MITIGATION OF CYPERMETHRIN INDUCED HISTOLOGICAL DAMAGE IN THE HEPATOPANCREAS OF FRESHWATER BIVALVE, PARREYSIA CYLINDRICA BY L-ASCORBIC ACID

Waykar Bhalchandra and Tambe Ram

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INTRODUCTION

Pesticides have become omnipresent contaminants of our environment and have been found in human and animal tissues all over the world (Anwar, 1997). Silent Spring, the book written by Rachel Carson, facilitated the ban of the pesticide DDT in 1972 in the United States and foretold of the poisoning of the planet by man (Paull, 2007). Since then, many countries have devised policies to reduce the use of pesticides. However, data of EU (1992–2003) statistics shows that consumption of pesticide did not decrease (Bjørling-Poulsen et al., 2008).

The use of various classes of insecticides as organophosphate, organochlorine, carbamate and pyrethroids have been increased many fold for the last 10 years (Wolansky et al., 2006). Synthetic pyrethroid insecticides target a wide range of insects and are highly toxic to aquatic invertebrate species. The incidence of major outcomes and fatalities attributable to pyrethroids are considerably less than organophosphates (Sudakin, 2006), however, their pathological effects have been encountered in experimental studies in different animals (Khan et al., 2009).

Excessive use of pesticides resulted in serious ecological and environmental problems as well as health hazards (Olea and Fernandez, 2007). Many pesticides are known inducers of oxidative stress by directly producing reactive oxygen species (ROS) and impede the natural antioxidant or oxygen free radical scavenging enzyme system (Geter et al., 2008). Pesticide disturbs the pro-oxidant–anti-oxidant system of the cells, thereby leading to the generation of free oxygen radical and reactive oxygen species (El-Gendy et al., 2010) causing oxidations in chain. All the bio-molecules of cell (nucleic acids, lipids, proteins, polysaccharides) are potential substrates of ROS (Manduzio et al., 2005), resulting in loss of membrane integrity, causing apoptosis, fibrosis and finally cell necrosis (Valko et al., 2006). In order to overcome such effects the most combative source would be support with exogenous antioxidant. Ascorbic acid is main dietary antioxidant. Ascorbic acid has potential role to prevent the oxidative damage (Sohini Singh and Rana, 2007). Many pesticides are known inducers of oxidative stress by directly producing reactive oxygen species (ROS) and impede the natural antioxidant or oxygen free radical scavenging enzyme system (Geter et al., 2008). Pesticide disturbs the pro-oxidant–anti-oxidant system of the cells, thereby leading to the generation of free oxygen radical and reactive oxygen species (El-Gendy et al., 2010) causing oxidations in chain. All the bio-molecules of cell (nucleic acids, lipids, proteins, polysaccharides) are potential substrates of ROS (Manduzio et al., 2005), resulting in loss of membrane integrity, causing apoptosis, fibrosis and finally cell necrosis (Valko et al., 2006). In order to overcome such effects the most combative source would be support with exogenous antioxidant. Ascorbic acid is main dietary antioxidant. Ascorbic acid has potential role to prevent the oxidative damage (Sohini Singh and Rana, 2007). Ascorbic acid is required for collagen and bone formation (Halver, 1957), and in wound healing (Gould, 1963). Ascorbic acid plays a very major role in tissue synthesis and growth processes and obviously, mediate rapid tissue repair in trauma and disease condition (Halver, 1972). 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 Illyas, 1994). In mollusc hepatopancreas is the main site of action, degradation and detoxification of pesticide, hence hepatopancreas are chosen as test organs.

MATERIALS AND METHODS

Medium sized healthy, live freshwater bivalve, Parreysia cylindrica were acclimatized for a week to dechlorinated tap water.

