RESEARCH PAPER | Science | Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Protective role of L-ascorbic acid on dichlorovos induced alterations in the protein contents of the fresh water bivalve, *Parreysia cylindrica*

KEYWORDS: Ascorbic acid, dichlorovos, organochlorine, carbamate and Parreysia cylindrica.

R. S. Tambe
Arts, Commerce and Science College, Satral, (Shirdi).
Arts, Commerce and Science College, Kholar

P. D. Pulate
Arts, Commerce and Science College, Satral, (Shirdi).
Arts, Commerce and Science College, Kholar

ABSTRACT *The increasing use of pesticides in order to improve the agricultural productivity to match the population growth rate is a global phenomenon. The use of various classes of insecticides as organophosphate, organochlorine, carbamate and pyrethroids have been increased many fold for the last 10 years. Accumulation of pesticides in lower organisms as well as in the tissues of fishes, birds and human have been recorded. Accumulated pesticides induce generation of reactive oxygen species (ROS). All the bio-molecules of cell like nucleic acids, lipids, proteins and polysaccharides are potential substrates of ROS. 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 many instigators. 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 Guttering, 1999: Agarwal , et.al. 2003). Ascorbic acid has potential role to reduce the activity of free-radical induced reactions (Holloway and Peterson, 1984). Therefore in present investigation attempts were made to observe whether treatment of ascorbic acid would aggravate or ameliorate the biochemical alteration in tissues induced by pesticide in model animal freshwater, bivalve.*

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 dichlorovos 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.

An investigation was undertaken to evaluate the effectiveness of ascorbic acid on Dichlorovos induced alteration on the ascorbic acid levels in an experimental model, the fresh water bivalve Parreysia cylindrica. The effect on bivalve was studied under five groups. Group A was maintained as control, group B bivalves were exposed to chronic dose of C4H7CI2O4P (0.04023ppm) while group C bivalve where exposed to respective chronic concentration of Dichlorovos with 50mg/L of L- ascorbic acid for 21 days. Were divided into D and E group. The D group bivalves were allowed to cure naturally while E group bivalves were exposed to 50mg/L of L-ascorbic acid for recovery. After every seven days bivalves from A, B, C, D & E were removed, and their tissue were separated and dried at 80 0c .From each powder, ascorbic acid level was estimated and presented as mg/gm of dry weight. Significant decrease was observed in ascorbic acid content on the exposure to Dichlorovos. However; depletion in the L-ascorbic acid level on the exposure to dichlorovos with L-ascorbic acid was minimum. Pre-exposed bivalves to dichlorovos showed fast recovery in the ascorbic acid exposed bivalves than those, which were allowed to recover naturally; the probable role of L- Ascorbic acid is discussed in the paper.

Introduction:

Water is one of the most important essential basic natural resource of the living kingdom. As an inevitable outcome aquatic, terrestrial and aerial life are being dangerously effected by the introduction of various undesirable and toxic components such as industrial and pesticide wastes. Since all industrial and agricultural sources discharge their effluents in the adjoin watery areas they not only frequently. Make Man caused serious hazards in the quality of water, but also to the aquatic life.

Organophosphate is more soluble extremely toxic nerve poisons, fast acting, quickly degraded do not toxic to many organisms, very dangerous to farm workers in agricultures, a number of organophosphate compounds were used for control of diseases pest all over the world in forestry practices, a bulk amount of pesticides was used in the past were plantation forestry was widely practiced.(S. C. Santra et,al.2001).Organophosphate compounds are neurotoxins that inhibit the breakdown of acetylcholine, thus they keep neurons in a state of excitement (Eto,1974). It is not acutely toxics to many crustaceans and other non-target organisms but is highly water soluble with a solubility of over 800,000 mg/L (PAN, 2008).

The step wise reduction of molecular oxygen to water during cellular respiration create highly reactive oxygen intermediates in all cells of aerobic organisms (Winston and Di Giulio,1991),Fridovich,1998.Halliwell and Gutteridge,(1999).Among these reactive oxygen species (ROS) are the superoxide (O_2) and hydroxyl radicals (OH), and the non-radical hydrogen peroxide (H_2O_2) . ROS can oxidize and disable essential cellular components like Lipids, DNA, and Proteins (Halliwell and Gutteridge1999). Oxygen radicals can be particularly harmful because they can form chain reaction that create more radicals. Exposure of organisms to xenobiotics that either promotes the production of ROS, the removal of ROS can lead to an imbalance in pro-oxidants and antioxidants result in oxidative damage.

