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Temperature stress induces neuronal damage via a mechanism leading to free radical production and altering endogenous antioxidant defences in freshwater Indian catfish *Heteropneustes fossilis*

Ashwani Kumar Dubey*

Research & Development Unit, Godavari Academy of Science & Technology, Chhatarpur, 471001, India

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Abbreviations:
Lipid peroxidation (LOP), Superoxide dismutase (SOD), Ascorbic acid (AsA), Polyunsaturated Fatty Acids (PUFA).

Corresponding Author:
Dubey A.K.*
Email: ashwani_0326@yahoo.com
Phone: +919425143654

Abstract
The effectiveness of antioxidant defence system in relation to the lipid peroxidation process is of particular interest in the case of aquatic animals such as fish. Lipid peroxidation is an indicator of free radical induced membrane damage resulting from the degradation of polyunsaturated fatty acids (PUFA). Neural degeneration is a frequent consequence of excessive free radical production. Brain is rich in PUFA and therefore it is susceptible to oxidative damage The aim of this study was to determine oxidative damage in the identification of specific responses that might serve as early warning detection signals of insuring pathology in the brain of freshwater Indian catfish *Heteropneustes fossilis* to elevated temperature. Results demonstrated that with increasing temperature from 25° C to 37° C, the lipid peroxidation and superoxide dismutase activity increased. Ascorbic acid content increased at 32° C and 37° C while no significant change was observed at 27° C. Lipid peroxidation with the increase in superoxide dismutase activity during temperature exposure demonstrated a continuous increase in free radical production in the brain. The increase in ascorbic acid may be due to its utilization in order to prevent oxidative damage. In conclusion, temperature stress induces neuronal damage via a mechanism leading to free radical production and altering endogenous antioxidant defence system.

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1. Introduction

Oxidation is a very crucial process of the aerobic life system. Thus free radicals are generated either naturally or due to some biological dysfunction. Extreme environmental conditions are known to exert stress on aquatic animals. Very low or high temperature elicits a series of physiological responses (Marai, 2012). Standard body metabolism increase continuously with increase in temperature till the attainment of lethal temperature limit and each species display its own characteristic rate of increase at a given range of temperature. Temperature has been known to alter enzyme activities (Shaklee et al., 1977; Parihar et al., 1997), oxygen consumption (Duthi and Houlihan, 1982), ascorbic acid (Parvatheswararao, 1967; Parihar et al., 1996), phospholipids (Parihar and Dubey, 1995), in fish. Blood flow and phagocytes activities are enhanced by even small increase in temperature (Cheville, 1983; Parihar and Dubey, 1995). These activated phagocyte cells in rainbow trout; Salmo gairdneri has been demonstrated to produce oxyradicals (Higson and Jones, 1984; Secombes et al., 1988). Free radicals and its excess have a harmfull effect, including lipid peroxidation, nucleic acid damage, enzyme inactivation and protein degradation (Borg and Schaich, 1984; Halliwell and Cross., 1994). Free radicals can also react with DNA, protein, lipid in the cell membrane and cause damage (Awkins et al., 2001).

Antioxidant enzymes play a vital role in protecting cellular damage by the harmful effect of reactive oxygen species (Dubey et al., 1997; Altan et al., 2003). Antioxidant matters protecting the cell membranes from lipid peroxidation (Havsteen, 2002; Hosnuter et al., 2004; Tatli Seven and Seven, 2008; Dubey 2012). An extensive literature appeared to oxidative stress and antioxidant activity on lipid metabolism (Ahmed and Siddqui, 2007), hepatocytes (Bukowska et al., 2000), red blood cells (Duchnowicz and Koter., 2003), nephrotoxicity (Sharma et al., 2011) while in vivo oxidative activity has been shown in fish (Ozcan et al., 2004).

Oxidative damage is a major contributor to the development of cardiovascular disease, cancer, and neurodegenerative disorders. In healthy individuals, the generation of reactive oxygen species is well balanced by the counterbalancing act of antioxidant defenses. Hence, an imbalance between reactive oxygen species generation and antioxidant status in favor of the former has been described as oxidative stress (Marubayashi et al., 1985). Brain is rich in PUFA and therefore, highly susceptible to peroxidation (Sun and Sun, 1974; Dubey et al., 1997).

The aim of this study was to determine oxidative damage in the identification of specific responses that might serve as early warning detection signals of insuring pathology in the brain of freshwater Indian catfish Heteropneustes fossilis to elevated temperature.

2. Materials and Methods

2.1 Objective of research
The experiment was conducted in accordance with fish health and In contrast, prolonged activation of the temperature stress response is damaging and leads to affect growth and reproductive dysfunction. Indicator LOP associated with the response to acute stress provide a potential source of information on the health status of the fish.