Experimental design
Plate 1a: Control-digestive gland

Plate 1b: After acute exposure to cypermethrin at 24h

Plate 1c: After acute exposure to cypermethrin at 96h

Plate 1d: After acute exposure to cypermethrin with 50mg/L of L-ascorbic acid at 24h

Plate 1e: After acute exposure to cypermethrin with 50mg/L of L-ascorbic acid with 96 h

Plate 1f: After acute exposure to cypermethrin with 100mg/L of L-ascorbic acid at 24h

Plate 1g: After acute exposure to cypermethrin with 100mg/L of L-ascorbic acid with 96 h

Plate 1: Microphotographs showing L. S. of digestive gland (X-400) of Parreysia cylindrica on acute exposure to cypermethrin alone and in combination with 50mg/L and 100 mg/L of L-ascorbic acid

Abbreviations: DTL-digestive gland lumen, DT-digestive tubule, BM-basement membrane, CT-connective tissue, V-vacuole, BBM-broken basement membrane DC-digestive cell SC-calcium secretory cell, DEC-degenerating epithelial cells, and DCT-degenerating connective cell. PN-pycnotic nucleolus, NU-nucleolus
were observed throughout the tubules. Peritubular area was connective tissue and irregularly distributed tubular lesions degenerative changes such as pycnotic nuclei, necrosis of cypermethrin. After 96 h exposure to cypermethrin, the damage of hepatopancreas progressed with longer exposure damaged at some places (Fig. b of plate 1). The severity of membrane of the epithelium of hepatic tubules was found cells showed very irregular vacuolated appearance, basement intertubular connective tissue and muscle fibres surrounding the tubules were damaged; the lumen of the tubules of hepatopancreas was reduced due to shrinkage. Some digestive cells, the selective calcium staining shows that the vacuolar cytoplasm, granular material was present. The brown pellets or spherule are also present in cytoplasm. The digestive cells, cum secretary cells, normally appear triangular with varying heights. Their cytoplasm is more or less homogeneous, having a conspicuous nucleus, which is much bigger than that of the digestive cells, the selective calcium staining shows that the brown pellets or spherule are also present in cytoplasm. The pellets at times are so big that most of the cells, containing them, have the nucleus pushed aside to the basal part of the cells. hepatopancreas under cypermethrin intoxication As compared to hepatopancreas of control Parreysia cylindrica, after acute exposure to cypermethrin for 24 h, the intertubular connective tissue and muscle fibres surrounding the tubules were damaged; the lumen of the tubules of hepatopancreas was reduced due to shrinkage. Some digestive cells showed very irregular vacuolated appearance, basement membrane of the epithelium of hepatic tubules was found damaged at some places (Fig. b of plate 1). The severity of damage of hepatopancreas progressed with longer exposure to cypermethrin. After 96 h exposure to cypermethrin, the degenerative changes such as pycnotic nuclei, necrosis of connective tissue and irregularly distributed tubular lesions were observed throughout the tubules. Peritubular area was necrotic at some places. In the tubules, epithelial cells were separated from the basement membrane and the number of epithelial cells was reduced. The tubules appeared to be collapsed due to damage of epithelial cells. Generalized reduction of cell and nuclear size were observed (Fig. c of plate 1).The result of microscopy showed that epithelial tissue was probably a primary target of the pesticide intoxication. In combined exposure to cypermethrin 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 tubular lumen (Fig. d of plate 1). After 96 h of exposure, histopathological changes in hepatic tubules 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 cypermethrin alone after 96 h of exposure (Fig. e of plate 1).

In combined treatment of cypermethrin together with 100mg/L L-ascorbic acid after 24 h exposure retained most of the normal histopathological structure of hepatic tubules. There were normal stratified epithelial cells arranged regularly on the basement membrane. There was slight shrinkage of tubules of the hepatopancreas along with epithelial cells; the epithelial cells were slightly taller with few atrophied changes. The tubules were more or less comparable to control (Fig. f of plate 1). After 96 h of exposure, histopathological changes were relatively more as comparable to those of 24 h exposure but less as compared to those exposed to same dose of cypermethrin after 96 h Severity of hepatopancreas damage was much reduced (Fig. g of plate 1).