Insecticides comprise a greater proportion of pesticides used in the tropic and sub tropics because of the higher intensity of pests in these climate conditions. Organophosphates are simple esters of monoflurophosphoric

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

acid highly toxic. Dichlorovos is synthetically produced insecticides, which is most effective, quick killing organophosphate insecticide widely used in the field for biting and sucking type of insects. Highly toxic, vaporized easily used as a fumigants, primarily affects the nervous system through cholinesterase inhibition, the blockage of an enzyme required for proper functioning render the organism to behave abnormally, leading to death the toxicant get dissolved. Though the organophosphate pesticides may disappear rapidly from the body either by hydrolysis or elimination long term and repeated exposure to these pesticides have accumulative effect on aquatic animals (Begum and Vijayaraghavan, 1996).

L-ascorbic acid is one of the most important water soluble metabolite in the animal life. It is an antioxidant vitamin acting as a co-enzyme to convert proline and lysine to hydroxyproline and hydroxyl sine, both important for the collagen (structural protein) synthesis, soluble toxic molecules cerate free radical sand generate cellular injury and disease. Ascorbic acid acts as a detoxifier and may reduce the side effects of drugs such as cortisone, aspirin and insulin. It may also reduce the toxicity of the heavy metals like lead, mercury and arsenic. Man, other primates and guinea pigs are scorbutic animals, which can not synthesize the ascorbic acid and hence need regular intake through the food. Generally ascorbic acid is metabolized into the oxalic acid and diketogulanic acid (Tolbert *et al*., 1967) and the vitamin is immediately destroyed. In animal ascorbic acid contents in tissue increase in stress conditions and during metal toxicsis (Rao and Chinoy, 1986) indicating positive role of ascorbic acid in detoxification. (Jadhav , 1996) observed that the ascorbic acid content of various tissues for the bivalve *Parreysia cylindrica* after chronic exposure to toxicants (Mahajan and Zambare, 2001).

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 after brushing up the algae from their shells, animals were acclimatized for a week to de chlorinated tap water. The medium size animals were selected for experiment. The animals were exposed to chronic concentration of dichlorovos (0.04023 ppm, LC_{50} /10 values of 96 hrs.) and profenofos along with 50 mg/L of L-ascorbic acid up to 21 days. Every day the solution was changed.

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 treatment of dichlorovos (0.04023 ppm, LC_{50} /10 values of 96 hrs.) up to 21 days.
C) Group 'C' Animals were exposed to chronic treatment of dichlorovos (0.04023 ppm) along with 50 mg/L of L-ascorbic acid.

Set – II-Experimental design for recovery studies -
1) Group 'B' animals from set – I were divided into two groups for recovery studies.

Animals pre-exposed to chronic dose (0.6191 ppm) of dichlorovos were allowed to self cure normally in untreated fresh water up to 21 days.

i) Animals pre-exposed to chronic dose (0.6191 ppm) of dichlorovos 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 7^{th},14^{th} and 21^{st} days of interval animals from set-I were taken out, dissected and tissues such as digestive glands, gills, foot, mantle were separated and whole body mass of remaining animals was taken. All tissues were dried at 70 – 80^0 C in an oven till constant weights were obtained. The dried powders of different tissues of control and experimental animals were used for estimation of total protein contents by spectrophotometric method proposed by 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 students" test (Bailey, 1965) and the percentage of decrease or increase over control was calculated for each value.

The percentage of decreased in protein content after chronic treatment of 7, 14 and 21 days with dichlorovos was 31.89, 38.90 and 43.22 in mantle, 17.25, 24.22 and 33.12 in foot, 37.53, 44.26 and 53.29 in gills, 43.10, 57.17 and 62.44 in digestive glands, 29.15, 36.61 and 41.31 in gonad and 22.57, 37.87 and 44.04 in whole soft body.

