

AMELIORATING EFFECT OF L-ASCORBIC ACID ON PROFENOFOS INDUCED ALTERATIONS IN THE PROTEIN CONTENTS OF THE FRESHWATER BIVALVE, *LAMELLIDENS MARGINALIS* (LAMARCK)

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KEY WORDS

L-Ascorbic acid
Protein
Profenofos
Lamellidens marginalis

Received on :
07.10.2011

Accepted on :
14.12.2011

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ABSTRACT

The freshwater bivalve *Lamellidens marginalis* were exposed to chronic dose of Profenofos, 0.6191 ppm (LC₅₀/10) alone and in combination with 50mg/L L-ascorbic acid for 21 days. Percent protein contents in the mantle, foot, gill, digestive glands and whole body of control bivalve, *Lamellidens marginalis* were 41.76 ± 1.50, 60.49 ± 1.30, 51.52 ± 0.62, 47.52 ± 0.53 and 57.14 ± 1.88 respectively. Percent protein contents in the mantle, foot, gill, digestive glands and whole body of bivalve, *Lamellidens marginalis* on Profenofos intoxication were 21.98 ± 2.54 (p < 0.001), 41.48 ± 1.87 (p < 0.01), 22.78 ± 2.04 (p < 0.01), 16.68 ± 1.03 (p < 0.05) and 30.77 ± 2.09 (p < 0.01) respectively. Percent protein contents in the mantle, foot, gill, digestive glands and whole body of bivalve, *Lamellidens marginalis* on exposure to Profenofos with 50mg/L L-ascorbic acid were 30.77 ± 2.04 (p < 0.001), 45.24 ± 1.45 (p < 0.01), 29.96 ± 1.25 (p < 0.001), 32.23 ± 1.52 (p < 0.05) and 35.56 ± 1.94 (p < 0.01) respectively. Protein contents in the mantle, foot, gill, digestive glands and whole body of profenofos and Profenofos with 50mg/L L-ascorbic acid exposed bivalve, *Lamellidens marginalis* 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. Exposure to pesticide Profenofos 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 exposure to chronic dose of Profenofos showed recovery in normal water and the protein contents were increased to 28.16 ± 0.5 (p < 0.001), in mantle, 48.56 ± 0.23 (p < 0.01), in foot, 30.27 ± 0.45 (p < 0.001), in gill, 24.77 ± 0.24 (p < 0.001), in digestive glands and 43.96 ± 2.05 (p < 0.001), in whole body while percent protein contents in presence of 50mg/L of L-ascorbic acid were increased to 34.63 ± 1.82 (p < 0.01), in mantle, 57.15 ± 2.24 (p < 0.01), in foot, 39.42 ± 0.46 (p < 0.01), in gill, 30.16 ± 0.79 (p < 0.05), in digestive glands and 52.04 ± 0.45 (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 Profenofos induced damage.

INTRODUCTION

Excessive use of pesticides has 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). Such an effect may be at cellular or molecular level but ultimately it would lead to physiological, pathological and biochemical disorders that may prove fatal to the organism (Jain and Kulshreshtha, 2000).

Biological complex antioxidant system includes antioxidant enzymes and non-enzymatic antioxidants such as carotinoids, vitamin E and C acting against intracellular oxidative stress (Agarwal et al., 2003). Ascorbic acid has potential role to

reduce the activity of free-radical induced reactions (Holloway and Peterson, 1984). Ascorbic acid prevents free radical induced protein damage (Halliwell and Gutteridge, 1999). 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).

The present study investigates the propensity of profenofos induced variation in protein level and its possible attenuation by vitamin C in a convenient model, the fresh water bivalve, *Lamellidens marginalis* after chronic exposure.

MATERIALS AND METHODS

Medium sized, healthy, fresh water bivalve, *Lamellidens marginalis* were collected from Girna dam, 48km away from Chalisgaon. Animals were brought in laboratory and were acclimatized for a week to 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

- Group 'A' was maintained as control.
- Group 'B' animals were exposed to chronic dose of profenofos (0.6191 ppm) upto 21 days.
- Group 'C' animals were exposed to chronic dose of profenofos (0.6191 ppm) along with 50 mg/L of L-ascorbic acid.

Experimental design for recovery studies

Set – II

Group 'B' animals from set-I after 21 days exposure to profenofos were divided into two groups for recovery studies.

- Animals pre-exposed to chronic dose (0.6191 ppm) of profenofos were allowed to self cure in normal fresh water upto 21 days.
- Animals pre-exposed to chronic dose (0.6191 ppm) of profenofos were allowed to cure in 50 mg/L of L-ascorbic acid added fresh water upto 21 days.

