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To study the effect of cadmium chloride on the level of oxidative stress markers in *Channa punctatus*

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Abstract
To know the effects of cadmium chloride concentrations on the level of Lipid peroxidation (LPO) and Superoxide dismutase (SOD) in Liver and Brain tissues of fresh water murrel, *Channa punctatus*.
The fishes were exposed to cadmium chloride with two different concentrations (20ppm and 40ppm) and the oxidative stress was assessed on 1st day (24hrs), 5th day & 10th day of exposure along with the control group of fishes.
LPO elevates significantly in liver tissue on 1st, 5th & 10th day followed by brain tissue, when compared to control group.
An increased LPO level is due to formation of more lipid radicals.
Superoxide dismutase (SOD) activity decreases initially on 1st day of exposure of fishes to cadmium chloride when compared with control group of fishes and later it increases in experimental group on 5th day & 10th day in liver tissue followed by brain tissue.
Thus, fluctuation in antioxidant enzyme SOD was seen.
The results indicate that cadmium chloride may induce toxic effects which can be assessed by the oxidative stress biomarker SOD and LPO in *Channa punctatus.*

Keywords: antioxidant, superoxide dismutase, oxidative stress

Introduction
Cadmium is one of the contaminants of ecosystem.
The pollution of heavy metals increases with human activities like agricultural activities and motor vehicles are all major sources of metal pollution. Metals can be found in water and sediments they are accumulated in aquatic organisms such as fish [12].
Fishes are considered to be an important nutrition to human, but those from contaminated sites when consumed are of potential risk to the human health.
Channa punctatus have a more commercial significance as a food fish (Parveen *et al*., 2021).
Murrel being typical "live fishes'' and soft flesh without fat are considered to have medicinal and nutritional value.
Fishes are the bio indicators of aquatic ecosystem.
The oxidative stress induced by the metal can be assessed by the oxidative damage [11].

Cadmium
Cadmium is one of the most toxic heavy metal found in the environment and it is extremely toxic even at low concentrations, it does not serve a beneficial biological function in higher organism [25].
Human activities like combustion of fossil fuels, agricultural land and mining waste, contribute to cadmium contamination in the environment [27].
Cadmium is used as a stabilizer of PVC, pigments, alloys and in the electroplating used to protect steel from corrosion [7, 29].
In aquatic ecosystem the presence of cadmium, is regarded as a potential hazard for the aquatic organisms.
Cadmium is readily absorbed by organisms directly from the water in its free ionic form Cd^{2+} [2].
It also affects antioxidant enzymes especially SOD and LPO [6].

Oxidative stress
Oxidative stress is due to the imbalance between the free radicals and the antioxidants LPO & SOD in living organisms [15].

Free radicals
Free radicals on reacting with other molecules can form new radicals.
Free radicals are harmful in high production, during physical exercise cause muscular fatigue, aging and many diseases [3].
Increased production of free radicals can cause oxidation of lipids, proteins and alterations of gene expression [11].

Antioxidants
An antioxidant is a substance that helps to reduce oxidative stress by forming a less reactive radical or by reducing the damage of FR chain reaction on substrates such as lipids, proteins, carbohydrates or DNA [4].
Antioxidants are of two types: Enzymatic and Non-Enzymatic [18].

Table 1: Types of antioxidants

Enzymatic antioxidants	Non-enzymatic antioxidants
Superoxide Dismutase (SOD) Lipid Peroxidase (LPO)	Vitamin A Vitamin E Flavonoids

Oxidative stress & Cadmium toxicity

Cd concentration increases in living organisms including human with age and diet [21].

The absorption and accumulation of these heavy metals depends upon the factors such as sex, age, health condition and exposed people (Satarug S *et al* 2004) [16].

Cd is one of the most toxic heavy metals. Its toxicity can be considered as multidirectional. Exposure of Cd leads to an increase in the production of reactive oxygen species (ROS) namely hydrogen peroxide, superoxide radicals and hydroxyl radicals. Oxidative stress is induced by Cd [14].