In combined treatment of pesticide along with 50mg/l L-ascorbic acid the total protein content in mantle, foot, gills, digestive glands, gonads and whole soft body tissues was less decreased as compared to bivalve treated with pesticide only.

The percentage of decreased in protein content after chronic treatment of 7, 14 and 21 days with dichlorovos with ascorbic acid was 19.81, 25.77 and 37.67 in mantle, 9.21, 18,42 and 23.91 in foot, 26.19, 34.73 and 39.06 in gills, 14.76, 28.53 and 41.21 in digestive glands, 21.85, 29.23 and 32.12 in gonad and 19.12, 28.04 and 34.85 in whole soft body.

Animals pre-treated to chronic dose of pesticide dichlorovos for 21 days and are allowed to cure in ascorbic acid medium the increase in protein content was noted. The percent decrease of protein content after 7, 14 and 21 days was 12.49, 18.21 and 32.65 in mantle, 6.32, 12.97 and 23.04 in foot, 11.21, 22.72 and 43.62 in gills, 15.50, 27.11 and 46.48 in digestive glands, 11.69, 17.54 and 27.23 in gonad and 13.39, 21.70 and 44.04 in whole soft body.

Animal pre-treated to chronic treatment of dichlorovos and allowed cure in 50mg/l L-ascorbic acid in water bivalve showed increase in protein content in all soft body tissues of experimental bivalves. The percent increase in protein content after 7, 14 and 21 days was 20.44, 45.22 and 71.30 in mantle, 14.17, 34.21 and 50.85 in foot, 25.51, 48.17 and 82.37 in gills, 30.34, 49.35 and 89.13 in digestive glands, 21.53, 40.71 and 68.18 in gonad and 23.73, 41.16 and 79.12 in whole soft body.

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Table No. 3.1.a. Total protein content in different soft body tissues of *Parreysia cylindrica* after chronic exposure to Dichlorovos without and with ascorbic acid.

Sr. No.	Tissue	Control (A)			Dichlorovos (B)			Dichlorovos + 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 ±1.32	43.68 ±1.58	43.14 ±1.97	30.56** ± 1.00 (-31.89)	26.67** ± 3.04 (-38.90)	24.50** ± 2.17 (-43.22)	35.98** ± 2.17 (-19.81)	32.42** ± 2.49 (-25.77)	26.89** ± 1.34 (-37.67)
2	Foot	63.47 ±2.38	63.23 ± 1.20	62.91 ± 1.28	52.52* ± 2.80 (-17.25)	47.91*** ± 3.71 (-24.22)	42.07*** ± 2.50 (-33.12)	57.63** ± 1.19 (-9.21)	51.58** ± 2.11 (-18.42)	47.87*** ± 3.26 (-23.91)
3	Gills	54.15 ±2.43	53.70 ± 1.60	53.20 ± 0.58	33.83** ± 3.06 (-37.53)	29.93*** ± 1.86 (-44.26)	24.85*** ±1.04 (-53.29)	39.97** ± 2.95 (-26.19)	35.05** ±3.45 (-34.73)	32.42*** ± 3.94 (-39.06)
4	Digestive glands	50.74 ± 1.21	51.16 ± 0.64	50.85 ± 0.50	28.87** ± 1.01 (-43.10)	21.91*** ± 4.98 (-57.17)	19.10*** ±1.37 (-62.44)	43.25* ± 1.32 (-14.76)	36.57** ± 2.94 (-28.53)	29.89*** ± 3.24 (-41.21)
5	Gonad	48.58 ± 0.82	48.21 ± 1.56	47.12 ± 0.85	34.41* ± 3.14 (-29.15)	30.56* ± 2.31 (-36.61)	27.65** ± 2.20 (-41.31)	37.96* ± 2 .81 (-21.85)	34.12* ±1.30 (-29.23)	31.98** ± 2.87 (-32.12)
6	Whole soft body	61.65 ± 2.93	61.12 ± 1.52	60.72 ± 1.85	47.74* ± 2.05 (-22.57)	37.97** ± 4.41 (-37.87)	33.97*** ± 1.62 (-44.04)	49.87* ± 2.95 (-19.12)	43.98 ± 1.13 (-28.04)	39.56** ± 3.08 (-34.85)

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)

Table No. 3.1.b. Total protein content in different soft body tissues of *Parreysia cylindrica* after chronic exposure to Dichlorovos and its subsequent recovery.

