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Research Article

DIETARY ALPHA-TOCOPHEROL PREVENTS PHENOL INDUCED OXIDATIVE STRESS IN BRAIN OF FRESHWATER CATFISH *HETEROPNEUSTES FOSSILIS*

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ABSTRACT

Neuron degeneration is a frequent consequence of excessive free radical production. Brain is rich with polyunsaturated fatty acids, is susceptible to oxidative damage. In addition to neurons are post mitotic and can not replace damaged cell; thus the brain is a primary target in which oxidants can wreak their havoc.

In the present investigation the effect of phenol 5ppp, 10ppm and 15ppm for 15 days on lipid peroxidation, superoxide dismutase and ascorbic acid in brain of freshwater catfish ***Heteropneustes fossilis*** were observed Alpha-tocopherol was supplemented and responses were observed against oxidative stress caused by phenol.

Findings indicate that the life span of individual is governed by free radical generation and counterbalancing act of antioxidant defense. These finding suggest that aging is caused by progressive accumulation of defects initiated by free radicals.

Keywords: Lipid peroxidation, Oxidative stress, Antioxidant, Xenobiotic, Alpha-tocopherol, *Heteropneustes fossilis.*

INTRODUCTION

In normal metabolism, a balance exists between the generation of free radicals and antioxidant defenses mechanism[1].However normal enzymatic defenses are stresses, secondary defenses such as vitamin A, C and E prevent the chain reaction of autoxidation. Major antioxidant enzymes are viz superoxide dismutase; catalase, glutathione peroxidase, reduced glutathione, melatonine, ceruloplasmin, and albumin are non enzymatic antioxidants[2].Antioxidant enzymes play a vital role in protecting cellular damage by the harmful effect of reactive oxygen species[3,4,5,6]. Antioxidant matters protecting the cell membranes from lipid peroxidation[7,8,9,10]. An extensive literature appeared to oxidative stress and antioxidant activity on lipid metabolism[11], hepatocytes[12], red blood cells[13], nephrotoxicity[14] while *in vivo* oxidative activity has been shown in fish[15].

Reactive oxygen species play an important role in the etiology of diverse human disease[16] such as carcinogenesis[17, 18,] irradiation injury[19] and tumor promotion[20] as well as the normal process of ageing[21]. Brain is rich in polyunsaturated fatty acids and therefore, highly susceptible to peroxidation[22].

One class of xenobiotic that has received a considerable attention is phenol. Its common environmental contaminants occasionally; a large amount of phenol gets into the waste water treatment plant in the phenol discharging industries creating shock loading conditions on activated slug system. Phenol and its vapors are corrosive to the eyes, the skin, and the respiratory tract[23].

During the past number of years the value of antioxidant therapy, has been investigated thoroughly. Despite this, it is still questionable whether antioxidant supplementation is beneficial to the tissues during oxidative stress in fish or not. Nutritional effects on antioxidant enzymes have been seen in fish but these have been very specific[24,25].

In the present investigation the effect of phenol 5ppp, 10ppm and 15ppm for 15 days on lipid peroxidation, superoxide dismutase and ascorbic acid in the Brain of freshwater catfish ***Heteropneustes fossilis*** were observed. Alpha-tocopherol was supplemented and responses were observed.

MATERIAL AND METHOD

Experimental Animal

Adult freshwater catfish ***Heteropneustes fossilis*** (weight 50 ±1.8 gm, length 17±1.2 cm) were obtained from local vicinity of Ujjain and acclimatized in laboratory conditions for two weeks in dechlorinated tap water at room temperature and a photoperiod of 12hr light-12hr dark cycle. After acclimatization fish were then transferred to separate exposure glass aquaria. Fish were fed with a commercially available pellet every day.

Experimental Design

Eight groups of ten fish each were maintained. First group was kept as control. Second group treated with alpha-tocopherol; third, fourth and fifth groups were exposed with phenol at 5ppm, 10ppm and 15ppm respectively. Sixth, seventh and eighth group were exposed with phenol along with alpha-tocopherol 8 mg/ Kg body weight for 15 days. Fish were sacrificed after 15 days exposure. Brain was dissected out for the biochemical estimation.

Biochemical Assay

Lipid peroxidation level was measured in terms of melondialdehide (MDA) by the method[26]as described by[27].Superoxide dismutase activity assay by the method[28].Ascorbic acid measured by the modification of the dinitrophenylhydrazine (DNPH) technique[29] as described by[30].Protein content was estimated by the Folin phenol reaction as described by[31].

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.

RESULTS

The effects of different concentrations of phenol (5, 10 and 15ppm) and supplementation of alpha-tocopherol (8 mg/Kg body weight) for 15 days on lipid peroxidation, superoxide dismutase and ascorbic acid in brain, of ***Heteropneustes fossilis*** were observed. Results are depicted in table.

The lipid peroxidation was increased in brain after phenol exposure at 10ppm and 15ppm. The values were statistically significant ($p<0.05$). Supplementation with alpha-tocopherol considerably reduced the lipid peroxidation in all the treated groups as compared with phenol exposed group. Superoxide dismutase activity in brain showed significantly increased ($P<0.05$) at 5ppm and 10ppm whereas decreased activity at 15 ppm phenol exposure. However, supplementation with alpha-tocopherol has resulted in significant ($P<0.05$) depletion of superoxide dismutase activity in brain as

compared with phenol exposed group. Ascorbic acid content was decreased in brain after exposure to different concentration of phenol. This decrease in ascorbic acid was significant (P<0.05) in brain as compared with control, whereas supplementation of alpha-tocopherol resulted in a significant (P<0.05) increase ascorbic acid contents in brain.

