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Malondialdehyde, Superoxide dismutase and Catalase responses in *Anabas testudineus* (BLOCH, 1792) exposed to Laundry Detergent

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Abstract
Anabas testudineus (BLOCH, 1792) fish was selected as experimental animal for the present study. The fishes in test groups were exposed to two sublethal concentrations of Surf Excel Quick Wash detergent for various time durations. Control groups were maintained free from detergent. Lipid peroxidation product- Malondialdehyde (MDA) and enzymatic antioxidants (Catalase and Superoxide dismutase) were analyzed in brain, gill, liver and muscle tissues of fish by following standard methods. Malondialdehyde showed a significant increase with increased stress. Brain tissues of Test 2 samples of 96 hours stress duration showed highest malondialdehyde content (5.4 ± 0.04millimoles/100 g wet tissue). Enzymatic antioxidants studied also showed a significant increase with increase in stress level and stress duration, pointing to the fact that the reactive oxygen species formations were in the range in which the antioxidants could resist. Gill, Liver and Muscle tissue samples showed an increase in tendency of Catalase activity while brain tissues showed a decline in activity. Superoxide dismutase activity of test 2 liver tissues with 96 hours of experimental duration showed the highest value of 4.8428±0.017 units/milligram wet tissue in the studied groups. This study establishes the role of Malondialdehyde as a stress indicator in fresh water fishes even in sub lethal stress levels. Present study confirmed that even sublethal concentrations of detergent stress can cause significant alterations in enzymatic antioxidants of fresh water fish. So dose specific use of laundry detergent is suggested to save the indigenous fresh water fish resources.

Key words: *Anabas testudineus, Detergent, Enzymatic antioxidants, Malondialdehyde.*

Introduction

Indigenous fresh water fishes are facing many anthropogenic threats. Detergents, including the biodegrading ones have been discovered to induce poisonous effects and osmo-regulatory imbalance in aquatic lives especially if present in concentration that exceed metabolic demand. Such xenobiotic compounds could be persistent and more mobile in soil and water. Hence they are known to be among of the most common terrestrial and aquatic contaminants (Ezemonge et al., 2007; Azizullah et al., 2012). The detergents create tissue damage and biochemical alterations in the organs of organisms due to their potential toxicity and can act as an environmental stress. So the organisms can respond to it by developing various methods including altering the antioxidant status to counteract the stress (Jawaharlal et al., 2015). The detergent along with water after use in washing machines is usually discharged directly to the drainage systems or to the fields, ultimately reaching the fresh water sources around. Eutrophication impacts of phosphate containing detergents in fresh water bodies are a widely discussed topic among researchers. Yet one could rarely notice studies on lipid peroxidation reactions and antioxidant mechanisms of fresh water fishes exposed to detergents. So the present study has tried to understand the detergent induced stress responses in *Anabas testudineus* by assessing selected biomarkers like lipid peroxidation product Malondialdehyde and enzymatic antioxidants such as Catalase and Superoxide dismutase.

Materials and Methods

Anabas testudineus (BLOCH, 1792) is an indigenous air breathing fish, commonly known as *'Climbing perch'* belongs to the family Anabantidae widely seen in fresh water ponds and streams is selected for the present study as experimental animal. Specimens of good health and average weight of 8 gram and average length of 81.8 mm were purchased from a local hatchery and treated with 0.5% $KMNO_4$solution. Fish were kept in glass tanks (400 L) with well aerated tap water at 27 ± 1^0C and exposed to natural day and night cycle for two weeks and fed with balance staple diet *ad libitum.* Surf Excel Quick wash- a phosphate containing laundry detergent was selected for the induction of stress, since it is widely using for washing clothes. LC_{50} at 96 hours was calculated after acclimatization period ie., 97mg/l. Considering the amount of chemicals used in field applications, its loss, degradation, spill over, run off and its ultimate mixing into the rivers, tanks and irrigation canals and of the volume of water present in these storage systems, it is more appropriate that the inhabitants of the fresh water ecosystems are more likely to be exposed to low concentrations than to high concentrations. Hence two sub lethal concentrations were selected for the present investigation ie., 1/3 and 2/3 of the LC_{50} value *ie.*, 32.33mg/l as Test 1 and 64.66mg/l as Test 2. Experiments were conducted during August 2016-May 2017. Glass tanks of 10 litre capacity were selected and arranged for the study and filled with tap water.

