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Effect of Neurotoxicant, Nickel Chloride in Zebra fish

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ABSTRACT

Background: Environmental pollutants and heavy metal toxicity is a major threat to the humans and there are several health risks associated with it. Of the heavy metals, Nickel chloride is released through environment via evolutionary activity, contaminants and natural sources. Environmental and occupational exposures to nickel chloride are vital factor for neurological dysfunction in humans. **Purpose**: To analyse the effect of heavy metal toxicant nickel chloride in the brain of the adult zebra fish**. Methods**: Nickel chloride (3 µl of 1 M $NiCl_2$) was injected intramuscularly in the adult zebra fish for 30, 40 and 50 days. The time-dependent changes were analysed in the adult fish by performing histo pathology, oxidant and antioxidant status and biochemical parameters. **Result:** Chronic stress due to $NiCl_2$ was found to affect the .pathology of fish in a duration-dependent manner. The response of tissue antioxidants as assessed by the activities of SOD,GST, Catalase and the content of reduced glutathione were found to be affected at 50th day of induction. The oxidative stress as assessed by the content of lipid peroxidation was found to be increased when compared to control (p<0. 01)). The activity of phospholipids and free fatty acids were significantly decreased (p<0.01) on 50th day. **Conclusion**: The study suggests that nickel chloride can alter the neuronal morphology, oxidant status and the biochemical parameters owing to possible defects and biochemical variation in the brain of the fish of Nickel chloride exposure.

KEYWORDS: Daniorerio , Nickel chloride, Neurotoxin, Toxicity, Heavy metal toxicant

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INTRODUCTION:

Heavy metal toxicant Nickel chloride is well known environmental pollutant too. The nickel in drinking-water occurs via leaching from metals in contact with drinking-water, such as pipes and fittings. [1,2] Its exposure also occurs via jewellery, cosmetics. Wholemeal products, beans, seeds, nuts and wheat bran contain nickel in high concentrations.[3] Vegetarians consuming large amount of nuts and beans have higher concentration of nickel in their body. Inhalation, dermal contact, drinking water, food items and gastrointestinal ingestion are the primary routes of exposure to nickel. Nickel is a potential toxicological hazard for living organisms because of its carcinogenic, genotoxic, neurotoxic and immunotoxic effects. [4] Acute or chronic toxicity of nickel chloride causes oxidative stress induced by free radical formation.[4]Neurological disorders (giddiness, weariness) were noticed in individuals exposed to nickel and boric acid in drinking water.[5] Nickel treated with rats for 3 months were shown neurological signs such as lethargy, ataxia, prostration.[6] *Daniorerio,* simplest vertebrate and telost whose genetic make up is 80% similar to that of mammals and it is an excellent model of neurological study because the organisation and function of zebra fish brain is similar to that of humans. [7] Zebra fish possess all major neuromediator systems, antioxidant enzymes, other biochemical enzymes of synthesis and metabolism, similar to those observed in humans and hence they have been used in our current study.[8] Zebra fish have been proven as a useful model organism to study the specific mechanisms of contaminants.[9] The study focused on exploring the exposure of neurotoxicant, nickel chloride in Zebra fish which has not been analyzed for its long-term effect.

MATERIALS AND METHODS:

Zebra fish: Twelve adult female zebra fish were housed at light/ dark cycle of 14/10; water temperature of 27 +/- 1 °C maintained with aeration and bio-filtration. Group size maintained at 50 numbers. Procurement of all the laboratory grade chemicals from Sigma Aldrich, Loba Chemie. All the stock solution prepared as per the manufacturer instruction. Zebra fish were collected from Kolathur and verified by Dr. Jaya sheer Tikal (Scientist D) Zoological Survey of India, Chennai.