Sr. No.	Tissue	Dichlorovos	Recovery in normal water (i)			Recovery in A.A.(50 mg/lit) (ii)		
		21 days	7 days	14 days	21 days	7 days	14 days	21 days
1	Mantle	24.50 (-43.22)	27.56^{NS} ± 9.13 (+12.49)	28.96* ± 1.07 (+18.21)	32.50** ± 2.92 (+32.65)	29.51** ± 2.56 (+20.44)	35.58*** ± 3.36 (+45.22)	41.97*** ± 4.14 (+71.30)
2	Foot	42.07 (-33.12)	44.73^{NS} ± 2.63 (+6.32)	47.52* ± 5.06 (+12.97)	51.76** ± 1.41 (+23.04)	48.03** ± 5.33 (+14.17)	56.46** ± 1.58 (+34.21)	74.23** ± 1.18 (+76.44)
3	Gills	24.85 (-53.29)	27.63^{NS} ± 4.80 (+11.21)	30.49* ± 1.27 (+22.72)	35.69* ± 3.28 (+43.62)	31.19** ± 1.92 (+25.51)	36.82** ± 3.16 (+48.17)	45.32** ± 6.93 (+82.37)
4	Digestive glands	19.10 (-62.44)	16.14* ± 9.13 (+15.50)	24.28** ± 1.07 (+27.11)	27.98*** ± 2.92 (+46.48)	24.89* ± 1.23 (+30.34)	28.52*** ± 2.83 (+49.35)	36.12** ± 6.28 (+89.13)
5	Gonad	27.65 (-41.31)	30.89* + 2.38 (+11.69)	32.51* ± 1.32 (+17.54)	35.19** ± 3.52 (+27.23)	33.61** ± 2.11 (+21.53)	38.91** ± 3.79 (+40.71)	48.02*** ± 3.8 (+73.67)
6	Whole soft body	33.97 (-44.04)	38.52 ^{NS} ± 4.01 (+13.39)	41.35* ± 2.79 (+21.70)	48.94** ± 3.38 (+44.04)	42.04* ± 1.23 (+23.73)	47.96** ± 2.83 (+41.16)	60.86*** ± 6.28 (+79.12)

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)

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

Results and discussion:

Ascorbic acid contents in different tissues of *Parreysia cylindrica* after exposure to dichlorovos (0.04023 ppm) along with and without ascorbic acid and during recovery have been summarized in table no. 1. The ascorbic acid contents in gills, foot, gonads, digestive glands and whole body of bivalve in presence of dichlorovos (0.04023ppm) decrease with the increase in exposure period. The ascorbic acid content were more in pesticide with ascorbic acid exposed bivalves as compared to those exposed to only pesticide for the corresponding period of exposure. The bivalves pre-exposed to pesticide show fast recovery in the alteration of ascorbic acid level in presence of ascorbic acid than those allowed to cure naturally.

Pesticides (dichlorovos) affect the metabolism of fresh water bivalve *Parreysia cylindrica*. Alterations in metabolic processes following exposure to pesticide stress have always been used as an indicator of stress. Such stressful condition variable from pesticide to pesticide and animal to animal.Generally ascorbic acidic an excellent reducing agent, which is able to serve as donor antioxidant in the free radical mediated oxidation process and is able to reduce pesticide (Buttner and Jerkewitz. 1996).Role of ascorbic acid is Ana outstanding antioxidant in red blood cells where complex system of ascorbic transport and recycling exist has been emphasized unit some recent publication (May, 1995, Mendiraffa 1977). L-ascorbic acid is strong antioxidant and may extended its protective effect by precipitating free radicals and removing form the system (Tajmir Riah, 1991). Ascorbic acid plays an important role in distribution and exertion of trace minerals and toxic pesticides (Lewin 1974).