Healthy fishes of uniform weight and length were selected and divided into Test 1 (T1), Test 2 (T2) and Control (C) groups. 15 fish in each group (5 fish per tank) were kept in glass tanks filled with tap water without feed for two days. The fishes in T1 and T2 were experimentally exposed to 32.33mg/l and 64.66mg/l of detergent respectively and control groups were maintained free from detergent. Replicate experiment for each group was also conducted. Test and control group fishes were sacrificed in the initial hour (0) and after 24 hours, 48 hours, 72 hours and 96 hours of experimental durations. The tissues (Brain, Gill, Liver, and Muscle) were collected in ice cold containers. Tissue homogenates were prepared in suitable buffers for each estimation. Lipid peroxidation in tissue samples were measured by estimating the Malondialdehyde (MDA), a biomarker in stress studies, by adopting the standard method of Buege and Aust (1978) based on Thio Barbituric Acid (TBA) reactivity. Enzymatic antioxidants such as Catalase and Superoxide dismutase were determined using spectrophotometer by adopting standard procedures of Aebi (1984) and Lin et al., (1993) respectively.

Statistical Analysis

Data were expressed as Mean ± Standard Deviation; n=6, Statistical significance of the data were determined by one-way ANOVA (Duncan's test) using SPSS 16 software, and the results were expressed with significance $P \leq 0.01$.

Results

Changes in Malondialdehyde in fishes exposed to detergent stress

Table 1 shows the changes in lipid peroxidation reaction product, Malondialdehyde in test 1 and test 2 group fishes in comparison with corresponding control groups. Changes in lipid peroxidation lead to destruction of membrane lipids and production of lipid peroxides and their byproducts such as malondialdehydes. The tissue samples of experimental fishes showed an increase in tendency of Malondialdehyde content in all test groups compared with corresponding controls. Test 2 tissue samples showed augmented Malondialdehyde content than Test 1 samples. Compared with corresponding control gills (1.9110 ± 0.025), gill tissues of the test 2 fishes with 96 hours of exposure duration showed many fold increase in Malondialdehyde content (4.764 ±0.043) and showed significant difference from its control at $P \leq 0.01$. The brain tissues of the fishes of Test 2 samples with exposure duration of 96 hours showed highest malondialdehyde content of 5.4425 ± 0.041millimoles/100 g wet tissue, which is significantly different from the corresponding control brain with 3.0382 ± .02 millimoles MDA/100 g wet tissue. All test tissue samples showed an increase in concentration of Malondialdehyde with increase in exposure duration. For example the Test 2 liver samples of less than one hour of experimental duration showed an MDA content of 1.8940 ±0.025millimoles / 100 gram wet tissue, showed an increase in MDA content of 2.048±0.02, 2.7860±0.016, 3.3892±0.01 and 4.2645±0.035 millimoles / 100 gram wet tissue during 24,48, 72 and 96 hours of exposure duration respectively.

Alterations of Catalase content in detergent exposed fish

Table 2 shows the changes in catalase activity in different fish tissue samples exposed to detergent stress. Gill, Liver and Muscle tissue samples showed an increase in tendency of Catalase activity. Brain tissues showed a decrease in catalase activity after the initial exposure hours. Test 2 Gill tissues and liver tissues were not showing any increase in catalase activity after 72 hours of experimental duration. There was a statistically significant increase ($P \leq 0.01$) in CAT activity of Test 1 and Test 2 muscles of 96 hours of detergent exposure and a significant decrease of catalase activity in brain tissues of same experimental duration compared with the corresponding controls.

Response of Superoxide dismutase in detergent exposed fish

Superoxide dismutase activities of different organs of experimental groups are given in Table 3. Test groups of exposure duration of 24 hours upto 96 hours showed significant increase with the corresponding controls. In the initial hour of experimental duration gill tissues only showed significant increase with the corresponding control. Superoxide dismutase activity of test 2 liver tissues with 96 hours of experimental duration showed the highest value of 4.8428±0.017 units/milligram wet tissue in the studied groups.

