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Lead Induced Thyroid Dysfunction and Lipid Peroxidation in the Fish *Clarias batrachus* with Special Reference to Hepatic Type I-5'-Monodeiodinase Activity

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Lead in high concentration has been well recognized as a cumulative general metabolic poison (WHO 1977). The toxic effects of lead poisoning in fishes have been reviewed from time to time with reference to both hematological and biochemical variables (Jackim 1973; Johansson- Sjobeck and Larsson 1979; Haux and Larsson 1982). In this group of vertebrates lead is known to inhibit neural activity (Weber et al. 1983) and perhaps the reproductive behavior patterns (Weber 1993). However, very little is known about the effect of lead on endocrine physiology, particularly on thyroid function in fish. One considering ^{131}I uptake by thyroid (Katti and Sathyanesan 1987) and other involving estimation of circulating thyroxine (T_4) only (Spieler and Weber 1991) and not Triiodothyronine (T_3) which is known to be biologically more potent (Oppenheimer et. al. 1970). The principal pathway of T_3 generation is the peripheral monodeiodination of T_4 which is primarily catabolised by the enzyme, Type I 5'-monodeiodinase (Eales 1985), which has not been studied in relation to lead toxicity in fish. Therefore, in the present investigation, a systematic study has been made to evaluate the chronic effects of lead nitrate in circulating T_3 and T_4 concentrations along with the hepatic lipid peroxidation (LPO) in *Clarias batrachus*. Mode of action of lead rendering thyroid dysfunction has also been worked out by estimating Hepatic Type I 5'- monodeiodinase (5'-D) enzyme activity.

MATERIALS AND METHODS

Adult fish *Clarias batrachus* weighing 25 ± 5g and 15 ± 2 cm in length were purchased from the local supplier in the month of October, 1994 and were acclimatized in clean aquarium containing tap water for 14 days. Four groups of 10 fishes each were established. All four groups

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were maintained under 12L:12D photoperiod in separate water tanks (size 43x28x24cm) containing 10 L of tap water (19°C; pH 7.5-8.0; hardness approx. 130 mEq $Ca(CO_3)_2$/ L) and were provided with chopped goat liver *ad libitum* as food.

Group I without any treatment served as control. Groups II, III, and IV were exposed to 5, 10, and 15 ppm of lead nitrate, respectively (Katti and Sathyanesan 1987). All control and experimental tanks were constantly aerated and the water replaced after every alternate day. After 25 days of exposure the blood samples were collected and fishes were sacrificed by decapitation, liver was then collected and processed for 5'D enzyme assay and LPO studies. Separate homogenates were prepared for 5'-D enzyme assay and LPO. 5'-D enzyme activity was determined following the method of Kahl et al. (1984) with little modifications. Liver was homogenized using Potter Elverhjem Teflon glass homogenizer in 5 volume (wt/volume) ice-cold buffer (0.1 *M* phosphate buffer, pH 7.2 with 0.25 *M* sucrose and 5m*M* EDTA). After centrifugation at 5000 rpm for 30 min at 4°C the supernatant was incubated with T_4 (4 µ*M*) for 1 hr and the amount of T_3 generated was measured by radioimmunoassay (RIA). Serum T_3 and T_4 concentrations were determined using a commercially available radioimmunoassay (RIA) kit supplied by BARC (Bhabha Atomic Research Center), Bombay, India.

LPO was estimated by the thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to peroxidation of lipids, according to the method of Ohkawa et al. (1979). Liver was removed, washed in 5 % wt /volume phosphate buffer (0.1*M,* pH 7.2) and homogenized. The homogenate was then centrifuged at 5000 rpm for 30 min and the supernatant was used for LPO assay.

Liver protein was estimated by the method of Lowry et al. (1951) both for 5'-D and LPO.

Data are expressed as mean ± standard error. Significance level between two groups was calculated using student's 't' test.

RESULTS AND DISCUSSION

Serum T_3, T_4 and hepatic 5'-D enzyme activity decreased significantly in 10 ppm ($P< 0.05$, $P< 0.01$ and $P< 0.05$ respectively) and in 15 ppm ($P< 0.01$, $P< 0.05$, $P< 0.01$ respectively) lead nitrate groups, when compared to the respective control values. However, LPO increased significantly by all the doses of lead nitrate ($P< 0.001$ compared to the control values).

