RESEARCH PAPER | Zoology | Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Curative Role of L-Ascorbic Acid on Dicofol Induced Alterations of The Protein Levels in The Freshwater Bivalve, Parreysia Cylindrica

KEYWORDS: L-ascorbic acid, protein, dicofol, recovery, Parreysia cylindrica

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ABSTRACT *The freshwater bivalve Parreysia cylindrica were exposed to chronic dose of dicofol, 0.04023 ppm (LC50/10) alone and in combination with 50mg/L L-ascorbic acid for 21 days. Percent protein contents in the mantle, foot, gills, digestive glands, gonad and whole soft body tissue of control bivalve, Parreysia cylindrica were 43.14±1.52, 62.91±1.32, 53.20± 2.67, 50.86± 2.55, 47.12± 1.93 and 60.72± 1.90 respectively. Percent protein contents in the mantle, foot, gills, digestive glands, gonad and whole body of bivalve, Parreysia cylindrica on dicofol intoxication were 21.22±2.17 (p<0.001), 38.17±2.50 (p<0.01), 22.85±2.04 (p<0.01),17.10± 2.37 (p<0.05), 24.65± 1.20 (p<0.01), and 31.08± 2.62 (p<0.05) respectively. Percent protein contents in the mantle, foot, gills, digestive glands, gonad and whole soft body tissue of bivalve, Parreysia cylindrica on exposure to dicofol with 50mg/L L-ascorbic acid were 24.22± 1.34 (p<0.001), 43.87±2.26 (p<0.01), 28.82±2.94 (p<0.001), 26.90± 2.24 (p<0.05),27.99±1.87 (p<0.01), and 35.06± 2.08 (p<0.01) respectively. Protein contents in all soft body tissue of dicofol exposed bivalve, Parreysia cylindrica showed remarkable decrease in protein content as compared to control. The higher depletion of protein was observed in digestive glands as compared to other tissues. Animal exposed to dicofol in combination with 50 mg/L of L-ascorbic acid showed considerable reduction in the depletion of protein levels. The pre-exposed bivalves for 21 days of exposure to chronic dose of dicofol showed recovery in normal water and the percent protein contents were increased to 29.95±2.92 (p<0.001) in mantle, 47.09±1.41 (p<0.01) in foot, 33.35± 2.28 (p<0.001) in gills, 25.32± 1.92 (p<0.001) in digestive glands, 34.46±2.52 (p<0.01 and 45.54± 2.38 (p<0.001) in whole body, while percent protein contents in presence of 50mg/L of L-ascorbic acid were increased to 38.79± 2.14 (p<0.01) in mantle, 67.10± 2.18 (p<0.01) in foot, 43.31±3.93(p<0.01) in gills, 33.97± 2.28 (p<0.05) in digestive glands,44.74± 2.8 (p<0.01) in gonad and 57.90± 2.28 (p<0.001) in whole body. Fast recovery of percent protein contents was observed in presence of L-ascorbic acid than the recovery in the normal freshwater. This study indicates the protective and curative property of the L-ascorbic acid against the dicofol induced damage.*

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 revised 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). Accumulated pesticides induce generation of reactive oxygen species (ROS). The ROS attack unsaturated fatty acids of the cell membrane that lead to the formation of LPO. Increased formation of LPO causes alterations in the levels of GSH and activities of the antioxidant enzymes, which leads to oxidative stress (Geter et al, 2008, El-Gendy et al, 2010). All the bio-molecules of cell like nucleic acids, lipids, proteins and polysaccharides are potential substrates of ROS (Manduzio et al, 2005). Such an effect may be at cellular or even at molecular level but ultimately it leads to physiological, pathological and biochemical disorders that may prove fatal to the organism (Jain and Kulshrestha, 2000). Many investigators reported a variety of wreckage in various metabolic processes in different species exposed to different kinds of pollutants. Toxic effects of pesticides on protein content of some aquatic animals are studied by Mohanty et al, (2005), Satyaparameshwar et al, (2006) and Pawar et al, (2009).

In order to overcome such effects the most combative source would be support with exogenous antioxidant. Ascorbic acid is an important dietary antioxidant and serves to protect against oxidative damage to macromolecules such as lipids, protein, DNA and RNA which are implicated in chronic diseases (Halliwell and Gutteridge, 1999: Agarwal et al, 2003). Ascorbic acid has potential role to reduce the activity of free-radical induced reactions (Holloway and Peterson, 1984).

The investigation regarding the biochemical changes after pesticide exposure and its subsequent recovery in non target aquatic species such as molluscs was insufficient. Hence in the present study an attempt was made to investigate the effect of chronic treatment of pesticide dicofol and its subsequent recovery by exogenous administration of L-ascorbic acid on the protein contents of different soft body tissues of fresh water bivalve, Parreysia cylindrica.

