fluctuations of the ecosystem (Akarte et al., 1987). Molluscs, the lamellibranch bivalves constitute the important aquatic invertebrate biota which is of considerable research interest since they are universally distributed and have specific ecological adaptations. Pathological biochemical disturbances in aquatic organisms like mollusc due to pesticide toxicity are well documented (Waykar and Lomte, 2002; 2004). Histopathological changes are mostly confined to organs directly involved in their metabolism and detoxification (Rashatwar and Ilyas, 1994). Lamellidens marginalis is an economically important bivalve. It circulates large quantity of water through respiratory surface to obtain food and oxygen. Thus, it suffers a great risk of pesticide poisoning. Therefore, it is considered as a bio-monitoring tool in toxicological studies. There are few reports on the effects of xenobiotics on respiratory rate and gill histology of bivalves with no published data on histopathological alterations in L. marginalis after lambda cyhalothrin exposure. When pesticide enter into the body of molluscs, it create physiological as well as histopathological changes in the body of molluscs. Therefore, a study was conducted to determine possible respiratory hazards to L. marginalis following sub-lethal exposure to lambda cyhalothrin. A simple but effective way to prevent degenerative changes would be to prevent oxidative damage. The cells major defense against ROS damage includes antioxidants like ascorbic acid. For different physiological acts Vitamins are essential.

**Materials and Method**

**Experimental Design: Set-I**

1. Group ‘A’ was maintained as control.
2. Group ‘B’ animals were exposed to subacute treatment (LC 50/2 values of 96 hrs) of lambda cyhalothrin (0.75 PPM) upto 96 hrs
3. Group ‘C’ animals were exposed to subacute treatment of lambda cyhalothrin (0.75 PPM) along with 50 mg / litre L-ascorbic acid upto 96 hours.
4. Group ‘D’ animals were exposed to subacute treatment of lambda cyhalothrin (0.75 PPM) along with 100 mg/ litre L-ascorbic acid upto 96 hours.
Experimental design for recovery studies:

Set- II

1. Group ‘B’ animals exposed to lambda cyhalothrin for 96 hours from set-I were divided into three groups for recovery study

2. Group ‘E’ animals pre-exposed to lambda cyhalothrin were allowed to self-cure normally in untreated fresh water up to 15 days.

3. Group ‘F’ animals pre-exposed to lambda cyhalothrin, were allowed to cure in 50 mg / litre ascorbic acid in fresh water up to 15 days.

4. Group ‘G’ animals pre-exposed to lambda cyhalothrin were allowed to cure in 100 mg/ litre ascorbic acid in fresh water up to 15 days.

During experimentation animals were fed on fresh water algae. After 24 and 96 hours interval, animals from set-I and after 5 days, 10 days and 15 days interval, animals from set -II were dissected and their gills were taken out. Tissues were fixed in the aqueous Bouin’s fluid for 24 hrs and were processed by usual microtechnique method and serial sections of six micron thickness were stained with Mallory's triple stain.

Results and Discussion

The gross histopathological effects of acute dose of Lambda cyhalothrin alone and with 50 mg/L and 100 mg/L of L-ascorbic acid and recovery responses studied in an experimental model fresh water bivalve, Lamellidens marginallis are shown in plate no 1 to 2.

Gills under Lambda cyhalothrin intoxication

As compared to gills of control Lamellidens marginallis, after acute exposure to Lambda cyhalothrin for 24 h, the Interlamellar connective tissue and muscle fibres surrounding the gill lamellae were damaged; hypertrophy of gill lamellae, loss of inter lamellar junctions, vacuolization of basal epithelium in the gills. Some cells showed very irregular vacuolated appearance, basement membrane of the epithelium of gill lamellae was found damaged at some places (Fig. b of plate 1). The severity of damage of gills progressed with longer exposure to Lambda cyhalothrin. After 96 h exposure to Lambda cyhalothrin, the degenerative changes such as pycnotic nuclei, necrosis of connective tissue and irregularly distributed tubular lesions were observed throughout the gill lamellae. In the gill lamellae, epithelial cells were separated from the basement membrane and the number of epithelial cells was reduced. The gill lamellae appeared to be collapsed due to damage of epithelial cells. Generalized reduction of cell and nuclear size were observed (Fig. b of plate 1).The result of microscopy showed that epithelial tissue was probably a primary target of the pesticide intoxication. In combined exposure to Lambda cyhalothrin along with 50mg/L of L-ascorbic acid after 24 h showed damages at few places in the basement membrane of the tubules, slight shrinkage of epithelial cells and gill lamellae lumen (Fig. c of plate 1). After 96 h of exposure, histopathological changes in gill lamellae were relatively more, as compared to those of 24 h of exposure, but the intensity of
damage was relatively less as compared to those exposed to Lambda cyathorin alone after 96 h of exposure (Fig. d of plate 1). In combined treatment of Lambda cyathorin together with 100 mg/L L-ascorbic acid after 24 h exposure retained most of the normal histopathological structure of gill lamellae. There were normal stratified epithelial cells arranged regularly on the basement membrane. There was slight shrinkage of tubules of the gill lamellae along with epithelial cells; the epithelial cells were slightly taller with few atrophied changes. The gill lamellae were more or less comparable to control (Fig. e of plate 1). After 96 h of exposure, histopathological changes were relatively more as compared to those of 24 h exposure but less as compared to those exposed to same dose of Lambda cyathorin after 96 h. Severity of gill lamellae damage was much reduced (Fig. f of plate 1).