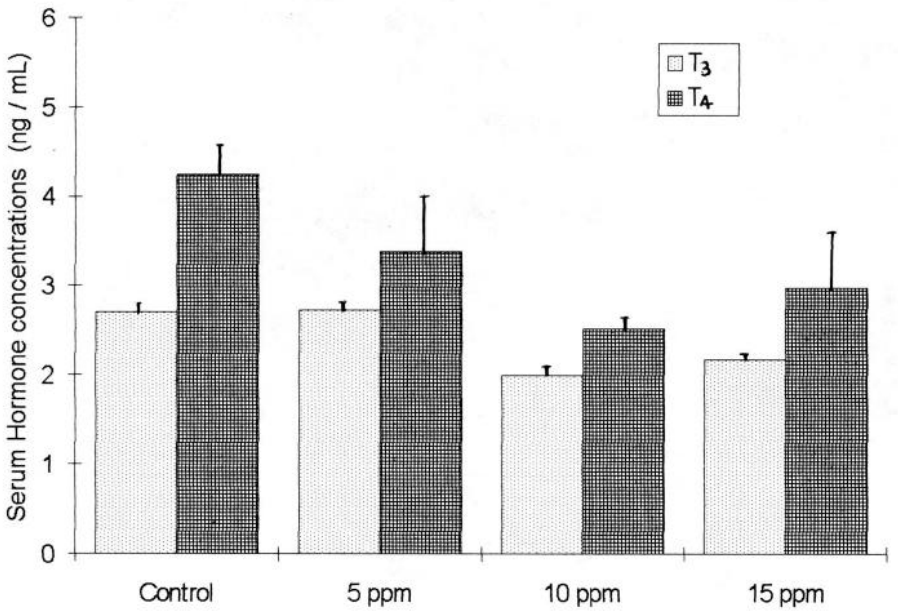

Figure 1. Effect of lead nitrate on serum thyroid hormone concentrations.

From these results, it is clear that with lead treatment serum thyroid hormone concentration decreases whereas LPO increases in dose dependent manner in *Clarias batrachus*. Decreased thyroid function based on thyroidal ^{131}I uptake and histology has also been reported earlier in this fish (Katti and Sathyanesan 1987). So far, only one report is available in fish that indicates lead induced alteration in circulating thyroid hormone concentration (Spieler and Weber 1991), that too, changes in T_4 concentration only has been studied. Thus, no report was available until today that investigated all three important parameters (Serum T_3, Serum T_4 and 5'-D activity) in relation to lead toxicity in fish.

In the present study, lower dose of lead nitrate was not effective with respect to thyroid function, whereas the two higher doses decreased serum T_4, T_3 concentrations and 5'-D enzyme activity indicating a dose specific response of lead in *Clarias batrachus*. Since in lead treated animals both T_4 and T_3 were decreased, it appears that lead primarily inhibits T_4 synthesis and/or release at the glandular level. One of the possibilities is that it might be inhibiting iodine uptake as reported earlier (Katti and Sathyanesan 1987). Another possibility is that, lead effect on thyroid gland is not the direct one rather indirectly through impairment of Thyrotropin-Releasing Hormone (TRH) as has been

