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Inhibition of Brain Na^+,K^+-ATPase Activity in Freshwater Catfish (*Channa punctatus* Bloch) Exposed to Paper Mill Effluent

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Paper and pulp mill effluent is a mixture of dissolved lignin and cellulosic degradation products, various other wood extracts and chlorinated organic compounds. It also contains high concentrations of heavy metals, total suspended solids, high pH, increased chemical oxygen demand and low dissolved oxygen that apparently increase the biological oxygen demand (Hamm et al., 1986; Suntio et al., 1988). Heavy metals detected in paper mill effluent include cadmium, chromium, copper, lead, mercury and zinc. Toxic potential of some of the paper mill effluent constituents has been reviewed by Owens (1991) and Perez-Alzola and Santos (1997). Earlier, we reported that the long-term paper mill effluent exposure adversely affected the fish phagocytes (Ahmad et al., 1998). The circulatory fish phagocytes were found to be over-activated in response to paper mill effluent exposure with increased reactive oxygen species (ROS) release. Furthermore, we observed that the over-activation of phagocytes was concomitantly associated with peroxidative damage of fish tissues (Fatima et al., 2000). While liver showed almost no damage, other organs (gills and kidney) showed higher levels of tissue damage as measured by lipid peroxidation (LPO). It is suggested that this selective response was due to differences in antioxidants status of various tissues (Ahmad et al., 2000).

Brain is the target of oxidative damage of environmental pollutants (Bano and Hasan 1989; Hai et al., 1997). The main factors that contribute to vulnerability of brain include high content of polyunsaturated fatty acids in brain membrane and low level of enzymatic and non-enzymatic antioxidants (Liu and Mori, 1993; Perez-Campo et al. 1993). Brain biomarkers thus may prove to be useful in pollution biomonitoring and may provide early warning signal to pollutant exposure (Peakall, 1992).

Na^+K^+-ATPase is a membrane bound, sulfhydryl-containing enzyme whose function is critical for the maintenance of cell viability. This enzyme carries out the transport of sodium and potassium ions against concentration gradient, resulting in the translocation of net charge. The enzyme acts as a current generator and contributes to the membrane potential of the nerve cells (Vizi and Oberfrank, 1992). This enzyme is known to be an early target for oxygen radical-induced damage to intact cell (Kim and Akera, 1987; Kako et al., 1988). Since

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altered membrane function is a major indicator of radical injury, we investigated effect of paper mill effluent on the activity of Na^+,K^+-ATPase in brain of freshwater catfish, *Channa punctatus* (Bloch). Besides, two other indicators of redox state viz., lipid peroxidation and total sulfhydryl (–SH) content of brain were also studied.

MATERIALS AND METHODS

Adult freshwater fish *Channa punctatus* (Bloch) of both sexes were collected from pollution-free water bodies and maintained in laboratory in 60 L glass aquaria. Prior to exposure, fish were acclimatized for 15 days and overall fish health under laboratory conditions was monitored. They were fed with autoclaved goat liver meal during the acclimatization and exposure. Fish (weight range 22-25g, length range 12-13 cm) were divided into five groups. Four groups of fish (n = 20 each group) were exposed to 1.0 % (v/v) of paper mill effluent. This concentration of effluent has been found to be optimum in inducing discernible responses in our previous studies (Ahmad et al., 1998; Fatima et al., 2000). Paper mill effluent was collected from discharge point of a paper mill installation. Some physico-chemical characteristics of paper mill effluent were as follows: pH 9.3 ± 0.404, temperature 26.08 ± 0.52 °C, biological oxygen demand (BOD) 320.33 ± 33.06 mg/L, chemical oxygen demand (COD) 418.84 ± 52.50 mg/L, total solid (TS) 656.25 ±128.44 mg/L, total dissolved solid (TDS) 404 ± 55.52 mg/L and total suspended solid 252.25 ± 85.32 mg/L. Fish were exposed for 15, 30, 60 and 90 days in such a way that all the fish were sacrificed on the 91st day. The brains of fish were removed on a chilled glass plate, washed and homogenised in 2 mL ice-cold 0.05 *M* tris-HCl (pH 7.4). The homogenates were used for Na^+,K^+-ATPase assay, lipid peroxidation and total –SH content measurement.