Materials and Methods

Medium sized, healthy, fresh water bivalve, Parreysia cylindrica were collected from Girna dam, 48 km away from Chalisgaon. Animals were brought in laboratory and were acclimatized for a week in dechlorinated tap water. The medium sized animals were selected for experiment.

Experimental design:

Set – I

For experimental studies the animals were divided into three groups

a) Group 'A' was maintained as control.
b) Group 'B' animals were exposed to chronic dose of dicofol (0.04023ppm, $LC_{50/10}$ values of 96 hrs) upto 21 days.
c) Group 'C' animals were exposed to chronic dose of dicofol (0.04023ppm), along with 50 mg/L of L-ascorbic acid upto 21 days.

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Experimental design for recovery studies - Set – II

1) Group 'B' animals from set I were divided into two groups for recovery studies.

i) Animals pre-exposed to chronic dose of dicofol (0.04023ppm) were allowed to self cure normally in untreated fresh water up to 21 days.

ii) Animals pre-exposed to chronic dose of dicofol (0.04023ppm) were allowed to cure in 50 mg/L of L-ascorbic acid added fresh water up to 21 days.

During experimentation animals were fed on fresh water algae. After every 7th,14th and 21st days of interval, animals from set-I and set-II were, dissected and tissues such as mantle, gills, foot, gonad, digestive glands and whole soft body tissues were separated and was dried at 80^0 C in an oven till constant weights was obtained. The total protein levels in dried powders of different tissues of control and experimental animals were estimate by the method of Lowry et al, (1951). The amount of total protein content was expressed in terms of mg of protein/100mg of dry weight of tissue.

Each observation was confirmed by taking at least three replicates. The difference in control and experimental animal group was tested for significance by using student't' test (Bailey, 1965) and the percentage of decrease or increase over control was calculated for each value.

RESULTS AND DISCUSSION

The data obtained regarding the protein contents in different soft body tissues after chronic exposure to dicofol with and without L- ascorbic acid and during recovery are summarized in the table no. 1 and 2. The obtained results demonstrated that, after chronic exposure to pesticide a marked depletion of protein contents in the mantle, foot, gills, gonad, digestive glands and whole soft body tissues of the experimental freshwater bivalve, Parreysia cylindrica were observed as compared to bivalves maintained as control. The results showed that, there was progressive decrease in the protein content as exposure period was increased. The results recorded in the present study are in harmony with the results of previous investigators (Waykar and Pulate, 2012; Pardeshi and Gapat, 2012).

The decrease in amount of protein content in all tissues after exposure to dicofol was attributed due to oxidative stress generated by pesticide. Pesticides are inducers of reactive oxygen species (Geter et. al., 2008). In the presence of reactive oxygen species (ROS), proteins can be damaged by direct oxidation of their amino acid residues and cofactors or by secondary attack via lipid peroxidation (Grune, 2000; Requena et al, 2003). Oxidative attack on proteins results in site-specific amino acid modifications, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge and increased susceptibility to proteolysis. The amino acids in a peptide differ in their susceptibility to attack, and the various forms of activated oxygen differ in their potential reactivity. Primary, secondary, and tertiary protein structures alter the relative susceptibility of certain amino acids. Oxidative modification of specific amino acids is one mechanism of marking a protein for proteolysis (Stadtman, 1986). In mollusk the proteases enzyme degrade oxidised proteins (Farr and Kogoma, 1991). Waykar and Lomte (2002) observed increased protease activity in experimental bivalves after exposure to pesticides.

Table No. 1. Total protein content in different soft body tissues of Parreysia cylindrica after chronic exposure to Dicofol without and with ascorbic acid.