The oxidative stress results in the oxidation and damage of Deoxyribonucleic acid (DNA), proteins and lipids and phospholipids of cellular membrane. Hence by lowering the mitochondrial potential Cd disrupts the synthesis of adenosine tri phosphate (ATP) and oxidative phosphorylation [28].

The ROS production is balanced by enzymatic (SOD and CAT) and non-enzymatic (Vit C, Vit E, GSH) antioxidant barriers.

Cd also damages DNA and also inhibits DNA repair enzymes [17].

It is also influenced with gene expression by disrupting cell signalling pathway. It also causes apoptosis and necrosis (Wang, Liu, *et al*., 2013).

Cadmium has an adverse effect on the respiratory, reproductive and haematological system in many fishes [24].

Oxidative stress biomarkers

Biomarkers serve an important role in bio monitoring of toxic metal pollution in aquatic ecosystem by indicating the oxidative stress in various organisms [23]. From a clinical point of view, the use of biomarkers to assess the degree of oxidative stress is very valuable.

Lipid per oxidation (LPO)

Lipid per Oxidation is an antioxidant enzyme and also a biomarker of pollutants exposure in various organisms [11].

Lipid peroxidation results in the formation of lipid radicals and this results in the formation of complex mixture of lipid degradation products [1].

Heavy metal pollution in aquatic organisms is induced by LPO [5].

The antioxidant enzyme protects against oxidative stress and also to tissue specific damage. These antioxidant enzymes are also a biomarker of ROS and a tool in environmental assessment risk factors [8].

The ROS can be protected and detoxified by antioxidant enzymes such as lipids, proteins and DNA damage [13].

Superoxide dismutase (SOD)

SOD is the antioxidant enzyme that can cause a catalytic reaction by dismutation of superoxide anion to O_2 and H_2O_2 and CAT reacts with H_2O_2 to form water and oxygen molecule [10, 13].

SOD can cause defence upon superoxide radicals and is the first line of defence against oxidative stress. The dismutation of O_2^- and formation of H_2O_2 can be catalyzed by the enzyme SOD.

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Aims & Objectives

Aim

To study the effect of cadmium chloride toxicity on the level of Oxidative stress markers LPO and SOD in liver and brain tissues of *Channa punctatus.*

Objectives

To study the effect of cadmium chloride on liver and Brain tissues of *Channa punctatus* by estimating the level of oxidative stress markers (LPO & SOD).

To study the effect of cadmium chloride on 1st day, 5th day and 10th day of exposure to fishes (*C. punctatus*) and compare the results obtained with control values.

Experimental design

Materials

Tubs
Net
Fishes
Dissection box

Collection of fish sample

Edible fish samples of *Channa punctatus* of same size and weight will be collected from nearby Fish market, Hyderabad, Telangana.

The fishes will be collected in air filled polythene bags with water for the Experiment to be estimated in the Laboratory, for further analysis.

Channa punctatus

It is a fresh water murrel, snake-headed belonging to family Channidae of the order perciformes.

Channa punctatus is a fresh water murrel lives in rivers, lakes, ponds, swamps and marshes.

They feed on invertebrates and small fishes (Narejo *et al*., 2005, 2010).

It is a bottom dweller geographically distributed important economic food source to human.

The presence of respiratory organ helps fish to survive in difficult conditions.

Murrel fingerlings are used for giving medicine every year in month of June on the eve of mrigasira karthi for allotment of asthma patients that gather all over the country in Hyderabad since long back.

It can be maintained *in vitro* condition. It is a good model for studying of biochemical effects of certain pollutants.

Channa punctatus has ability to respond to environmental pollution.

Channa punctatus was selected because of its ecotoxicological characteristics

Fresh water murrel can be easy acclimatization to laboratory conditions and availability throughout the season [9].

Methodology

To Estimate the effect of cadmium chloride on oxidative stress biomarkers by

a. Lipid peroxidation method by Garcia *et al*. (2005).
b. Superoxide dismutase by Marklund and Marklund (1974).