Discussion

Changes in lipid peroxidation lead to destruction of membrane lipids and production of lipid peroxides and their byproducts such as malondialdehydes. In the present study the tissue samples of experimental fishes showed an increasing tendency of Malondialdehyde content in all test groups compared with corresponding controls. As stress level increases, the malondialdehyde content also on a rise, same way with increased exposure duration. Test 2 samples showed an increase compared with the test 1 samples. Among the tissue samples studied, test 2 brain samples with detergent exposure duration of 96 hours showed the highest MDA content. Sreejai and Jaya (2010) reported increased Malondialdehyde content in brain and kidney tissues of *Oreochromis mossambicus* Peters exposed to hydrogen sulphide stress. Uner et al., (2005) also reported an increase in tissue MDA content in fishes (*Oreochromis nitoticus)* exposed to Etoxasole. MDA is formed from the breakdown of polyunsaturated fatty acids and it serves as a convenient index for determining the extent of lipid peroxidation. It can be considered as a biomarker of effect representing the state of membrane lipid peroxidation. Continuous exposure to environmental stresses lead to the generation of reactive free radicals which can even overwhelms the function of antioxidant defence mechanisms. So that lipid peroxidation of the cell membrane occurs and causes disturbances in cell integrity and might lead to cell damage/death. Lipid peroxidation is considered as a complex self propagating process producing high levels of cell degradation, increases the rigidity (decreases the fluidity) of cellular membranes. Early warning signs of environmental pollution are frequently noted as biochemical responses in organisms against environmental stress and are known as biomarkers of exposure. The production and elimination of reactive oxygen species (ROS) typically exist in a dynamic balance in organisms and antioxidant enzymes like superoxide dismutase and

Table 1. Changes in Malondialdehyde content (Milli moles/100gram wet tissue). *Values are Mean ± S.D, No. of fishes = 6; *P≤0.01; SD - Standard Deviation*

Table 2: Changes in Catalase activity in fish tissues. Values are Mean ± S.D, No. of fishes = 6; *$P \leq 0.01$; SD - Standard Deviation

Malondialdehyde content (Milli moles/100gram wet tissue)

Time interval Hrs)	Brain			Gill			Liver			Muscle		
	Control	T1	T2	Control	T1	T2	Control	T1	T2	Control	T1	T2
0	2.8198 ±0.055	2.7785 ±0.024*	2.8962 ±0.025*	1.8582 ±0.024	1.8820 ±0.023*	1.9275 ±0.012*	1.8990 ±0.029	1.8892 ±0.025	1.8940 ±0.025	3.0912 ±0.022*	3.3510 ±0.029*	3.3820 ±0.016*
24	2.7620 ±0.026	2.9058 ±0.025*	3.4228 ±0.043*	1.8488 ±0.025	2.4012 ±0.039*	3.3028 ±0.043*	1.8198 ±0.023	1.9640 ±0.028*	2.0480 ±0.020*	3.1105 ±0.038*	3.3198 ±0.032*	3.8030 ±0.043*
48	2.8678 ±0.197	3.1605 ±0.157*	4.2738 ±0.025*	1.8728 ±0.032	2.7715 ±0.042*	3.4878 ±0.073*	1.7960 ±0.024	2.0408 ±0.021*	2.7860 ±0.016*	3.0698 ±0.009*	3.2642 ±0.012*	3.8845 ±0.020*
72	2.8680 ±0.064	3.3865 ±0.016*	4.0938 ±0.479*	1.8918 ±0.021	3.0985 ±0.038*	3.0960 ±0.034*	1.8580 ±0.036	1.9855 ±0.037*	3.3892 ±0.019*	3.2788 ±0.021*	3.3725 ±0.016*	3.8582 ±0.025*
96	3.0382 ±0.020	3.9683 ±0.021*	5.4425 ±0.041*	1.9110 ±0.025	3.1848 ±0.021*	4.7640 ±0.043*	1.8532 ±0.028	3.3268 ±0.035*	4.2645 ±0.035*	2.9352 ±0.091*	3.3988 ±0.019*	3.923 ±0.020*