Recovery study Animals pretreated to cypermethrin when allowed to cure in normal water and with L-ascorbic acid showed the restoration of normal structure of hepatopancreas. 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 intertubular connective tissues and muscle fibres surrounding the tubules 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 cypermethrin were allowed cure in 50 mg/L of L-ascorbic acid in freshwater, hepatopancreas of bivalve showed the restoration of normal structure of the hepatopancreas. Histological sections of hepatopancreas after 5 days of recovery showed normal shape and size of epithelial cells. Regeneration of intertubular connective tissues and muscle fibres surrounding the tubules was observed. In some regions of tubules, necrotic changes were observed (Fig. d of plate 2). After 10 days of recovery more restoration of histology of hepatopancreas 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 cypermethrin were allowed to cure in 100 mg/L of L-ascorbic acid in freshwater for 5 day of recovery, normal architecture of hepatopancreas, with slight tubular lesions was

RESULTS AND DISCUSSION

The gross histopathological effects of acute dose of cypermethrin 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, Parreysia cylindrica are shown in plate no 1 to 2.

Histology of the normal hepatopancreas of Parreysia cylindrica

-Hepatopancreas is composed of tubules, having different shapes and sizes, and are surrounded by the intertubular connective tissues with some muscles, collagen fibres and amoebocytes. The epithelium of the tubules is placed on a thin basement membrane (Fig. a of plate 1). The epithelial cells have basally situated nuclei. Tubular epithelium comprises of two types of cells, the digestive and the secretary cells, the digestive cells are highly vacuolated and elongated, roughly cylindrical with the spherical nucleus at the base. In the vacuolar cytoplasm, granular material was present. The second, more or less pyramidal cells, known as the calcium cum secretary cells, normally appear triangular with varying heights. Their cytoplasm is more or less homogeneous, having a conspicuous nucleus, which is much bigger than that of the digestive cells, the selective calcium staining shows that the brown pellets or spherule are also present in cytoplasm. The pellets at times are so big that most of the cells, containing them, have the nucleus pushed aside to the basal part of the cells.

Set I: The animals were exposed to acute dose (0.4992 PPM LC50, value of 96 h) of cypermethrin, cypermethrin in same dose along with 50 mg/L of L-ascorbic acid and cypermethrin in same dose along with 100mg/L of L-ascorbic acid for 96 h.

Set II: The animals pretreated to cypermethrin (0.4992 PPM) for 96 h were divided in three groups. First group was allowed to cure in normal water, second in 50 mg/L of L-ascorbic acid and third in 100 mg/L of L-ascorbic acid for 15 days. Control animals were maintained in normal water. During experimentation, animals were fed on fresh water algae and every day solutions were changed. After 24 and 96 h of exposure, animals from set-I and after 5 days, 10 days and 15 days from set -II were dissected, hepatopancreas were fixed in aqueous Bouin’s fluid for 24 h, processed by usual way, serial sections were cut at six micron thickness and were stained with Mallory’s triple stain.

Animal pretreatment and cypermethrin intoxication Animals pretreated to acute and sub acute dose of cypermethrin were allowed to cure in normal water and with L-ascorbic acid showed the restoration of normal structure of hepatopancreas. 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 intertubular connective tissues and muscle fibres surrounding the tubules 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).

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When animals pretreated to acute dose of cypermethrin were allowed cure in 50 mg/L of L-ascorbic acid in freshwater, hepatopancreas of bivalve showed the restoration of normal structure of the hepatopancreas. Histological sections of hepatopancreas after 5 days of recovery showed normal shape and size of epithelial cells. Regeneration of intertubular connective tissues and muscle fibres surrounding the tubules was observed. In some regions of tubules, necrotic changes were observed (Fig. d of plate 2). After 10 days of recovery more restoration of histology of hepatopancreas 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 cypermethrin were allowed to cure in 100 mg/L of L-ascorbic acid in freshwater for 5 day of recovery, normal architecture of hepatopancreas, with slight tubular lesions was
Plate 2a: Curing digestive gland in freshwater at 5 days

Plate 2b: Curing digestive gland in freshwater at 10 days

Plate 2c: Curing digestive gland in freshwater at 15 days

Plate 2d: Curing digestive gland in 50 mg/L L-ascorbic acid in freshwater at 5 days

Plate 2e: Curing digestive gland in 50 mg/L L-ascorbic acid in freshwater at 10 days

Plate 2f: Curing digestive gland in 50 mg/L L-ascorbic acid in freshwater at 15 days