2.2 Experimental animal and design
Adult freshwater Indian catfish Heteropneustes fossilis (weight 50±1.8 gm, length 17±1.2 cm) were procured from local vicinity of Ujjain (MP) and acclimatized in laboratory conditions for two weeks in dechlorinated tap water at room temperature. A photoperiod of 12-hr light: 12-hr dark cycle maintained. After acclimatization fish were randomly transferred into separate exposure glass aquaria. Four groups were maintained, of 30 each. First group was kept as control (25° C). The second; third and fourth groups were kept for temperature exposure.

2.3 Temperature exposure
Experimental fish were exposed to temperature raised from 25° C (control) 27° C, 32° C and 37° C for 60, 120, 180, and 240 minutes using a thermostatic water heater. The elevated temperature was attained to the required level within 10-15 minute and then maintained for varying periods.

2.4 Tissue homogenization
Brains were dissected out weighed and homogenized in a Polytron homogenizer at a speed of 13,000 rpm. The buffers for homogenization of tissue were: 50 mM sodium phosphate buffer (pH 7.4) for lipid peroxidation assay; chilled 50mM Tris HCl (pH 8.2) containing 1mM DTPA for superoxide dismutase activity assay and ice cold 0.25 M perchloric acid for ascorbic acid assay.

2.5 Biochemical assay
Lipid peroxidation level was measured in terms of malonaldehide equivalents (MDA) by the thiobarbituric acid test Okhawa et al., (1979) using a molar extinction coefficient of 1.56 X 105/min/cm.as described by Parihar et al., (1996). Superoxide dismutase activity was assayed by the method of Marklund and Marklund (1974). Ascorbic acid measured by the modification of the dinitrophenylhydrazine (DNPH) technique of Terada et al., (1978) as described by Thomas et al., (1982). Protein content was estimated by the Folin phenol reaction as described by Lowry et al., (1951) using bovine serum albumin as a standard.

2.5.1 Statistical analysis
All data are expressed as mean ± SEM. Control and treatment values were compared by Student's t-test. The ($p < 0.05$) level was selected as the point of minimal statistical significance in every comparison.

3. Results

Changes in lipid peroxidation, superoxide dismutase activity and ascorbic acid content in brain after temperature exposure for varying periods are summarized in table.

3.1 Effect of temperature on lipid peroxidation
Lipid peroxidation level was significantly increased in brain with increase in temperature from 25°C and 37°C. Its levels were

significantly increased when temperature periods extended from 60 to 240 min.

3.2 Effect of temperature on superoxide dismutase activity

Superoxide dismutase (SOD) activity was significantly ($P<0.05$) increased at 32° C and 37° C elevated temperature at various time 60 to 240 min. in comparison to control. Whereas no significant change ($P<0.05$) was observed at 27° C.

3.3 Effect of temperature on Ascorbic acid

Ascorbic acid (AsA) content was increase at 32° C and 37° C significantly at extended time period, while no significant change was observed at 27° C for varying time periods.

Table: Changes in the concentration of Lipid peroxidation, Superoxide dismutase and Ascorbic acid during temperature exposure from 25° C (control) to 37° C at various times 60 min. to 240 min. in brain of freshwater Indian catfish *Heteropneustes fossilis*

Experiment	Time Period (Minute)	lipid peroxidation (n moles MDA/mg protein	superoxide dismutase (unit/gm wet tissue)	ascorbic acid (µg/wet tissue)
Control(25° C)	60	6.23±0.50	10987±316	85.99±7.71
	120	6.34±0.50	11026±338	88.97±7.69
	180	6.24±0.48	10900±318	89.88 ±7.69
	240	6.28±0.49	10890±313	89.98±7.61
27° C	60	7.38±0.52*	11038±314*	115.21±8.11
	120	7.78±0.53*	11836±311*	118.39±8.08
	180	7.21±0.58*	11948±308*	121.32±8.04
	240	7.55±0.59*	11998±302*	124.31±8.31
32° C	60	12.35±0.43*	12473±310#	123±10.84#
	120	12.85±0.41*	12800 ±312#	128±8.62#
	180	13.65±0.49*	12880±313#	136±9.23#
	240	14.63±0.50*	12890± 312#	140±7.91#
37° C	60	20.50±0.48#	12899±528#	116.66±9.58#
	120	20.68±0.49#	13.680±529#	129±9.79#
	180	21.67±0.47#	13990±531#	139.79±8.23#
	240	22.68±0.48#	14580±533#	180.08±8.21#

Values are expressed as mean ± SE (N = 10); statistically significant level *$p < 0.05$, # $p<0.001$ difference between control (25° C) and treatment groups (27° C to 37° C) at various times 60 to 240 minutes.

4. Discussion

In the present study describes the protective effect of enzymatic and non-enzymatic antioxidants against temperature induced oxidative damage in the brain of freshwater Indian catfish *Heteropneustes fossilis*. Results revealed a significant increase in brain oxidative damage compared to controls during temperature stress as evidence by an increase in the level of lipid peroxidation.

