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The effect of chloramphenicol on ascorbic acid contents of *Lamellidens corrianus* (lea) and *Parreysia cylindrica* (Annandale and Prashad).

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Abstract

Lethal and sublethal exposure of chloramphenicol, one of the broad spectrum antibiotics, changed the ascorbic acid level in various tissues of the freshwater bivalves, *L. corrianus* and *P. cylindrica.* The acute dose of chloramphenicol ($LC_{50/2}$) 470.37PPM was given to *L. corrianus* and 369.09PPM to *P. cylindrica* up to 96 hours. The chronic ($LC_{50/10}$) concentrations used were 94.07 PPM and 73.81 PPM for *L. corrianus* and *P. cylindrica* respectively. up to 21 days. The ascorbic acid contents were estimated in mantle, gill, foot, testis, ovary, whole body and digestive gland after 24 and 96 hours of acute and 7, 14 and 21 days of chronic exposure.The level of ascorbic acid showed decrease in gill and whole body exposed to chloramphenicol in *L. corrianus*. The maximum decrease was observed in foot of *L. corrianus* while in *P. cylindrica* there was increase in ascorbic acid contents after chronic exposure of chloramphenicol. The maximum ascorbic acid content was observed in mantle and lowest in digestive gland. In *P. cylindrica* maximum decrease was showed by digestive gland after chronic exposure of chloramphenicol.

Keywords: Ascorbic acid, Chloramphenicol, *Lamellidens corrianus, Parreysia cylindrica.*

INTRODUCTION

With the great demand for the protein rich food, one has to rely on fishes, shellfishes, bivalves and oyster like sources, as it provides many of the nutrients. The study of these nutrients is possible by the biochemical analysis of the different biomolecules in general and with the altering induced conditions in exposed animals in particular.

The biochemical changes occurring in the body gives first indication of stress. During the stress, to overcome this altered situation extra energy is needed. The biochemical composition varies according to the situation like seasonal changes, environmental factors (Temperature, salinity), starvation and toxicants added to water as a result of different anthropogenic activities.

The bivalves resist against such unwanted conditions by its own way and try to minimize the effect of the altered situation by removing the toxicant or by biotransformation. Effects of toxicants result from their interaction with certain receptors of the organisms. Thus the impact of toxicant is exerted not only on cell but also on the cell content. Different toxicants affect the metabolic activities which are expressed in terms of different changes that occurred in bivalve [1,2 and 3] Ascorbic acid, being important constituent in cellular metabolism, the interactions of the biomolecules gives proper idea of toxicant stress and its effect.

For different physiological acts vitamins are essential, although required in trace amount. Most of the animals can synthesize this water soluble vitamin by their own, but not the human being. Hence in case of human beings it is introduced through the diet.

Ascorbic acid helps to maintain the oxidation-reduction potential of the cell at the stabilized level. Ascorbic acid works in a chain of DPNH and the cytochrome of electron transport through hydroxylation system.

Ascorbic acid acts as an essential factor for normal growth in rainbow trout, *Salmo gairdneri* [4,5 and 6]. In terrestrial animals the dietary ascorbic acid has role in the host defense systems. Though the complete prevention of viral infection is not possible, high doses of ascorbic acid reduces potency of the viral diseases [7]. The accumulation of ascorbic acid at the site of wound healing was found by Gould (1963) [8]. Interferons get enhanced in circulatory system after ascorbic acid ingestion through diet [9]. Lymphoidal tissue regeneration and their differentiation occurred under the influence of ascorbic acid [10]. Siddique (1967) [11] found the increase in ascorbic acid in liver, gonads and serum of *Ophiocephalus punctatus* with increase in temperature.

Chinoy and Seethalakshmi (1977) [12] showed impact of ascorbic acid on steroidogenesis in gastropod. Navarre and Halver (1989) [13] suggested that the diet fed with ascorbic acid reduced the mortality rate and is due to the faster recovery rate offered by ascorbic acid, which resists diseases caused by the bacteria, *Vibrio anguillarum*. Thus ascorbic acid has a central position in curing the impaired condition occurred by the pathogenic attack and resists against the diseases in organisms. The impact of chloramphenicol on ascorbic acid content was studied in bivalves, *L. corrianus* & *P. cylindrica* in the present study. So far the side effects of the antibiotics on the bivalves, oysters and other invertebrates are not yet studied.