From the above references conclusion may be drawn that ascorbic acid acts as detoxifying agent. As a result, the levels of the ascorbic acid in the tissue decrease. When ascorbic acid is supplied externally, some of the ascorbic acid is absorbed by the gills, Gonads, Foot, Digestive gland and whole body hence the level of the ascorbic acid recovered and hence it minimizes the toxic load of the toxicant. During the toxic stress, animals needed more energy and hence most of the tissue glucose is used for the energy and hence most of the tissue glucose is used for the energy purpose therefore there may be poor synthesis of the ascorbic acid as its synthesis needs the glucose.

REFERENCE

Azarich, (2002): Effect of organophosphate (Nvan) on protein contents of gills and liver in the fish, Rasbora daniconius. Him. J. Env. Zool., 19 (1): 63 – 64. | Bailey, N.T.J. (1965): Statistical methods in Biology. ELBS. English University Press, London. | Begum G and Vijayaraghavan S (1996): alteration in protein metabolisms of muscle tissue in the fish clarius batrachus (Linn) by commercial grade dimethoate, Bull. Environ. Contain, Topical. 57: 223-228. | Chinoy, N.J. (1972b): Ascorbic acid level in mammalian tissues and its metabolic significance. Comp. Biochem. Physiol., 42(A); 945-952. | Chinoy, N.J. and Garg A.N. (1978): Proc. All over India Symp. Environ. Biol. Trivandrum, India. Abst. N.41; Pp.24. | Deshmukh, Shrikant. (2012): Effect of L- ascorbic acid on copper induced alterations in protein, ascorbic acid and DNA content of freshwater bivalve, Indonaia caeruleus. National Journal of Life Sciences, 9(11); 37-41. | Dhār R. and K. Biswas (1997): Ground water arsenic calamity in Bangladesh. Curr. Xci, 48-49. | Eto (1974): Dept of Ecology. Eco toxicology of Natural and Anthropogenic Extreme Environments. | Fridovich (1998): Dept of Ecology. Ecotoxicology of Natural and Anthropogenic Extreme Environment. | Halliwell, B. and Gutteridge, JMC (1999): Free radicals in biology and medicine. Oxford University Press, Oxford. | Halver J. (1972): The role of ascorbic acid in fish diseases and tissue repair.Bull jopsoc. Sci fisheries, 35(1) 79-92. | Jadhav S. M., Sontakke Y. B. and Lomte V. S. (1996). Effect of carbaryl on ascorbic acid contents in the selected tissues of Corbicula striatella. J. Ecotoxicology env. Monitoring. 6(2): 109-112. | Jadhav Sunita, Sontakke, Y. B. and Lomte, V.S. (1996): Effect of carbaryl on ascorbic acid content in the selected tissues of Corbicula striatela. J. Ecotoxicol. Env. Monit., 6(2); 109-112. | Lewin S (1974) Vitamin C: Its molecular biology and medical potential. Academic Press, London, UK. | Lowry, O.H., Rosenberger, N.J., Farr, A.L. and Randall, R.J.(1951): Protein measurement with folin-phenol reagent. J. Biol. Chem. 193; 265-275. | Mahajan, A.Y. and Zambare, S.P. (2001): Ascorbate effect on CuSo4 and HgCl2 induced alteration of protein levels in fresh water bivalve Corbicula striatella. Asian J. Micro. Biotech. & Environ. Sci. 3 (1-2); 95-100. | Mendiratta S. W. May J. M. (1997): Enzyme dependant thyroxin reeducates. Free Red. Med. Q4:789. | PAN. (2008) Dept of Ecological Science. Exotoxicology of Natural and Anthropogenic Extreme Environments. | Rao, M. V. and Chinoy N. J. (1986): Effect of estaradiol benzoate on rat testis and adrenal. Exp. Clin. Experimentally 23:55-60. | Ror J. H. (1967): methods of biochemical analysis (ED. Bick) Intersicence Nesw York. Vol.5.44-45. | S. C. Santra (2001) (2001) de3pt of Environmental Science university of Kalyani, Nadia. West Bengal. | Tolbert, B. M. A. W. Clem, E. M. Bell, and E. M. Baker (1967): Metabolism of Lascorbic 4-H acid in Man, Amir, J. clin, Nutr. 20: 250-261. | Winston and Digiwlio (1990): Dept of Ecology, Ecotoxicology of Natural and Anthropogenic Extremes Environment. |