In the present study the fishes were treated with 0.05% $KMnO_4$ solution for 20 min to avoid any dermal infections and after that the fishes were acclimatized for 1week under laboratory conditios before start of the experiment to

remove the residual effects of other chemicals (Pandey *et al*., 2006).

Stored water was used to allow the chlorine to settle as it will also cause stress to fishes. water is changed daily to maintain same concentrations of cadmium**.**

Three tubs were taken and they were filled with 5 litres of water, 15 fishes were taken, they were divided in to three groups, with 5 fishes in each group. One group is taken as control, second group and third groups are taken as experimental in which fishes were exposed to cadmium chloride with two different concentrations for a time period of the 1st day, 5th day, & 10th day.

On each experiment day, fish was sacrificed and dissected for the removal of target organ and the experiments was performed to know the level of LPO and SOD in liver and brain tissues of *Channa punctatus.*

Procedure

Lipid peroxidation (LPO)

Lipid peroxidation in tissue was measured by modified method of Garcia *et al*. (2005). 1 mL of 10% homogenate was added to 1 mL of 20% TCA and heated at 70 0C for 10 min, and cooled at room temperature and centrifuged at 3000 rpm for 10 min. 400 µL of supernatant was mixed with 200 µL of 0.5% TBA reagent in a test tube covered with a glass marble and heated in a boiling water bath for 10 mins and the tubes cooled to room temperature. The absorbance of the pink coloured trimethine condensation product was measured at 533 using a spectrophotometer. The results were expressed as nano mole MDA/gm weight of tissue.

Superoxide dismutase (SOD)

Superoxide dismutase activity in tissue was estimated by modified protocol of Marklund and Marklund (1974). The assay system in a final volume of 1.0 mL consisting 600 µL of 83.3 mM Tris–Hcl buffer, (Ph 8.2), 100 µL of 0.5 mM DETPA, 50 µL of enzyme, 50 µL of Tris-EDTA, 50 µL of 0.01 N HCl, 100 lL of H2O2 was mixed well and the reaction was initiated by adding the 50 µL of 3.97 mM Pyrogallol. Increased absorbance was read at 420 using a spectrophotometer. The enzyme activity was expressed as Units/mg protein.

Result

LPO in liver

Table 2: LPO level in Liver of *Channa punctatus*

Tissue	Days	Control	Experimental 20 ppm 40 ppm	
Liver	1st	0.20	0.28	0.30
	5th	0.26	0.42	0.44
	10th	0.23	0.68	0.72