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MATERIALS AND METHODS

The freshwater bivalves, *L. corrianus* and *P. cylindrica* were collected from Girna dam, Dist: Nasik, M.S. The animals were acclimatized to laboratory conditions for 4 days prior to experimentation. During experimentation only those animals showing good movements and in apparent good health, were used for investigation. The animals were divided into five batches, two for acute and two for chronic exposures of chloramphenicol and one batch was maintained as control in each case.

a) Acute exposure to Chloramphenicol

The healthy bivalves, *Lamellidens corrianus* were exposed to acute treatment ($LC_{50/2}$) of chloramphenicol 470.37PPM, while *Parreysia cylindrica* were exposed to chloramphenicol 369.09PPM concentration up to 96 hrs.

b) Chronic exposure to Chloramphenicol

The acclimatized *L. corrianus* were exposed to ($LC_{50/10}$) concentration of chloramphenicol 94.07 PPM while *P. cylindrica* were exposed to chronic concentration of chloramphenicol 73.81 PPM up to 21 days.

During exposure period, no special food was provided and the water with required concentration of chloramphenicol was changed daily in the experimental set. Control set was provided with dechlorinated water only without addition of chloramphenicol.

After 24 and 96 hours of acute exposure and 7, 14 and 21 days of chronic exposure, the mantle, gill, foot, testis, ovary, digestive gland and the whole body flesh were isolated, blotted to remove excess water and dried in oven at 80°C till constant weight was obtained. All tissues were ground separately into fine powder, from which ascorbic acid contents were estimated.

Ascorbic acid content was estimated by using Hydrazine reagent by the method as given by Roe (1967) [14]. The calibration curves were drawn by plotting concentrations of standard against optical density to determine the corresponding value of ascorbic acid content from tissues after acute and chronic exposure to chloramphenicol. The results were expressed in mg per 100 mg of dry tissue. The % variations were also calculated to find out the antibiotic induced stress to the biochemical substances undertaken for study and the test of significance was applied.

Results and Discussion

After acute and chronic exposures of chloramphenicol there was a marked decrease in ascorbic acid contents in almost all tissues of *L. corrianus* and *P. cylindrica*. Chloramphenicol induced stress decreased ascorbic acid contents maximum in the digestive gland (75.0%) after acute exposure in *L. corrianus* while in the foot (66.66 %) after chronic exposure to chloramphenicol in *P. cylindrica*. The reverse trend was found in gill where 27.99 % increase in ascorbic acid was observed after chronic exposure (Table 1).

After 24 hrs of acute exposure to chloramphenicol in *P. cylindrica* showed incline pattern of ascorbic acid content in gill 35.89 % and then gradually decreased up to 22.58 %. The similar type of result was also found in foot after chronic exposure to chloramphenicol. The maximum decrease was found in the digestive gland 55.26 % after acute and in gill 46.66 % after chronic exposure to chloramphenicol. The overall effect in ascorbic acid contents was significant at $P < 0.001$, $P< 0.01$ or $P < 0.05$ level for acute and chronic exposure while non-significant in some of the cases (Table 2).

Table 1. Impact of Chloramphenicol on ascorbic acid content of *Lamellidens corrianus* after acute and chronic exposure