During experimentation animals were fed on fresh water algae. After every 7th, 14th and 21st days interval, animals from set-I and set-II were, dissected and tissues such as digestive glands, gills, foot, mantle were separated and whole body mass was dried at 80°C in an oven till constant weights were 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 tissues after chronic exposure to profenofos with and without

L- ascorbic acid and during recovery are given in the Table 1 and 2. The total protein levels of all tissues after chronic treatment of profenofos were decreased. The higher depletion of protein in digestive gland than mantle, foot, gill and whole body, might be due to high metabolic potency and efficiency of the gland as compared to other tissues. The digestive gland may be the site of action of pollutant in the body of bivalves or it seems to be main site of degradation and detoxification of pesticide and hence has the largest demand of the energy for the metabolic processes resulting into increasing utilization of protein to meet energy demand. Muley and Lomte (1995) and Waykar and Lomte (2001) supported the higher loss of protein in digestive gland. The severity of protein depletion increases with increase in exposure period to profenofos.

A marked fall in the protein level in all the tissues after exposure to pesticide is attributed to the impairment of protein synthesis and indicates a rapid initiation of breakdown of protein to amino acids which may enter in to TCA cycle through aminotransferase to cope up with the high energy demands and the stress conditions. These results are in agreement with the earlier findings (Parthasarathy and Joseph, 2011) which indicated that the decreased protein content might also be attributed to the destruction/necrosis of cells and consequent impairment in protein synthetic machinery. Catabolism of proteins and amino acids make a major contribution to the total energy production. Jha (1988) supported the idea of consumption of amino acids for metabolic processes as energy source.

According to Sivaprasad and Raman Rao (1980), depletion of protein in pollutant treated animal might be due to enhanced proteolytic activity. Increase in protease activity also supported depletion of protein content (Srinivas and Purushottam Rao, 1987). Waykar and Lomte (2002) observed increased protease activity in fresh water bivalve, *Parreysia cylindrica* after exposure to pesticide.

Waykar and Lomte, (2001); David *et al.* (2004) and Bhide *et al.* (2006) suggested that the pesticide stress influences the conversion of tissue protein into soluble fraction that reaches in the blood for utilization. In long term exposure to profenofos much of the energy must have been used up to compensate

Table 1: Total Protein content in different tissues of *Lamellidens marginalis* after chronic exposure to profenofos without and with ascorbic acid

Treatment	Tissue	7 days	14 days	21 days
Control(A)	Mantle	43.96 ± 1.40	42.86 ± 1.61	41.76 ± 1.50
	Foot	61.54 ± 2.40	61.23 ± 1.24	60.49 ± 1.30
	Gill	52.75 ± 2.45	51.62 ± 1.63	51.52 ± 0.62
	Digestive gland	48.35 ± 1.23	47.52 ± 0.65	47.52 ± 0.53
	Whole body	60.81 ± 3.01	58.15 ± 1.56	57.14 ± 1.882
Profenofos (0.6191 ppm)(B)	Mantle	30.77* ± 1.95(-30.00)	26.37* ± 1.31(-38.46)	21.98* ± 2.54(-47.36)
	Foot	49.82** ± 2.03(-19.05)	45.23** ± 1.88(-26.12)	41.48** ± 1.87(-31.41)
	Gill	31.92** ± 1.56(-39.48)	27.36* ± 1.61(-47.00)	22.78** ± 2.04(-55.79)
	Digestive Gland	26.37*** ± 1.03(-45.46)	18.58* ± 2.03(-60.89)	16.68* ± 1.03(-64.89)
	Whole body	45.42*** ± 2.03(-25.31)	35.16*** ± 1.29(-39.52)	30.77* ± 2.09(-46.15)
Profenofos (0.6191 ppm) + A.A. (50 mg/L)(C)	Mantle	34.56** ± 1.88(-21.37)	31.04* ± 1.30(-27.56)	30.77* ± 2.04(-26.32)
	Foot	54.21** ± 1.68(-11.90)	48.44** ± 2.06(-20.88)	45.24** ± 1.45(-25.20)
	Gill	38.35* ± 2.08(-27.29)	32.05* ± 1.42(-37.92)	29.96* ± 1.25(-41.86)
	Digestive Gland	43.86** ± 1.24(-9.30)	38.16** ± 0.89(-19.68)	32.23*** ± 1.52(-32.16)
	Whole body	48.35** ± 0.58(-20.49)	38.10* ± 2.01(-34.48)	35.56** ± 1.94(-37.77)

Values expressed as mg/100mg dry wt. of tissue (+) or (-) indicate percent variation over control ± indicate S.D. of three observation, Values are significant at *p < 0.001, ** p < 0.01, *** p < 0.05