The activity of Na^+,K^+-ATPase was determined as inorganic phosphorus (Pi) production using the method of Svoboda and Mossinger (1981). Pi was estimated according to the method of Fiske and Subbarow (1925). The Na^+,K^+-ATPase activity was expressed as the inorganic phosphorus produced per hour per mg protein.

Lipid peroxidation was estimated using method of Utley et al. (1967) with slight modification (Fatima et al., 2000). The rate of lipid peroxidation was expressed as nanomoles of thiobarbituric acid reactive substance (TBARS) formed per hour per milligram of protein using extinction coefficient $1.56 \times 10^5\ M^{-1}cm^{-1}$.

Total sulfhydryl content of brain was estimated according to the method of Sedlak and Lindsay (1968). Total –SH content per mg protein was calculated using extinction coefficient of 13,100 $M^{-1}cm^{-1}$.

Protein content of each sample was determined using the method of Lowry et al. (1951). Data were statistically analyzed using one way factor ANOVA to assess the significant differences between experimental and control groups. The significance was set at $P< 0.05$.

RESULTS AND DISCUSSION

Paper mill effluent inhibited Na^+,K^+-ATPase activity, decreased –SH content and increased lipid peroxidation in brain tissue of fish. Fig. 1 shows the effect of paper mill effluent on Na^+,K^+-ATPase activity. Significant inhibition on Na^+,K^+-ATPase activity was observed at 15 ($P < 0.05$) and 30 ($P < 0.001$) days of exposure while 60 and 90 days exposure showed restoration on its activity. ATPase is a membrane bound enzyme and its activity depends closely on the structural integrity of the membrane. Synaptosomal or microsomal phospholipid are essential for the Na^+,K^+-ATPase activity and their degradation lead to the inhibition of the enzyme Sun, 1972; Charnock et al., 1973; Wheeler et al., 1975). In addition, microsomal membranes contain phospholipids with high degree of unsaturation (Cotman et al., 1969). These double bonds play a critical role in determining the functional activity of the Na^+,K^+-ATPase (Barnett and Palazzotto, 1974). Marked elevation of brain tissue lipid peroxide formation was observed in fish exposed to paper mill effluent for 15 and 30 days time intervals ($P < 0.05$ and $P < 0.001$, respectively) while 60 and 90 days exposure showed decrease in the lipid peroxidation (Fig 2).

It is well known that heavy metals induce the radical chain degradation of membrane and thereby facilitate LPO (Matta et al., 1999). Our results demonstrate an inverse correlation between the Na^+,K^+-ATPase activity and lipid peroxidation which is in conformity with the finding of Sovoboda and Mossinger (1981) on rat brain. Initial increase in the lipid peroxidation with concomitant decrease in Na^+,K^+-ATPase activity may be attributed to the toxic action of heavy metals and other organic toxicants of paper mill effluent. Partial restoration in the activity of Na^+,K^+-ATPase and slowing down of lipid peroxidation process after 30 days may partly be due to the fact that fish might have developed some adaptive mechanism such as induction of enzymatic and non-enzymatic antioxidants. Such an adaptive response to toxicant exposure has been observed in several freshwater and marine fishes (Otto and Moon, 1995).

A significant time-dependent depletion in total –SH content was observed at 30 days of exposure ($P < 0.05$). On the other hand, recovery was observed at 60 and 90 days (Table 1). Our findings are in conformity with those of Mather-Mihaich and DiGiulio (1986) who reported an initial decrease in glutathione level, a –SH group containing tripeptide, in channel catfish exposed to bleached kraft mill effluent with subsequent recovery after long-term exposure. Protective role of glutathione against oxidative stress-induced toxicity is well established in aquatic animals (Hasspieler et al., 1994). The higher LPO values in fish exposed for 15 and 30 days may be due to the decrease in –SH (glutathione) content. Adaptive responses have been observed in various fish species (Otto and Moon, 1995) and most common mechanism involved in adaptive response is the induction of antioxidants (Filho, 1996). Although antioxidant system in the brain of paper mill effluent exposed fish has not been studied, our observations on other organs provide enough support for an adaptive response to paper mill effluent exposure in fish (Fatima et al., 2000). Furthermore, use of rat brain Na^+K^+-ATPase assay