Tissues	24 h		96 h		7 d		14 d		21 d	
	Control	Chlor	Control	Chlor	Control	Chlor	Control	Chlor	Control	Chlor
M	1.5644 ±0.2332	1.5111 ±0.200 -3.409NS	1.5822 ±0.0817	1.4577 ±0.0522 -7.865*	0.9066 ±0.1111	0.8760 ±0.0998 -3.372NS	1.2977 ±0.1332	1.114 ± 0.1051 -14.109**	1.0341 ±0.0503	0.6577 ±0.0105 -36.396**
G	0.7288 ±0.1166	0.6400 ±0.0288 -12.195NS	0.4800 ±0.0101	0.7822 ±0.0288 -62.96***	0.2133 ±0.0059	0.3224 ±0.0101 -51.12***	0.3022 ±0.0817	0.4266 ±0.0293 +41.176*	0.4444 ±0.0503	0.3200 ±0.0290 +27.999**
F	0.4266 ±0.0817	0.4184 ±0.0377 -0.192NS	0.9244 ±0.0220	0.6548 ±0.0296 -21.16***	0.6044 ±0.0288	0.5238 ±0.0267 -13.33***	0.3200 ±0.0089	0.2258 ±0.0101 -29.41***	0.2666 ±0.0290	0.0888 ±0.059 -66.666**
O	0.3555 ±0.0101	0.2311 ±0.0293 -35.00***	0.3911 ±0.0148	0.4266 ±0.0288 -9.090*	0.4266 ±0.0290	0.3022 ±0.0105 +29.16****	1.4400 ±0.0886	1.991 ±0.3771 +38.271*	0.4800 ±0.0288	0.2311 ±0.1051 -51.85***
T	0.3656 ±0.0872	0.3370 ±0.0767 -7.819NS	0.5155 ±0.0503	0.4622 ±0.0883 -10.344NS	0.5155 ±0.0101	0.4088 ±0.0105 -20.68***	0.6724 ±0.0133	0.5108 ±0.0288 -24.02***	0.4622 ±0.0290	0.3377 ±0.0101 -26.92***
WB	0.3911 ±0.0817	0.2844 ±0.0267 -27.27***	0.106 ±0.0059	0.0711 ±0.0293 -33.33***	0.6755 ±0.0675	0.693 ±0.0101 +2.631**	0.3733 ±0.0101	0.6222 ±0.1332 +66.666*	0.3377 ±0.0171	0.3733 ±0.0288 +10.526*
DG	0.3555 ±0.0503	0.1471 ±0.0059 -58.620**	0.5155 ±0.0477	0.1288 ±0.0377 -75.00***	0.2311 ±0.0089	0.218 ±0.0105 -50.263NS	0.3377 ±0.0290	0.2338 ±0.0089 -30.769**	0.4622 ±0.0220	0.2844 ±0.0471 -38.461**

M = Mantle; G = Gill; F = Foot; O = Ovary; T = Testis; WB = Whole body; DG = Digestive gland.
Values are expressed as mg/100mg dry weight of tissue.
± indicates standard deviation of three independent replications.
+ or - indicates % variation over control.
Significance: * P < 0.05; ** P < 0.01; *** P 0.001; NS = Non-significant.

Table 2. Impact of Chloramphenicol on ascorbic acid content (mg%) of *Parreysia cylindrica* after acute and chronic exposure

Tissues	24 h		96 h		7 d		14 d		21 d	
	Control	Chlor	Control	Chlor	Control	Chlor	Control	Chlor	Control	Chlor
M	1.208 ±0.1332	1.0062 ±0.0818 -16.764*	1.76 ±0.377	1.3902 ±0.1051 .-21.010*	1.3866 ±0.1716	1.1733 ±0.0817 -15.384*	1.315 ±0.0817	0.9635 ± 0.05516 -26.756**	1.28 ±0.1332	0.8355 ±0.0767 -34.722*
G	0.6933 ±0.0817	0.9422 ±0.0767 +35.897***	0.551 ±0.0675	0.6755 ±0.0288 +22.580*	0.7111 ±0.1332	0.5511 ±0.0511 -22.499NS	0.4266 ±0.0675	0.3022 ±0.2908 -29.166*	0.5333 ±0.0220	0.284 ±0.0998 -46.666**
F	0.6044 ±0.0872	0.3022 ±0.0503 -50.00**	1.155 ±0.0872	0.9777 ±0.0101 -15.384*	0.5866 ±0.0872	0.6755 ±0.0377 +15.151NS	0.3733 ±0.0290	0.4622 ±0.0101 +23.809**	0.4088 ±0.02936	0.4977 ±0.016 +21.739**
O	0.2133 ±0.0553	0.1955 ±0.2050 -8.333NS	0.302 ±0.0502	0.2701 ±0.0290 -10.596NS	0.4622 ±0.0101	0.3840 ±0.0059 -16.923***	1.244 ±0.0767	0.9422 ±0.05516 -24.285***	0.5511 ±0.05817	0.4088 ±0.0290 -25.806**
T	0.4622 ±0.0503	0.2844 ±0.0 -38.461**	0.502 ±0.0288	0.2944 ±0.0059 -41.353***	0.800 ±0.20	0.7529 ±0.0503 -5.882NS	0.9066 ±0.0293	0.8059 ±0.0767 -11.111*	0.4444 ±0.0288	0.3200 ±0.0767 -27.999*
WB	0.6755 ±0.0872	0.5629 ±0.0050 -16.666NS	0.106 ±0.0050	0.0639 ±0.0101 -40.028***	0.7642 ±0.0293	0.7466 ±0.0290 -2.325***	0.533 ±0.0101	0.4266 ±0.0105 -20.00***	0.6755 ±0.0501	0.4088 ±0.0105 -39.473**
DG	0.3733 ±0.08728	0.3541 ±0.0817 -5.142NS	0.3377 ±0.0293	0.1511 ±0.0089 -55.263***	0.3200 ±0.0769	0.2133 ±0.0059 -33.333*	0.3911 ±0.0029	0.2133 ±0.089 -45.45*	0.9599 +0.0293	0.4977 ±0.1716 -48.148**