Catalase Activity in fishes (Micromoles of H_2O_2 decomposed/ gram tissue/minute)

Time interval (Hrs.)	Brain			Gill			Liver			Muscle		
	Control	T1	T2	Control	T1	T2	Control	T1	T2	Control	T1	T2
0	1025.4 ±288.11	1068.4 ±247.89	1159.4 ±203.94	1159.4 ±203.94	1348.4 ±275.67	1348.4 ±275.67	1135.8 ±646.99	1348.4 ±275.67	907.40 ±124.98	710.825 ±125.44	805.250 ±122.92	858.375 ±180.9
24	1049.0 ±272.394	1280.9 ±343.991	1348.4 ±275.678	1017.8 ±157.220	1546.5 ±495.857	1348.4 ±275.678	1425.1 ±505.121	1280.9 ±343.991	1159.4 ±203.945	720.925 ±110.739	1017.8 ±157.220	1159.4 ±203.945
48	1025.4 ±288.118	2660.0 ±3896.98	891.30 ±149.804	1159 ±203.945	1458.0 ±554.875	1348.4 ±275.678	1159.4 ±203.945	1454.6 ±469.882	1381.2 ±787.706	720.925 ±110.739	1159.4 ±203.945	1106.3 ±258.103
72	1068.4 ±247.893	746.225 ±84.523	818.850 ±101.791	1135 ±239.489	1348.4 ±275.678	2727.1 ±1117.73	1348.4 ±275.678	1348.4 ±275.678	2727.1 ±1117.73	783.450 ±160.655	1159.4 ±203.945	1348.4 ±275.678
96	1068.4 ±247.893	685.50 ±71.341*	685.50 ±71.341*	1159 ±203.945	1614.0 ±394.649	2727.1 ±1117.73	1318.8 ±319.361	2018.7 ±616.162	2727.1 ±1117.73	720.925 ±110.739	1159.4 ±203.94*	1398.9 ±261.05*

Table 3: Changes in Superoxide dismutase activity in fish tissues. Values are Mean ± S.D, No. of fishes = 6; *$P \leq 0.01$; SD - Standard Deviation

Super Oxide dismutase Activity (Units/milligram wet tissue)

Time (Hours)	Brain			Gill			Liver			Muscle		
	Control	T1	T2	Control	T1	T2	Control	T1	T2	Control	T1	T2
0	1.5878 ±0.0098	1.5920 ±0.0168	1.6112 ±0.0132	2.4705 ±0.0072	2.4910 ±0.0109*	2.4985 ±0.0102*	2.9275 ±0.0346	2.9495 ±0.0104	2.9188 ±0.0269	1.0220 ±0.0109	1.0485 ±0.010	1.0380 ±0.021
24	1.5820 ±0.0084	1.9682 ±0.0172*	1.9182 ±0.0265*	2.4735 ±0.0104	2.0798 ±0.0230*	2.9393 ±0.0153*	2.9422 ±0.0145	3.1345 ±0.0200*	3.1238 ±0.1206*	1.0322 ±0.0177	1.1322 ±0.0195*	1.3145 ±0.013*
48	1.5920 ±0.0168	2.0798 ±0.02307*	2.4792 ±0.01511*	2.4748 ±0.0098	2.9188 ±0.0269*	3.1300 ±0.0104*	2.9495 ±0.0104	3.2047 ±0.0120*	3.4782 ±0.0151*	1.0292 ±0.0196	1.3145 ±0.0133*	1.9270 ±0.01*
72	1.5920 ±0.01687	2.4898 ±0.02112*	2.9452 ±0.01087*	2.4985 ±0.01025	2.9495 ±0.01041*	3.4018 ±0.01733*	2.9422 ±0.01457	3.3753 ±02016*	3.7310 ±0.01296*	1.0307 ±0.01850	1.9360 ±0.00775*	1.9550 ±0.009*
96	1.5920 ±0.01687	2.9422 ±0.00981*	3.1402 ±0.04146*	2.4882 ±0.02006	3.1302 ±0.01457*	3.7145 ±0.01578*	2.9318 ±0.03732	3.4295 ±0.01025*	4.8428 ±0.01756*	1.0352 ±0.01320	2.0798 ±0.02307*	1.9800 ±0.01*

catalase can eliminate ROS within a short period of time. When this balance is destroyed by an exogenous contaminant, superfluous ROS may lead to oxidative stress, lipid peroxidation, and cellular apoptosis (death). Malondialdehyde (MDA), a final product of lipid peroxidation, is often used to evaluate the oxidative damage apparent in organisms along with antioxidant enzymes (Weili et al., 2015).