Study Design: To induce metal toxicity, adult female zebra fish were exposed to various concentrations (0.01 M,0.1 M and 1 M) of Nickel chloride for one week. After the exposure, the smear test was done to determine the effective dosage followed by the survival and mortality analysis. Based on the data(not shown),3.0 µl(477 µg) of 1M Nickel Chloride was injected to the fish intramuscularly using a Hamilton syringe daily for 30 days,40 days and 50 days.

Group Distribution:

Group I-Control Adult Zebra fish

Group II –Adult Zebra fish subjected to Nickel chloride for 30 days .

Group III- Adult Zebrafish subjected to Nickel chloride for 40 days.

Group IV- Adult Zebra fish subjected to Nickel chloride for 50 days

Histo Pathology: Adult fishes were euthanized and dissected as per ethical guidance. Fish were anesthetized with 15 °C water and sacrificed by a cut between the brain and the spinal cord to remove the head separately. The brain was dissected with a pin and knife. A thin layer smear of brain tissue was made on a glass slide and stained with Haematoxylin & Eosin respectively for 2 minutes each followed by PBS washes. Slides were viewed at 45X magnification using labomed lx 400 microscope.

Antioxidant enzymes:

Lipid Peroxides Test: The level of Lipid peroxides estimated by Thiobarbituric acid reaction method described by Ohkawa et al.[10] To 0.2 ml of fish brain cells extract, 0.2 ml of SDS, 1.5 ml of acetic acid and 1.5 ml of Thiobarbituric acid were added. The mixture was made up to 4.0 ml with water and then heated in a water bath at 95°C for 60 minutes. After cooling, 1.0 ml of water and 5.0 ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the organic layer was taken,and its absorbance was read at 532 nm. The activity of lipid peroxides expressed as nanomoles.

Superoxide Dismutase: The SOD activity was measured by the method of Misra and Fridovich.[11] The activity of SOD of the fish brain cells was assayed by their capacity to compete with native or partially succinylatedferricytochrome c for free radicals generated by the xanthine/xanthine oxidase system. The reduction of ferricytochrome c was monitored with a micro plate reader by using the wavelength pair 550 nm minus 557 nm.

***Catalase*:** Catalase activity was measured by the method of Aebi.[12]0.1 ml of fish brain cell extract was added to a cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reactionwas started by the addition of 1.0 ml of freshly prepared 30mM Hydrogen peroxide. The rate of decomposition of H_2O_2 was measured spectrophotometerically by changes in absorbance at 240 nm. The activity of catalase expressed as units/mg protein.

Glutathione peroxidase: The method of Wood, J.L assayed glutathione peroxidize.[13]About2.0 ml of phosphate buffer (75 mmol/L, pH 7.0), 50µl of (60mmol/L) glutathione reductase solution, 50µL of (0.12 mol/L) NaN_3, 0.1 ml of (0.15mmol/L) Na_2 EDTA ,100µL of (3.0 mmol/L) NADPH, and 100µL of fish brain cell extract were mixed in a tube. Water was added to make a total volume of 2.9 ml. The reaction started by the addition of 100µL of (7.5 mmol/L) H_2O_2,

and the conversion of NADPH to NADP monitored by a continuous recording of the change of absorbance at 340 nm at 1-min interval for 5 min.

Glutathione Reductase: Glutathione reductase was assayed by the method of Stahletal.[14].1.0 ml phosphate buffer, 0.5 ml EDTA, 0.5 ml Glutathione oxidized and 0.2 ml of NADPH was made up of 3.0 ml with distilled water. After the addition of 0.1 ml of fish brain cell extract, the change in optical density at 340 nm was monitored for 2 minutes at 30 seconds interval.

Assay For Phospholipids**:** Whole brain of single fish was teased in chloroform and Methanol solution, 5 ml in the ratio, 7:3 for 15 minutes. The methanol layer that extracted the free fatty acid was transferred to a fresh 15 ml tube and allowed to evaporate at 45°C until concentrated to pure phospholipids. The phospholipids were dissolved in 2.0 ml of chloroform, added to 2.0 ml of ammonium ferrothiocyanate in a test tube, and intimately mixed for 1 min on a rota mixer. Following phase separation the lower chloroform phase was removed with a Pasteur pipette and the optical density measured at 488 nm.