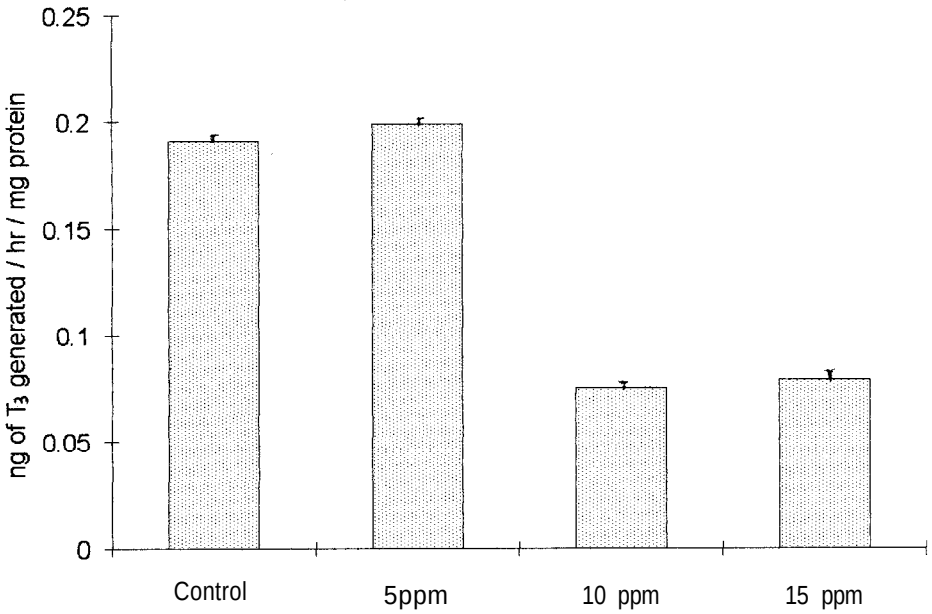

Figure 2. Effect of lead nitrate on Hepatic type I 5'- monodeiodinase activity.

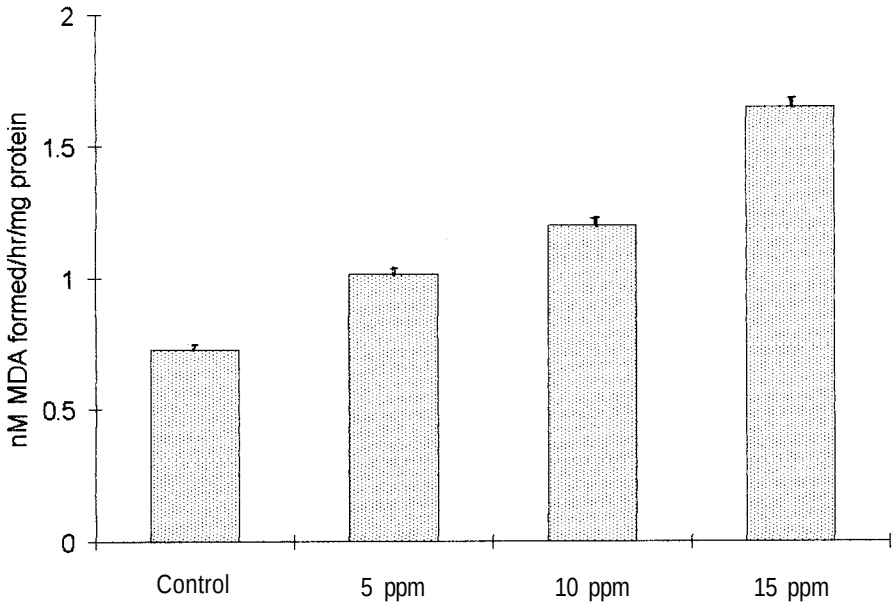

Figure 3. Effect of lead nitrate on Hepatic Lipid Peroxidation.

reported in occupationally exposed children (Huseman et al. 1987). This is true at least with respect to serum T_4 that is synthesized mainly in the thyroid gland. However, for T_3 the reason could be different. The lead induced decrease in serum T_3 concentration is because of the inhibition of 5'-D enzyme activity (as observed in the present study), which is primarily responsible for the conversion of T_4 to T_3, the major pathway of T_3 generation (Oppenheimer et al. 1970). Decrease in 5'-D enzyme activity has also been reported in rat with respect to another heavy metal, i.e., cadmium (Yoshida et al. 1987).

Increased LPO has been associated with lead toxicity in rat (Sifri and Hockstra 1978). Therefore, in the present investigation , lead induced increased LPO is the indication of the hepatotoxic effects of the metal. In *Clarias*, increased LPO was observed in all lead treated groups, indicating that lead nitrate is hepatotoxic to this fish even at very low dose (5 ppm). Since LPO is a complex phenomenon that involves several polyunsaturated fatty acids, catalysts, oxidants and reducing substances, involvement of one or more of these factors, that might be playing significant role in increasing LPO potential of lead in *Clarias batrachus* requires further investigation.

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