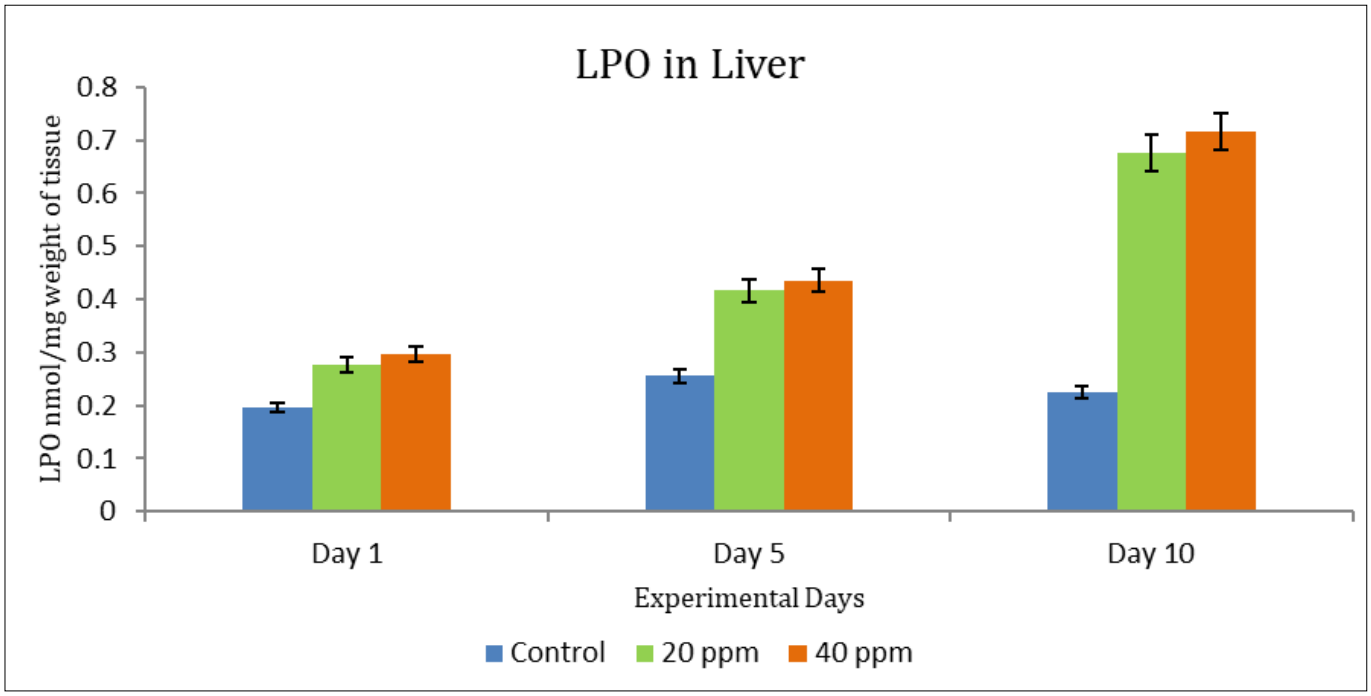

Fig 1: Comparative chart of LPO level in liver of *Channa punctatus*

LPO in brain

Table 3: LPO level in Brain of *Channa punctatus*

Tissue	Days	Control	Experimental	
			20 ppm	40 ppm
Brain	1st	0.20	0.22	0.25
	5th	0.26	0.36	0.38
	10th	0.23	0.44	0.47