Table: Effects of phenol (5ppm, 10ppm and 15ppm) and supplementation of alpha-tocopherol (8mg /Kg body weight) for 15 days in brain of *Heteropneustes fossilis.*

Group	Lipid peroxidation (n moles MDA/mg protein)	Superoxide dismutase (unit/gm wet tissue)	Ascorbic acid (µg/wet tissue)
Control	10.69 ± 0.81	12473 ± 606	90.37 ± 4.63
alpha-tocopherol	10.27 ± 0.71	12306 ± 580	89.49 ± 4.30
5ppm phenol	10.88 ± 0.82	20644* ± 646	67.41* ± 6.47
10ppm phenol	31.63* ± 0.51	20186* ± 631	42.72* ± 6.10
15ppm phenol	16.82* ± 0.64	18897* ± 635	14.87* ± 0.82
5ppm phenol+alpha-tocopherol	6.22* ± 0.63	6241* ± 64	70.17* ± 17.29
10ppm phenol+alpha-tocopherol	6.85* ± 0.81	6578* ± 58	74.74* ± 7.04
15ppm phenol+alpha-tocopherol	8.80 ± 0.80	7822* ± 64	82.21* ± 0.61

Values are expressed as mean ± SE from 10 fish in each group.

* Significant p <0.05 difference between control and treatment group at different concentration.

DISCUSSION

Certain classes of xenobiotic molecules may represent particularly prolific sources of oxygen radicals because of their abilities to be reduced to their corresponding radicals via many NAD(P)H-dependent reductases and then undergo redox-cycling[32,33,34]. Therefore, xenobiotic agents through redox-cycling have the potential to produce quantities of oxyradical, hydrogen peroxide and hydroxyl radical that overcome the protection afforded by antioxidants thereby leading to oxidative damage[35] which is manifested by damage to tissue macromolecules including DNA, proteins and lipids[36]. In the present study, phenol exposure resulted in, an increase in lipid peroxidation in brain. Lipid peroxidation largely result from free radical reaction in biological membranes which are rich in polyunsaturated fatty acids are considered to be an important feature in cellular injury[37], Impaired membrane function, structural integrity, decreased membrane fluidity and inactivation of several membrane-bound enzymes[38]. The observation is in agreement with the view that the enhancement of peroxidation of an essential functional membrane lipid could damage the biological membranes. Besides this the results presented in this study can very well explained by assuming of toxicity.

Membranes are not only highly important for maintaining the structure and building a permeability barrier between the interior of the cell but also a place where several functional proteins essential for cellular viability, are located. As a result of their high accumulation potential, relatively low concentration of lipophilic xenobiotics in the aquatic environment may lead to major changes in the structural and functional properties of membranes, which result in severe effects can on the whole cell. Increasing experimental evidences suggest that xenobiotic induced toxicity may also be mediated by oxygen radical intermediates[33,39,40,10].

Level of superoxide dismutase activity was increased with exposure to phenol, which taken together with the changes in lipid peroxidation, indicated an increased oxidative damage and possibly an increased oxyradical generation in brain. In higher concentration of phenol however, superoxide dismutase activity was decreased possibly due to general stress imposed on the cells. Superoxide dismutase activity is an important factor in preventing oxidative damage. Information about the effect of xenobiotics on antioxidant enzymes and lipid peroxidation in other fish species is limited and some what variable[41, 10]. Similarly in the mechanism of metabolism of these compounds by fish microsomes and cytosol to that of mammalian systems suggests that the severe health consequence by exposure to these environmental contaminants in mammals[42] might also have implications for the health of aquatic animals. Industrial uses of several monocyclic hydrocarbons viz., benzene, toluene, xylenes and other solvents have raised an important issue in industrial medicine and occupational health.

Nutritional effects on antioxidant enzymes have been seen in fish but these have been very specific, e.g., glutathione peroxidase and superoxide dismutase activities in rainbow trout were reduced respectively by diets deficient in selenium[24] and manganese[25.] Less is known on the impact of dietary antioxidants on endogenous antioxidant enzymes in fish. Supplementation of alpha-tocopherol has resulted maintain the superoxide dismutase activity at normal level, compare with controls group reducing lipid peroxidation. Similarly[35] have been reported the ability of alpha-tocopherol to inhibit oxidative stress in rat liver cell.

The present study clearly demonstrated the association of ascorbic acid content depletion with the increase in lipid peroxidation and superoxide dismutase activity. Similar trend of ascorbic acid content depletion associated with changes in various oxidant defense indices has been demonstrated in humans[43,44] and in fish[45]. Supplementation of alpha-tocopherol was able to improve the ascorbic acid content in tissues. It not only protects against oxyradicals that might initiate lipid peroxidation of cell membrane but may also serve as a scavenger of chain-propagating free radicals such as lipid hydroperoxyl radicals[46]. Ascorbate in combination with alpha-tocopherol can result in synergistic inhibition of oxidative damage to cell membranes[47], ß- Carotene exerts protective effects against singlet oxygen[33].

CONCLUSION

The present investigation shows an increase in lipid peroxidation by the treatment of phenol in brain. However, supplementation with alpha-tocopherol significantly ameliorated the toxic effects of phenol. These finding suggests that alpha-tocopherol can be used as an effective antioxidant against phenol neurotoxicity.

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