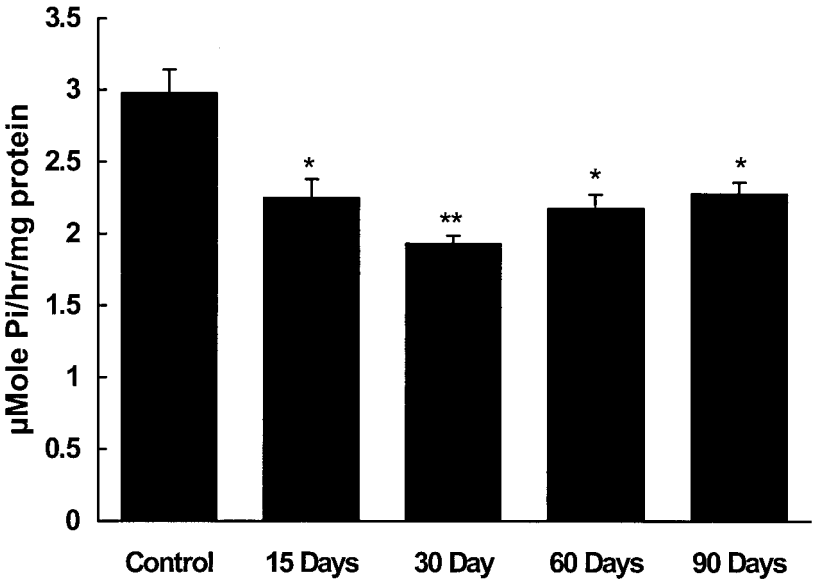

Figure 1. Na^+,K^+- ATPase activity in brain of freshwater fish *Channa punctatus* Bloch exposed to 1% paper mill effluent for 15, 30, 60 and 90 days and control fish. Data are expressed as mean ± S.E. (n=6). Significant changes vs control * $P < 0.05$, ** $P < 0.001$.

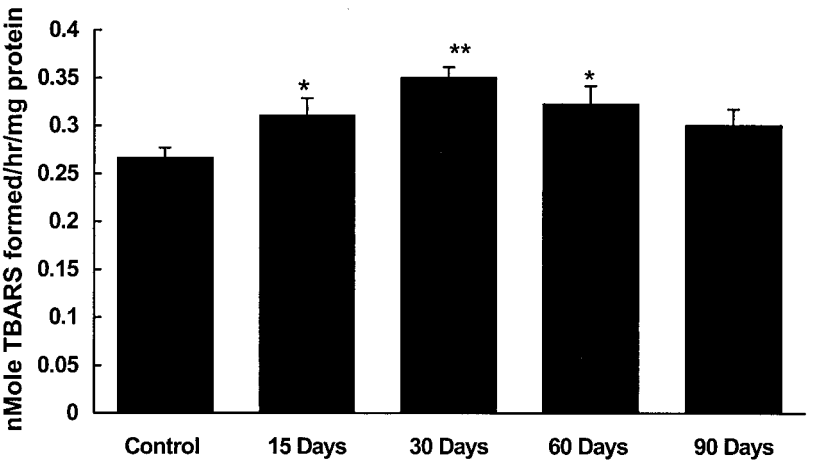

Figure 2. Lipid peroxidation level in brain of freshwater fish *Channa punctatus* Bloch exposed to 1% paper mill effluent for 15, 30, 60 and 90 days and control fish. Data are expressed as mean ± S.E. (n=6). Significant changes vs control * $P < 0.05$, ** $P < 0.001$.

Table 1. Effect of paper mill effluent on total -SH content of fish brain.

	Days of exposure				
	Control	15day	30 day	60 day	90 day
Total –SH content (μmole/mg protein)	67.39 ± 5.39	56.05 ± 1.12	47.43 ± 2.27*	54.91 ± 4.39	59.90 ± 3.23

* = $P < 0.05$

has been reported to be useful in assessing the effectiveness of biological treatment of paper mill effluent (Araujo et al., 1994). Our studies demonstrate that measurement of Na^+K^+-ATPase activity in fish could prove to be a sensitive biomarker of toxic effect of environmental pollutants on brain biomembranes.

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