The first line of defense mechanism against damaging effects of Reactive Oxygen Species is antioxidant enzyme such as Catalase which directly scavenges the superoxide radicals and hydrogen peroxide, converting them to less reactive species. In the present study also Gill, Liver and Muscle tissue samples showed an increase in tendency of catalase activity. Brain tissues showed an increase in catalase activity in the initial experimental duration followed by a decrease. Increased rate of malondialdehyde accumulation in brain tissues as per this study might be the suppressing factor of catalase in brain. The activities of the endogenous enzymes to remove the continuously generated free radicals initially increase due to induction but later enzyme depletion occurs, resulting in oxidative cell damage. Increased level of MDA and a reduction in the activity of CAT were observed in brain tissue of cypermethrin exposed fish. Similar findings have been reported by several researchers (Orun et al., 2008; Ventura-Lima et al., 2009; Oliva et al., 2012).

Under stress conditions, body mechanisms are altered to combat the effect of the pollutants/stressors in order to stabilize the organism. Oxidative stress is the crucial manifestation of a multi-step pathway, culminating in an imbalance between prooxidant and antioxidant defence mechanisms due to the functional deterioration of antioxidants, or the excessive accumulation of super oxide radicals, or both, which leads to tissue damage. It has been demonstrated that exposure to various contaminants including detergents could produce ROS which cause various organ lesions. MDA, a lipid peroxidation marker, was used to assess the levels of oxidative stress. The significant increase in MDA level indicated the generation of superoxide radicals in fish exposed to detergent stress. Due to the inhibitory effects on oxy-radical formation, the SOD-CAT system provides the primary safeguard against oxygen toxicity (Kavitha & Rao, 2009; Livingstone, 2001). The most important enzymes for the detoxification of reactive oxygen species in all organisms are superoxide dismutase (SOD), catalase and glutathione peroxidases (GPXs) (Di Giulio & Meyer, 2008).

Atli et al., (2006) reported that superoxide dismutase catalyzes the dismutation of O_2 to H_2O_2, and Catalase reduces H_2O_2 to $2H_2O$. The Superoxide dismutase activity in the present study showed an augmented rate in the detergent treated fish and were significantly higher from the corresponding controls. Excessive reactive oxygen species may have induced the synthesis of more SOD or increased its activity to protect against oxidative stress. Increased SOD activity in organisms indicated that ROS levels were still in the range in which SOD could resist the oxidative stress. In the present study the exposure concentrations of fish to detergent were in sublethal levels. Previous research demonstrated that SOD activity was activated under mild adverse stress and declined under more intense stress (Shao et al., 2012; Kumar, 2016).

Conclusion

The results of the current study establish the role of Malondialdehyde as a stress indicator in detergent stress induced fresh water fishes. In order to overcome the stress effect, the enzymatic antioxidants play an important role in fish body. Even though the concentration of detergent applied is in sublethal level, long term exposure creates detrimental effects in fish. This study puts forward the scope of considering Malondialdehyde and selected enzymatic antioxidants as stress biomarkers for higher aquatic organisms like fish. Unabated discharge of detergent containing effluents from households and other sources including laundry service providers to the fresh water bodies damages the fish population. This obviously points to a possible threat due to unbridled influx of detergents to other living bodies in the fresh water aquatic system. Hence there is urgent need to frame appropriate policies to contain the discharge of detergent containing drains into fresh water system. Since major stakeholders in this domain are unorganized individual households and small scale laundry service providers, there needs wider sensitization generated at grassroots to avoid an environmental danger that can save fresh water fish treasure. A new culture of dose-specific use of laundry must be inculcated among people. There needs an action plan to phase out detergents containing phosphates and promoting eco-friendly detergents.

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