Plate 2g: Curing digestive gland in 100 mg/L L-ascorbic acid in freshwater at 5 days

Plate 2h: Curing digestive gland in 100 mg/L L-ascorbic acid in freshwater at 10 days

Plate 2: Microphotographs showing L.S. of digestive gland (x 400) of pre-exposed Parreysia cylindrica to cypermethrin during recovery in normal fresh water and, 50 mg/L and 100 mg/L of L-ascorbic acid in freshwater

Abbreviations: DTL-digestive gland lumen, DT-digestive tubule, BM-basement membrane, CT-connective tissue, V-vacuole, BBM-broken basement membrane DC-digestive cell SC-calcium secretary cell, DEC-degenerating epithelial cells, and DCT-degenerating connective cell. PN-pycnotic nucleus, NL-nucleolus
observed (Fig. g of plate 2). After 10 day of recovery almost all
damages were recovered and structure of hepatopancreas
was like those of control animals (Fig. h of plate II). The
histopathological changes as observed on cypermethrin
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 cypermethrin along with 50mg/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.
The mechanism through which ascorbic acid played a
protective role could also be attributed to its free radical
scavenging, antioxidant and apoptosis inducing nucleophilic
properties as supported by Surjiyo and Anisur (2004);
Zongyuan et al. (2009); Saraswathy et al. (2010). Probable
cause of epithelial damage in cypermethrin poisoning is the
destruction of basement membrane, which mainly contains
collagen. Darr et al. (1993) reported that pesticide generate
the super oxide radical anion (O²-) leading to tissue
destructions and marked fibrosis. They also stated that
pesticide inhibits collagen synthesis. Snawder and Chamber
(1993) reported that pesticide inhibits activities of lysyl oxidase
and proline hydroxylase and alter posttranslational
modification of collagen resulting in morphological defect in
connective tissue. Reason for collagen damage can be due to the
decrease in ascorbic acid content during acute
cypermethrin exposure. Number of workers has reported that
ascorbic acid content is decreased due to pesticide intoxication
in mollusc (Jadhav et al., 1996; Waykar and Lomte, 2004).
Collagen is a fibrous connective tissue protein, which is
abundant in basement membrane. The stability of collagen
depends upon its triple helix structure, which in turn depends
on the presence of the unique amino-
acid the hydroxyproline. Ascorbic acid stimulates collagen
synthesis by stimulating hydroxylation of proline, which is
catalyzed by prolyl hydroxylase. The function of ascorbic acid
for the stimulation of collagen synthesis is to keep the non-
hem iron of prolyl-4-hydroxylase in the active state (Kivirikko
and Myllyla, 1987). During the catalytic reaction a highly,
reactive iron-oxygen complex, ferryl ion, is produced, which
subsequently hydroxylates an appropriate proline residue
(Tschank et al., 1994). However, the generation of ferryl ion
also proceeds without proline hydroxylation in uncoupled
reaction cycles. Ascorbate is utilized as a specific alternative
acceptor of the ferryl in these uncoupled reaction cycle. In
the absence of ascorbate, prolyl-4-hydroxylase is rapidly
inactivated by self-oxidation (Tschank et al., 1994). Ascorbic
acid is known to participate in several biosynthetic reactions
as source of electron energy (Gorbunova, 1966). Probable
reason behind the poor rate of recovery of hepatopancreas in
animals pretreated to acute treatment of cypermethrin in fresh
water may be due to low concentration of ascorbic acid due
to cypermethrin stress. Proline of collagen synthesized in low
concentration of ascorbic acid is insufficiently hydroxylated.
This abnormal collagen cannot properly form trilamellar fibres
and thus do not give normal structure to basement membrane
so as to maintain epithelial layers. Skin lesions are found to be
the principal effect of pesticide toxicosis and hence ascorbic
acid can have protective and curing capacities on such action
of pesticide. This study clearly indicated protective and wound
healing property of ascorbic acid in experimental animals.
Thus it is evident that vitamin C not only confirm protection
against pesticide toxicity but can also perform therapeutic
role against pesticide toxicity.

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REFERENCES