Lipid peroxidation level was significantly increased in brain with increase in temperature from 25° C and 37° C. Its levels were significantly increased when temperature periods extended from 60 to 240 min. Brain combines the presence of high percentage of PUFA with high free radical generating system. Fish PUFA are characterized by high degree of instauration than that of terrestrial organism.

Since temperature exposure induced the lipid peroxidation which is considered to be the consequence of free radical activity in those biological membrane which are rich in PUFA (Cheeseman, 1982), its increase level in brain can have serious consequence for the whole organism taking into the account of main co-ordinary regulatory functions of the brain during temperature stress. Process of lipid peroxidation has been linked to production of oxygen radical and tissue damage (Aitken and Fisher, 1994). In our previous study temperature may mediate the production of reactive oxygen species capable to inducing lipid peroxidation and oxidative breakdown in membranes (Parihar and Dubey, 1995). Recently, other study has been investigated the lipid peroxidation largely result from free radical reaction in biological membranes which are rich in PUFA are considered to be an important factor in cellular injury (Cheesman, 1982), Impaired membrane function, structural integrity, decreased membrane fluidity and inactivation of several membrane bound enzymes (Gutteridge and Halliwell, 2000). The observation is an agreement with the view that the enhancement of peroxidation of an essential functional membrane lipid could damage the biological membranes. Therefore, further attention is need to asses the free

radical and the lipid profile in the relation of temperature stress.

In the brain SOD activity was significantly ($P<0.05$) increased at 32° C and 37° C elevated temperature at various time 60 to 240 min. in comparison to control. Whereas no significant change ($P<0.05$) was observed at 27° C. Extending the period of temperature exposure from 60 to 240 min. showed various responses at the different elevated temperature. In the present study increased lipid peroxidation with the increase in superoxide dismutase activity during temperature exposure demonstrated a continuous increase in free radical production in the brain. Temperature exposures consistently revealed a relationship between the SOD activities and lipid peroxidation in brain of Heteropneustes fossilis. The very marked increase in SOD activity with exposure to temperature is almost certainly due to generation of free radical which resulted in peroxidation of lipids in biomembranes. Similar increase in SOD activity with increase in lipid peroxidation was also reported by (Radi et al., 1985), in fish exposed to certain pollutants. Oxidative stress might be associated with elevated or depressed SOD activity. It might be elevated owing to induction by the stressor or depressed as a toxic response to the stressor (DiGulio et al., 1989). In the case of temperature exposure, the first process i.e. induction of SOD activity might occur. Greater increase is SOD activity during temperature exposure indicates increase dismutation of superoxide anion to hydrogen peroxide (Farber, 1994).

Ascorbic acid (AsA) content was increase at 32° C and 37° C significantly at extended time period, while no significant change was observed at 27° C for varying time periods. There is growing evidence which indicate that antioxidant vitamin might at as protective agents against free radicals. Because AsA role as an antioxidant it is important to examine its level in brain as an indicator of the oxidative stress. In conditions of AsA deficiency symptoms of oxidative stress including cell damage, might be expected to appear as the oxidant defense system are challenged to compensate for the lack of AsA (Bendich et al., 1986). In our previous study AsA deficiencies were observed in respiratory organs, gill and air sac during temperature increase (Parihar and Dubey, 1995) in fish. These finding indicated that oxidative stress reflected to AsA deficiency under temperature exposure in respiratory organs and in these reported by (Henning et al., 1991) by an increase in lipid peroxidation. AsA contents was increased significantly ($P<O.05$) in brain at each temperature exposure for varying periods as compare with control. Studies with human subjects showed that elevated level if AsA increases phagocytic activity (Krault et al., 1988; Goetzl et al., 1974) and enhance humoral immune response/antibody production in channel catfish (Li and lovell., 1985). As temperature exposure resulted in significantly increase in lipid peroxidation, the increase of AsA was probably due to the reducing power of AsA in brain during temperature dependent free radical generation. Nevertheless, the study of lipid profile such as total lipid and phospholipids pattern have not been measured in relation to free radical activity in the present study. It may be important in understanding the animal health monitoring. The intracellular membranes contain a high proportion of individual phospholipids which are particular sensitive to oxidative stress (Vliet and Bast., 1992).

Conclusion

In conclusion, an increase in lipid peroxidation is indicative of increased oxidative damage in brain during temperature stress. A high level of SOD activity suggested that fish brain act as effective free radical scavenging. The increase AsA was probably due to the reducing power of AsA in brain during temperature dependent free radical generation. Given this relation between oxidative damage and antioxidant defenses suggested that this information would help in an integrated program in fish management, involving also measurement of neural damage and animal health.

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