Table 2: Total Protein content in different tissues of *Lamellidens marginalis* during recovery after chronic exposure to Profenofos

Treatment	Tissue	Bivalves pre-exposed to pesticide up to 21 days 7 days	Recovery 14 days	21 days
(i) Recovery in normal water	Mantle	21.98* ± 2.54(-47.36)	24.37** ± 0.45(+10.90)	28.16* ± 0.50(+28.15)
	Foot	41.48** ± 1.87(-31.41)	42.82*** ± 0.4(+3.23)	48.56** ± 0.23(+17.06)
	Gill	22.78** ± 2.04(-55.79)	23.98** ± 0.45(+5.26)	30.27* ± 0.45(+32.86)
	Digestive Gland	16.68* ± 1.03(-64.89)	18.05** ± 0.46(+8.19)	24.77* ± 0.24(+48.48)
(ii) Recovery in A.A. (50 mg/L)	Whole body	30.77** ± 2.09(-46.15)	32.77** ± 1.23(+6.50)	43.96* ± 2.05(+42.86)
	Mantle	21.98* ± 2.54(-47.36)	27.49*** ± 1.85(+25.06)	34.63** ± 1.82(+57.56)
	Foot	41.48** ± 1.87(-31.41)	45.39*** ± 1.76(+9.41)	57.50*** ± 2.24(+38.61)
	Gill	22.78** ± 2.04(-55.79)	26.38** ± 2.06(+15.78)	39.42** ± 0.46(+73.05)
Digestive Gland		16.68* ± 1.03(-64.89)	21.98* ± 0.94(+31.75)	30.16*** ± 0.79(+80.78)
	Whole body	30.77** ± 2.09(-46.15)	38.10*** ± 1.05(+23.80)	52.04* ± 0.45(+69.14)

Values expressed as mg/100mg dry wt. of tissue, (+) or (-) indicate percent variation over control, ± indicate S.D. of three observation, Values are significant at * p<0.001, ** p<0.01, *** p<0.001, **** p<0.05

the stress, resulting in to the depletion of protein. Sahib *et al.* (1984) suggested that the animal exposed to pesticides may compensate any possible protein loss by increasing its protein synthesis. Gill *et al.* (1990) concluded that compensatory production for reduced enzyme as a result of tissue necrosis or to meet increased demand to detoxify the pesticides might have necessitated enhanced synthesis of enzymes. The toxicants may affect the hormonal balance which could directly or indirectly affect the tissue protein levels (Singh *et al.*, 1996).

Both the protein degradation and synthesis are sensitive over a wide range of conditions and how changes to a variety of physical and chemical modulators. The degradation due to oxidative stress (Hai *et al.*, 1995) is a characteristic of organophosphate compounds; oxidative stress also induces changes in free radical production. When the free radical production overwhelms the endogenous antioxidant levels, they cause considerable cell damage and death. All the major biomolecules like lipids, proteins and

nucleic acids may be attacked by free radicals (Cheeseman and Slater, 1992). The physiological and biochemical alterations observed in an animal under any physiological stress can be correlated with the structural and functional changes of cellular proteins. Proteins occupy a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways (Lehninger, 2008). Oxidative modification of specific amino acids is one mechanism of marking a protein for proteolysis (Stadtman, 1986).

In combined exposure to profenofos with 50mg/L of L-ascorbic acid the severity of protein depletion was much reduced. Antioxidants are capable of counteracting the damage. Oxidative stress occurs when the production of harmful molecules called free radicals is beyond the protective capability of the antioxidants defenses. Antioxidants block the process of oxidation by neutralizing free radical. In doing so, the antioxidants themselves become oxidized.

The bivalves pre-exposed to pesticide profenofos showed fast recovery in the protein level in presence of L-ascorbic acid than those allowed to cure naturally. This study indicates that the use of L-ascorbic acid protect the tissues from oxidative damage caused by pesticide. 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 is already highlighted (Griffiths and Lunec, 2001). The stimulatory action of ascorbic acid is indicated by increase in cell population, protein content and level of lysosomal enzymes, antioxidants and enhanced capacity for phagocytosis (Agarwal *et al.*, 2003). 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). Mahajan and Zambare (2006) studied the protection by ascorbic acid against the arsenic induced alterations in ascorbic acid levels in freshwater bivalve, *Lamellidens marginalis*. Waykar (2006 and 2007) studied L-ascorbic mediated protection against the pesticide induced toxicity in fresh water bivalve, *Parreysia cylindrical* and found the similar 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 is 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 is evident that vitamin C not only confirm protection against pesticide toxicity but can also perform therapeutic role against pesticide toxicity in mollusk.

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