Assay For Free Fatty Acids**:** Whole brain of single fish was teased in chloroform and acetic acid solution, 5 ml in the ratio, 7:3 for 15 minutes. The chloroform layer that extracted the free fatty acid was transferred to a fresh 15 ml tube and allowed to evaporate at 45°C until concentrated to 100µl. 10µl of the chloroform solution was added to a 0.5% solution of a mixture of diphenylcarbazone and diphenylcarbazide (5:95) in methanol and read in a microplate reader, Robonikat 550 nm against a reagent blank.

RESULTS:

Statistical comparisons were made using Graph Pad software. Two-way ANOVA test with a 95% confidence interval was performed at an alpha = 0.05 (95% confidence interval).

Effect of $NiCl_2$ on the pathology of the brain of the Zebra fish

Necrotic damage was observed in the brain of adult fish (Fig1) which was not evident in the case of control fish , in a dose-dependent manner.

Group I- represents the normal architecture of the brain cells.

Group II,III, IV represents the mild shrunken cells, necrotic cells and distortion in the purkinghe layer

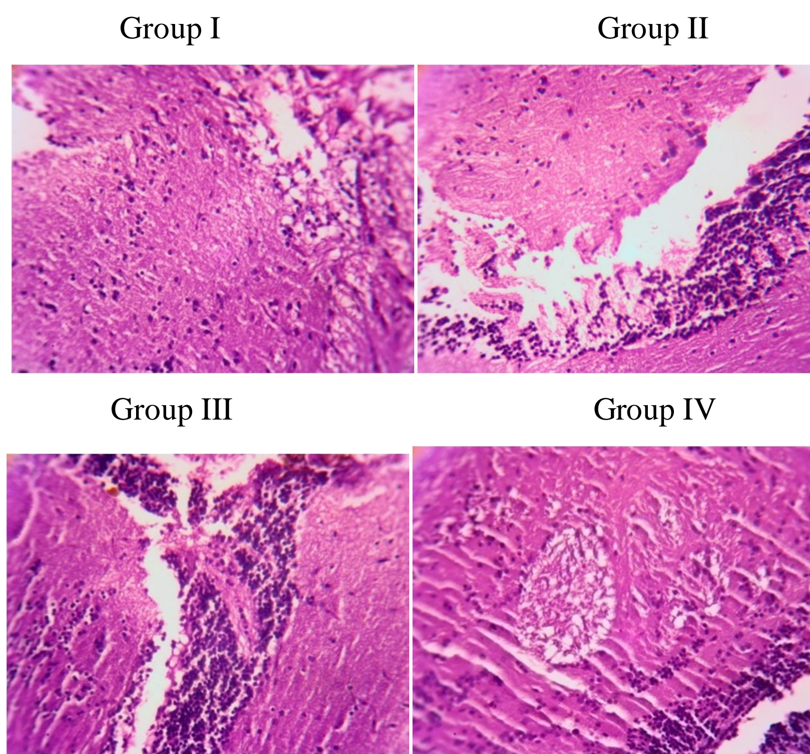

Fig 1: Microscopic appearance of cerebrum portion of brain of Zebra fish that were subjected to $NiCl_2$ exposure.

Effect of Nickel Chloride on Oxidant and Antioxidant Activities:

The status of oxidant/antioxidant in the brain of Zebra fish exposured to Nickel chloride presented in Fig2. It was evident that while the level of peroxides was significantly elevated ($p<0.001$),the SOD and CAT activity were drastically reduced in the 50^{th-}day group when compared to that of control. The activities of GR and Gpx also showed a significant decrease in 50 days of Nickel chloride exposures concerning control.