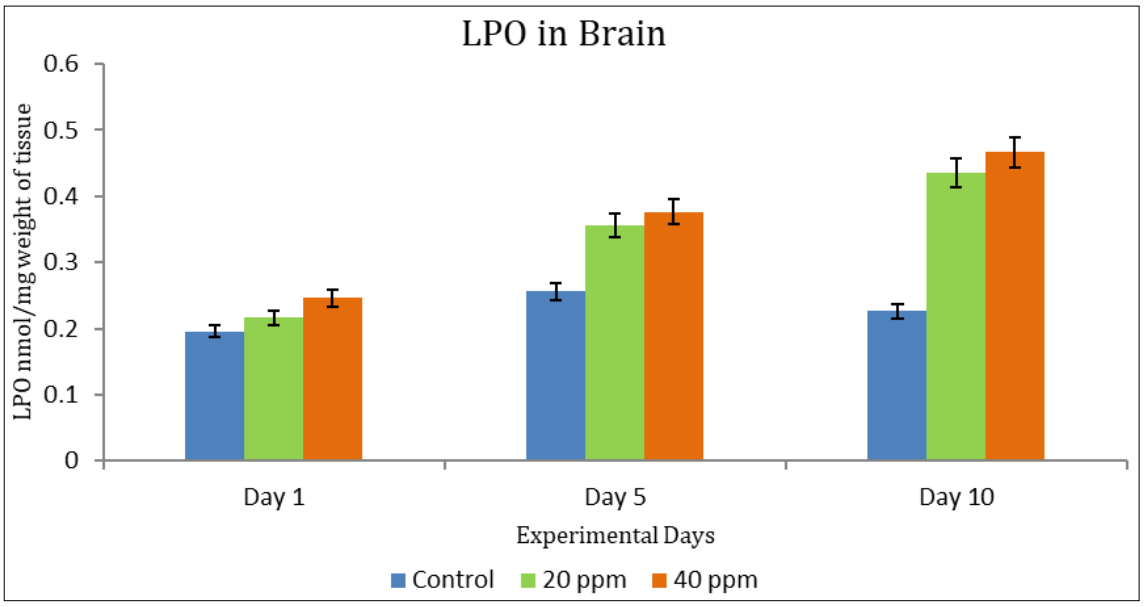

Fig 2: Comparative chart of LPO level in brain of *Channa punctatus*

SOD in Liver

Table 4: SOD level in liver of *Channa punctatus*

Tissue	Days	Control	Experimental	
			20 ppm	40 ppm
Liver	1st	0.25	0.18	0.26
	5th	0.22	0.20	0.35
	10th	0.24	0.22	0.37

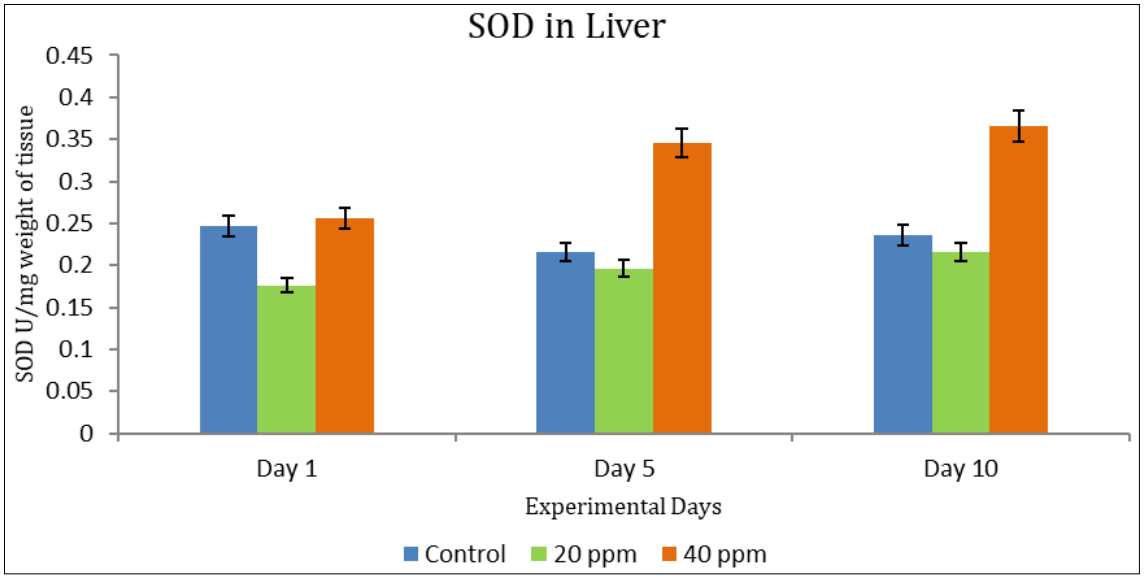

Fig 3: Comparative chart of SOD level in liver of *Channa punctatus*

SOD in Brain

Table 5: SOD level in Brain of *Channa punctatus*

Tissue	Day	Control	Experimental	
			20 ppm	40 ppm
Brain	1st	0.25	0.14	0.21
	5th	0.22	0.17	0.27
	10th	0.24	0.19	0.34