Sr. No.	Tissue	Control (A)			Dicofol (B)			Dicofol + A.A.(50 mg/lit) (C)		
		7 days	14 days	21 days	7 days	14 days	21 days	7 days	14 days	21 days
1	Mantle	44.87 ± 1.42	43.68 ± 1.16	43.14 ± 1.52	29.96*±1.56 (-33.22)	23.88*±3.04 (-45.32)	21.22**±2.17 (-50.81)	33.92*± 1.17 (-24.40)	26.62**±2.49 (-39.05)	24.22***± 1.34 (-43.85)
2	Foot	63.48 ± 2.42	63.23 ± 1.26	62.91 ± 1.32	47.83**±2.80 (-24.65)	43.22**±2.71 (-31.64)	38.17**±2.50 (-39.32)	53.63***±1.19 (-15.51)	47.98***±2.11 (-24.11)	43.87***± 2.26 (-30.26)
3	Gills	54.16 ± 2.48	53.70 ± 1.65	53.20 ± 2.67	29.83**±2.06 (-44.92)	25.93**±3.86 (-51.71)	22.85***±2.04 (-57.04)	36.94***± 1.95 (-31.79)	32.05***± 2.45 (-40.31)	28.82***±2.94 (-45.82)
4	Digestive glands	50.74 ± 1.25	51.16 ± 2.68	50.86 ± 2.55	25.87*±3.01 (-49.01)	17.92**±2.98 (-64.97)	17.10***±2.37 (-66.37)	41.95*± 1.32 (-17.32)	36.87*± 1.94 (-27.93)	26.90**± 2.24 (-47.10)
5	Gonad	48.58 ± 2.88	48.21 ± 1.62	47.12 ± 1.93	38.36*±2.14 (-21.03)	32.56**±1.31 (-32.46)	24.66**±1.20 (-47.66)	38.87***± 1.90 (-19.98)	38.12***± 1.30 (-20.92)	27.99***± 1.87 (-40.59)
6	Whole soft body	61.66 ± 3.04	61.12 ± 1.58	60.72 ± 1.90	43.74**±1.05 (-29.06)	34.98**± 2.41 (-42.76)	31.08***±2.62 (-48.81)	46.27***± 2.95 (-24.95)	39.18***± 1.13 (-35.89)	35.06***± 2.08 (-42.25)

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Table no. 2. Total protein content in different soft body tissues of Parreysia cylindrica after chronic exposure to Dicofol and its subsequent recovery.

Sr. No.	Tissue	Dicofol	Recovery in normal water			Recovery in A.A.(50 mg/lit)			
		21 days	7 days	14 days	21 days	7 days	14 days	21 days	
1	Mantle	21.22**± .17 (-50.82)	24.47^{NS}±3.13 (+15.31)	25.81**±1.07 (+21.63)	29.95**± 2.92 (+41.14)	27.14*± 2.56 (+27.18)	32.46**±3.36 (+52.25)	38.79***±2.14 (+82.79)
2	Foot	38.17**± 2.50 (-39.32)	42.57^{NS}±2.63 (+11.52)	45.18^{NS}±3.06 (+18.36)	47.09 *±1.41 (+25.67)	48.62^{NS}±2.33 (+27.37)	54.81*±1.58 (+43.59)	67.10***±2.18 (+75.79)
3	Gills	22.85***±2.04 (-57.05)	26.02^{NS}±3.80 (+13.87)	27.81*±1.27 (+21.70)	33.35**±2.28 (+45.95)	29.22**±1.92 (+27.87)	35.24**±2.16 (+54.22)	43.31***±3.93 (+89.54)
4	Digestive glands	17.10***±1.37 (-66.37)	19.84*±2.13 (+16.02	22.11*±1.07 (+29.29)	25.32**±1.92 (+48.07)	23.43**±1.23 (+37.01)	27.80**±1.83 (+62.57)	33.97***±2.28 (+98.65)
5	Gonad	24.66**± 2.20 (-47.67)	27.65*±2.38 (+12.12)	29.72*±1.32 (+20.51)	34.46**±2.52 (+39.74)	31.15**±1.91 (+26.31)	36.35**±2.79 (+47.40)	44.74***±2.8 (+81.42)
6	Whole soft body	31.08***± 2.62 (-48.82)	35.54^{NS}±2.9 (+14.35)	38.35*±1.79 (+23.39)	45.54**±2.38 (+46.52)	39.12*±1.23 (+29.08)	47.21**±2.83 (+51.89)	57.90***±2.28 (+86.29)

1. Values expressed as mg/100mg dry wt. of tissue, 2. (+) or (-) indicate percent variation over control, 3. ± indicate S.D. of three observation, 4. Values are significant at *P<0.001, **P<0.01, ***P<0.05
5. NS (Not significant)

The decrease in amount of protein content in different soft body tissues after chronic exposure to pesticide, indicate a rapid initiation of breakdown of protein which ultimately results in increase in the free amino acid pool, which may be fed to TCA cycle through aminotransferase probably to cope up with the high energy demands under toxic stress (Vincent et al, 1995; Waykar and Lomte, 2001, Pottinger et al, 2002). Jha (1988) supported the idea of consumption of amino acid for metabolic processes as energy source. Catabolism of proteins and amino acids make a major contribution to the total energy production. Claybrook (1983) reported that structural proteins are used as energy source under stressful conditions. The depletion of protein content in the tissues was might be due to diversification of energy, to meet the impending energy demand under toxic stress (Vincent et al, 1995) and to prevent fatigue due to pesticide toxicity (Parate and Kulkarni, 2003).

The results of total protein contents in all tissues clearly indicate that digestive glands was the most affected organ followed by gill, whole body, mantle, gonads and foot. The higher depletion of protein in the digestive glands might be due to high metabolic potency and efficiency of the glands, when compared to other soft body tissues of the bivalve. The digestive glands seems to be the main site of degradation and detoxification of pesticides and hence has the largest demand of energy for the metabolic processes resulting into increasing utilization of protein in digestive glands provides better indication of the extent of toxicity. Waykar and Lomte (2001), and Waykar and Pulate (2012) supported the most alteration of protein contents in digestive glands of freshwater bivalves.