M = Mantle; G = Gill; F = Foot; O = Ovary; T = Testis; WB = Whole body; DG = Digestive gland.
Values are expressed as mg/100mg dry weight of tissue.
± indicates standard deviation of three independent replications.
+ or - indicates % variation over control.
Significance: * P < 0.05; ** P < 0.01; *** P 0.001; NS = Non-significant

The antioxidant role of ascorbic acid is a well-known phenomenon, which protects the tissues from the superoxide radical generated due to different toxicological effects. Changes in the environment cause alteration in the ascorbic acid content.

The varied functions of the ascorbic acid make it dynamic. Any alteration in the surrounding water due to the contamination of water also alters ascorbic acid contents. Different pollutant stress has its impact on the concentration of ascorbic acid [15 and 16]. Ascorbic acid content increase during stress [17] and after metal intoxication indicating its role in detoxification process.

The curing response against methyl mercury damage was seen in the reproductive organs of guinea pig after ascorbate administration [18].

Wedemeyer (1969) [19] observed that the stress-induced release of cortisol occurred concomitant with a decrease in the ascorbic acid in the kidney of salmonids.

In higher animals (vertebrates) the reduced exogenous requirement of ascorbic acid may be a result of its lower need for biochemical functions with age or an increased storage capacity combined with more efficient endogenous reuse. Jadhav et al. (1996) [20] showed a decreased level of ascorbic acid content after pesticidal stress in *Corbicula striatella*. Waykar (2000) [21] reported a decrease in the ascorbic acid level in various tissues of *Parreysia cylindrica* on exposure to pesticide.

Clarkson et al. (1988) [22] and Rao et al. (1994) [17] reported that the oxygen radical formed due to methyl mercury forms reactive oxygen intermediates with ascorbic acid. Daine et al. (1994) [23] showed the recovery from chromium intoxication by ascorbic acid treatment. Sometimes vitamin C and vitamin E acts in combination for detoxification. Mahajan and Zambare (2001) [24] found that the reduction in protein depletion due to $CuSO_4$ and $HgCl_2$ was recovered by ascorbate treatment in *Corbicula striatella*.

Mouse peritoneal macrophages when elicited by the antioxidant ascorbic acid have been found to be significantly stimulatory, exhibiting significant enhancement in protein content, lysosomal acid hydrolase levels and capability to phagocytise [25]. The ascorbic acid supply may boost the macrophage activity, helping to remove intracellular free irritant. These results indicate the positive role of ascorbic acid in toxicant stress.

The depleted level of ascorbic acid is a vivid response against chloramphenicol to cope up the toxic stress caused by exposure to antibiotics. The changed level of ascorbate reflects the great interaction among the biomolecules present in the cell cited in the present paper.

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