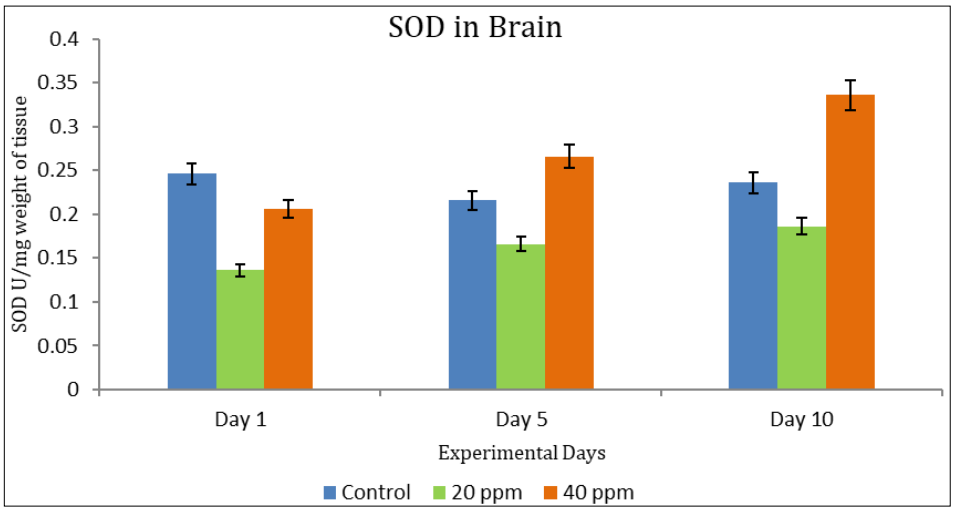

Fig 4: Comparative chart of SOD level in Brain of *Channa punctatus*

Discussion

LPO

It is one of the main causes for the oxidative damage which is induced by metals [6].

It is used as biomarker of pollution [22].

Cd causes an enhancement in ROS formation, which leads to the peroxidation damage in liver and brain [26].

Liver shows an increased level of LPO on exposure to cadmium chloride due to an increased production of ROS.

Liver shows an increased level of LPO in 20ppm on 1st, 5th& 10th days 0.28<0.42<0.68 when compared with control (0.20).

Liver shows an increased level of LPO in 40ppm on 1st, 5th & 10th days 0.30<0.44<0.72 when compared with control (0.20).

Brain shows an increased level of LPO in 20ppm on 1st, 5th &10th days 0.22<0.36<0.44 when compared with control (0.20).

Brain shows an increased level of LPO in 40ppm on 1st, 5th & 10th days 0.25<0.38<0.47 when compared with control (0.20).

Therefore, LPO was found to be low in control, further increased LPO was seen in experimental in liver followed by brain this is due to highest peroxidative damage.

SOD

It is an antioxidant enzyme and also a group of metallo-enzymes, constitute a defense system against the natural or chemically induced production of ROS [19].

Liver shows decreased level of SOD in 20ppm on 1st day and increased in level was observed on 5th day &10th day 0.18<0.20<0.22 when compared with control (0.22).

Liver shows decreased level of SOD in 40ppm on 1st day & increased in level was observed on 5th day & 10th day 0.26<0.35<0.47 when compared with control (0.22).

Brain shows decreased level of SOD in 20ppm on 1st day & increased in level was observed on 5th day & 10th day 0.14<0.17<0.19 when compared with control (0.22).

Brain shows decreased level of SOD in 40ppm on 1st day& increased in level was observed on 5th day & 10th day0.21<0.27<0.34 when compared with control (0.22).

SOD: was found to be high in control and fluctuations was seen in experimental in liver followed by brain.

The result indicates that increased in LPO level and fluctuation in antioxidant defence system in fishes may be due to cadmium chloride toxicity.

Therefore the effect of Cadmium chloride on LPO & SOD depends on the Cd chloride concentrations (20ppm & 40ppm) and also with different time intervals (1st day, 5th day, & 10th day).

Conclusion

Cadmium has an adverse effect on the respiratory, reproductive and haematological system in many fishes [24].

The present study revealed that Cadmium chloride toxicity is responsible for the oxidative stress in liver and brain tissues of *Channa punctatus* as shown by increase in LPO and in response, the antioxidant (SOD) defense mechanism were induced.

Oxidative stress may cause aging processes and also diseases like Parkinson's, Cancer and Alzheimer's.

Therefore it is advised for the patients who are suffering from diseases to take foods rich in Antioxidants to overcome with oxidative damage.

Abbreviations

DETPA: Diethylenetriaminepentaacetic acid
DNA: Deoxyribonucleic acid
EDTA: Ethylenediaminetetraacetic acid
HCL: Hydrochloric acid
LPO: Lipid peroxidation
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TBA: Thiobarbituric acid
TCA: Trichloroacetic acid

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