**Recovery study**

Animals pretreated to Lambda cyathorin when allowed to cure in normal water and with L-ascorbic acid showed the restoration of normal structure of Gills. In histological section after 5 days of recovery in normal water, exhibited regeneration of connective tissue, basement membrane, epithelial cells and reduction in necrosis and vacuolization (Fig. a of plate 2). This is more evident after 10 days of recovery. The interlamellar connective tissues and muscle fibres surrounding the gill lamellae were regenerated. The tubular lesions occurred at all exposure times and persisted even after 10 days of recovery (Fig. b of plate 2). After 15 days of recovery, more restoration of epithelial cells with normal shape and size were observed but still necrotic lesions were seen (Fig. c of plate 2). When animals pretreated to acute dose of Lambda cyathorin were allowed cure in 50 mg/L of L-ascorbic acid in freshwater, Gills of bivalve showed the restoration of normal structure. Histological sections of Gills after 5 days of recovery showed normal shape and size of epithelial cells. Regeneration of interlamellar connective tissues and muscle fibres surrounding the lamellae was observed. In some regions of lamellae, necrotic changes were observed (Fig. d of plate 2). After 10 days of recovery more restoration of histology of Gills was observed (Fig. e of plate 2). After 15 days of recovery histological section exhibit normal histological structure likes that of control animals (Fig. f of plate 2). When animals pretreated to acute treatment of Lambda cyathorin were allowed to cure in 100 mg/L of L-ascorbic acid in freshwater for 5 day of recovery, normal architecture of Gills, with slight tubular lesions was observed (Fig. g of plate 2). After 10 days of recovery almost all damages were recovered and structure of Gills was like those of control animals (Fig. h of plate 2). The histopathological changes as observed on Lambda cyathorin exposure were reported after exposure to different pesticides (Thoser et al., 2001; Omiama, 2004; Waykar, 2006; Saraswathy et al., 2010). In present study on combined exposure to acute dose of Lambda cyathorin along with 50 mg/L and 100 mg/L of L-ascorbic acid showed reduction in damages, indicating the protective effect of L-ascorbic acid. Thus the result of the present study
clearly demonstrates protective ability of ascorbic acid against pesticide toxicity.

Plate 1a. L.S. of gill X 400 - Control gill

Plate 1b. L.S. of gill X 400 - After acute exposure to lambda cyhalothrin after 96hrs

Plate 1c. After sublethal exposure to lambda cyhalothrin with 50 mg/l ascorbic acid at 24 hr

Plate 1d. After sublethal exposure to lambda cyhalothrin with 50 mg/l ascorbic acid at 96 hr

Plate 1e. After sublethal exposure to lambda cyhalothrin with 100 mg/l ascorbic acid at 24 hrs

Plate 1f. After sublethal exposure to lambda cyhalothrin with 100 mg/l ascorbic acid at 96 hrs.
Plate 1: Microphotographs showing L. S. of Gills (X-400) of Lamellidens marginallis on acute exposure to Lambda cyalothrin alone and in combination with 50mg/L and 100 mg/L of L-ascorbic acid

**Recovery Study**

Plate 2a. Curing gills in normal fresh water in 5 days

Plate 2b. Curing gills in normal fresh water at 10 days

Plate 2c. Curing gills in normal fresh water at 15 days

Plate 2d. Curing gills in 50mg/l ascorbic acid in fresh water at 5 days

Plate 2e. Curing gills in 50mg/l ascorbic acid in fresh water at 10 days

Plate 2f. Curing gills in 50mg/l ascorbic acid in fresh water at 15 days
Plate 2g. Curing gills in 100mg/l ascorbic acid in fresh water in 5 days

Plate 2: Microphotographs showing L. S. of pre-exposed Lamellidens marginallis to Lambda cyalothrin during recovery in normal fresh water, 50mg/L and 100 mg/L of L-ascorbic acid in freshwater.

Abbreviations
C- Ciliary’s border, CR-Chitinous rods, GF-Gill filament, IFS-Interfilamental space, N- Nucleus, Nu- Nucleolus, RE-Respiratory epithelium, DRE-Degenerating respiratory epithelium, DCR- Degenerating chitinous rods, SW- Swollen tip of gill filaments IC- Interfilamental space

Conclusion
This study clearly indicated protective and wound healing property of ascorbic acid in pesticide induced tissue damage in experimental model. Thus it is evident that Vitamin C not only confirms protection against pesticide toxicity but can also perform therapeutic role against pesticide toxicity.

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