Large body of literature reported that, pesticide stress caused depletion of protein content. Satyaparameshwar et al, (2006) reported decrease in the protein contents of freshwater bivalve, Corbicula striatella after heavy metal stress. Waykar and Pulate (2012) reported decreased protein contents in different soft tissues of freshwater bivalve, Lamellidens marginals (L) after chronic exposure to profenofos and reported highest decrease in protein contents in digestive glands. Pardeshi and Gapat (2012) reported a marked decrease in protein content in different soft tissues of the freshwater bivalve, Lamellidens corrianus after chronic exposure to nickel chloride.

In combined exposure to dicofol with 50mg/l of L-ascorbic acid the severity of protein depletion was much reduced. Pesticides are known to enhance the formation of reactive oxygen species. The ROS have the capacity to cause damage to biomolecules such as proteins, nucleic acids etc. Ascorbic acid usually acts as an antioxidant. It typically reacts with oxidants of the reactive oxygen species such as the hydroxyl radical formed from hydrogen peroxide Such radicals are damaging to animals at the molecular level due to their possible interaction with proteins, and nucleic acids. Sometimes these radicals initiate chain reactions. Ascorbic acid can terminate these chain radical reactions by electron transfer. Ascorbic acid functions as a reductant for many free radicals, thereby minimizing the damage caused by oxidative stress. Thus this study indicates that the use of L-ascorbic acid protect the tissues from oxidative damage caused by pesticide.

Many investigators reported the similar results. Mahajan and Zambare, (2001) reported the protection by ascorbic acid against the heavy metal induced alterations in protein levels in fresh water bivalve, Corbicula striatella. The bioregulatory role of ascorbic acid to protect extracellular protein function through gene expression was highlighted by Griffiths and Lunec, (2001). Waykar and Pulate (2012) and Pardeshi and Gapat (2012) reported the role of ascorbic acid in amelioration of protein alteration induced by toxicants in bivalves. The present results showed that ascorbic acid, one of the most important antioxidant, spares the other oxidants by forming the first line defense against free radicals and peroxides that are generated during cellular metabolism (May, 2000).

Recovery study

In present study, the bivalves pre-exposed to chronic concentration to dicofol showed fast recovery in protein level in presence of 50 mg/L L-ascorbic acid than those allowed curing naturally (table no.2). This study indicates that the use of L-ascorbic acid protect the tissues from oxidative damage caused by pesticide. The results recorded in the present study are in harmony with the results of previous investigators (Mahajan and Zambare, 2006; Pardeshi and Gapat, 2012;

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Waykar and Pulate, 2012).

Ascorbic acid has promising antioxidant property. L-Ascorbic acid play a curative role against pesticide induced biochemical alteration and cures structural damages caused by pesticides in the animal body. Many studies have demonstrated that vitamin C, can readily scavenge ROS, reactive nitrogen species and prevent oxidative damage to many important biological macromolecules such as DNA, lipids and proteins (Carnes et al, 2001; Konopacka, 2004). According to Wu et al, (2004) and Vishwanatha Swamy et al, (2011) at cellular level, ascorbic acid has been mitigate the deleterious effect of ROS directly by increasing antioxidant enzyme activities of cells and indirectly by reducing oxidized form of vitamin E and GSH. Hence the preventive effect of vitamin C on tissue damage induced by pesticides may be associated with its antioxidants capacity or as free radical scavenger that inhibits lipid peroxidation (Carr and Frei, 2000). In addition to scavenging of ROS and reactive nitrogen species, ascorbic acid can regenerate other small molecule antioxidants, such as a-tocopherol, GSH, urate, and β-carotene from their respective radical species (Englard and Seifter, 1985). These properties of ascorbic acid make it a suitable antidote for pollutant toxicity in rodents and possibly in human subjects (Sohini and Rana, 2007). Mahajan and Zambare (2006) showed faster recovery by ascorbic acid against the toxicants induced alterations in protein, ascorbic acid, DNA and RNA levels in freshwater bivalve.

CONCLUSION

From the obtained results it may be concluded that the physiological disturbances arising in animals after exposure to pesticides exhibits trends towards normalization and this rate of recovery from pesticide induced damage was faster on exposure to L-ascorbic acid indicating the preventive and curative property of the L-ascorbic acid against the pesticide induced damage. Thus it was evident that vitamin C not only confirm protection against pesticide toxicity but can also perform therapeutic role against pesticide toxicity in mollusc.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Zoology, Rashtriya Arts, Commerce and Science College Chalisgaon for providing laboratory facility for this work.

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