Results were expressed as mean ± standard deviation. SOD, Superoxide dismutase; CAT, Catalase; GR, Glutathione reductase; GPx, Glutathione peroxidase . Significance was tested statistically by two way ANOVA with a 95% confidence interval was performed at an alpha = 0.05 (95% confidence interval) within the same exposure concentration between different durations of exposure of $NiCl_2$. All asterisks represents statistically significant

**P<0.05; **P<0.01; as compared to control values.*

Comparisons

were made between Group I vs Group-II,III and IV

Table 1: Oxidant and antioxidant status in the brain of the fishes subjected to Nickel chloride,

S.No	Parameters	Group I	Group II	Group III	Group IV
1	Superoxidedismutase(μ moles per mg)	173±4	187±6*	210±6*	230±6**
2	Lipid peroxidases(μ moles per mg)	51.9±3	56.1±3*	63.5±4*	69.4±4**
3	Catalase(μ moles per mg)	81.31±4	87.89±4*	98.71±5*	108.12±5**
4	Glutathione peroxidases(μ moles per mg)	75.32±3	71.31±3	65.32±3*	62.35±3**
5	Glutathione reductases(μ moles per mg)	56.24±3	52.03±3	46.24±3*	42.36±3**

Effect of Nickel chloride on phospholipids and free fatty acids

The activity of phospholipids and free fatty acids were lowered in 50 days of Nickel chloride exposure compared to control.

Table 2 The activities of Phospholipids and free fatty acids in the Danio rerio subjected to Nickel chloride exposure for different days

	Parameters	Group I	Group II	Group III	Group IV
1	Free fatty acid(nanomoles per ml)	48±2	44±2*	42±2*	36±2**
2	Phospholipid(nanomoles per ml)	86±2	83±4*	75±4*	60±4**

Results are expressed as mean ± standard deviation. Significance was tested statistically by two way ANOVA with a 95% confidence interval was performed at an alpha = 0.05 (95% confidence interval) within the same exposure concentration between different durations of exposure of $NiCl_2$. All asterisks represent statistically significant

**P<0.05; **P<0.01;* as compared to control values.

Comparisons were made between Group I vs Group-II, III and IV,

DISCUSSION:

$Nicl_2$ Affects The Purkinje Layer Of The Zebra fish:

Histopathological studies would give a potential toxicological effect of heavy metals in marine organisms. Brain demyelination and necrotic changes were observed in rainbow trout when exposed to nickel chloride of concentration 1 mg/L and 2 mg/L for 21 days.[15] In our present study, necrotic changes were observed in the cerebrum regions.

Nickel Chloride Affects The Oxidative Status:

It has been reported that Nickel is responsible for generation of Reactive oxygen species which results in increased oxidative stress. [16] An antioxidants enzyme quenches the free radicals and neutralizes the reactive oxygen species generated by nickel hazard. In our study, antioxidant enzymes such as sod and cat were depleted .There was significant increase in SOD activity in 50th day group compared to the control in our present study. In another study in Alyssum mantinum under nickel treatment[17], SOD activity also increased significantly. Heavy metal toxicity also results in increased accumulation of Lipid peroxidation product.[18]The accumulation of LPO may be due to increased ROS formation by heavy metal toxicant , Nickel chloride. Similar to our study, exposure to nickel chloride in Oncorhynchus mykiss*(rainbow trout),*was found to cause abnormal biochemical enzymes.[19]

Nickel Chloride Can Affect The Phospholipids And Free Fatty Acids:

Ni toxicity leads to increased production of reactive oxygen species, thereby damaging the membrane lipids by per oxidation.[20] As a result, phospholipids and free fatty acids in the brain tissues were decreased compared to control

CONCLUSION

This study can provide useful information for understanding of Ni-induced toxicity. The study suggested that $NiCl_2$ have significant impact by affecting the nervous system. This study is alarming given chronic exposure to nickel chloride, even if it is not widely prevalent.

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