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Cadmium Toxicity Induced Changes on Antioxidative Enzymes Level in Fresh Water Catfish Channa Punctatus (Bloch)

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Abstract

Aquatic ecosystem of India is being affected by various heavy metals. In present, cadmium contamination in aquatic organism as well as its accumulation in food chain has become a global environmental concern. Cadmium exposure to fishes alter the various enzymatic and histopathologic profiles. Such alteration can be act as a bioindicator for aquatic environment. The present study has been planned to observe the effect of cadmium chloride on commercially important fish *Channa punctatus* (body weight 27.04±0.19 g and length 16.7±0.20 cm). For Invitro assessment of cadmium effect in term of enzymatic and nonenzymatic assay have been done. Fishes were acclimatized and treated separately with different concentration of $CdCl_2$. Dissected out the liver and homogenized in 0.1 M sodium phosphate buffer (pH 7.4). Biochemical analysis was done for lipid peroxidation, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) and protein estimation. The results were shown as mean and standard error (Mean± SEM). The analysis of variance (ANOVA) was analysed and significantly applied for the Dunnett test for multiple comparison of groups against the control which determine the significant differences among the groups. This study is for academic importance and will provide valuable information regarding cadmium toxicity. At the same time, it will address the public health issue as for as consumption of the fishes from contaminated water is concerned.

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Introduction

Global environmental issues by heavy metal pollution and its detrimental effect on aquatic and human life have shown the vast and varying attention to researchers. The continuously exposure of heavy metals into aquatic ecosystem by natural and anthropogenic activities (Butnariu, 2022, Gupta *et al.*,2023). Aquatic ecosystem primarily serves the purpose of food demands so, it become the primary target for heavy metal pollutants (Ali *et al.*,2019, Kumar *et al.*, 2020). Aquatic body act as a sink for adsorption of heavy metal and continuously increase in its concentration by releasing from point and nonpoint sources which deteriorates the water quality and fish health (Pandey and Kumari, 2023, Kumar *et al.*, 2023).

Cadmium is a biologically non essential heavy metal that has given great importance from the toxicological and ecotoxicological point of view (Genchi,2020, Gupta *et al.*,2023). From its natural reservoir, this metal is continuously decreased by anthropogenic processes and natural processes and distribute it among different environmental compartments, with aquatic ecosystems being important sites for final disposal of its soluble forms. Cadmium has been listed in the “Black list” of European community (Ali *et al.*,2018). Cadmium plays no important role in biological function in an organism and has a particularly more bioaccumulation tendency (Rajeshkumar and Li,2018).

Cadmium (Cd) enters the human body via food, particularly through leafy vegetables, grains, and cereals. Cd is toxic to several tissues, most notably causing hepatotoxicity upon acute administration, as well as nephrotoxicity upon chronic exposure (Genchi,2020, Kumar *et al.*,2020, Varsha *et al.*,2022). It accumulates in the liver and kidneys and has a long biological half-life of 17 to 30 years in human (Rahimzadeh *et al.*,2017). The liver is the primary target organ following acute systemic Cd exposure. The uptake of Cd into the liver is critical for the development of overall toxicity induced by the heavy metal. Approximately half of Cd absorbed systemically is rapidly accumulated in the liver which results in the reduced availability of Cd to such organs as the kidneys and testes, which are more sensitive to its toxic actions (Charkiewicz *et al.*,2023). Production of reactive oxygen species and oxidative tissue damage due to Cd have been associated with hepatotoxicity (Yang *et al.*,2021). It has been demonstrated that Cd produces both dose and time-dependent increases in intracellular glutathione concentration during chronic environmental or occupational exposure at low doses (Nemmiche, 2017).

Cadmium occurs naturally in ores together with zinc, lead and copper. Metallic cadmium has mostly been used as an anticorrosion agent (Ballen et a., 2021). It is also present as a pollutant in phosphate fertilizers which may lead to contamination of soil and can enter into aquatic bodies through sewage sludge and runoff from agricultural lands and producing deleterious effects on flora and fauna by affecting various physiological, biochemical, and cellular processes (Nino-Savala,2019). The major sources of contamination include electroplating, paper, PVC plastic, pigment and ceramic industries, battery, mining and smoldering units and many other modern industries (varsha *et al.*, 2017).

Evaluation of the effects of cadmium on fish is of particular interest due to its significance in the contamination of aquatic ecosystems. When fishes are exposed to stress condition, their physiological, biochemical and behavioural parameters among others, may become changed and this alteration can be used as early biomarkers of toxicity (Sharma *et al.*, 2019). In biological organisms, cadmium binds with enzymes having sulfhydryl groups, rupture cell membrane permeability,

accumulate in cell organelles, binds with nucleic acids and also induces endocrine disruption at hormone level (Anurag *et al.*,2012, Jancic and Stosic,2014). At high concentration, it causes adverse changes in the soft tissues of liver and leading to adverse effects in neurological, behavioural and physiological functions (Ungureanu and Mustatea, 2022). There are strong evidences suggesting the involvement of oxidative stress in the mechanism of cadmium toxicity. A number of antioxidants exist to balance the cellular production of ROS, maintaining the intracellular redox balance by preventing the cellular damage caused by ROS.

Materials and Methods

Experimental animals and chemicals

The fresh water fish *Channa punctatus* (with mean weight 27.04±0.19 g and length 16.7±0.20 cm) collected from local market of Lucknow city were used for biochemical and enzymes assays. Selected specimens were acclimated to laboratory conditions for one month prior to the experiments. The specimens were given prophylactic treatment by bathing them twice in 0.05% KMnO4 solution for 2 minutes to avoid any dermal infections. The $CdCl_2$ concentration was taken as 35mg/L and 70mg/L. Dissected out the liver and homogenized in10% (w/v) chilled sodium phosphate buffer (0.1 M, pH 7.4) by using a Potter Elvehjem homogenizer. A part of this homogenate was used for biochemical estimations and the other part was centrifuged at 9,000 rpm for 30 minutes at 4°C. Supernatant were taken for analysis of SOD, CAT, GPx, GST and protein estimation.

Biochemical Assays

Lipid peroxidation (LPO)

Tissue LPO was measured using the method of Ohkawa et al. (1979). Absorbance was recorded at 530 nm, and the results were expressed as n moles MDA / hr / mg tissue.

Reduced glutathione (GSH)

GSH concentration was measured in brain tissue using the method of Ellman (1959). The absorbance of GSH-DNTB conjugate was determined at 412 nm and the concentration (nM GSH/mg protein) was calculated using standard calibration.

Superoxide dismutase (SOD)

SOD activity was analyzed using the method of Kakkar et al. (1984). Colour intensity of the chromogen was measured at 560 nm. The results were expressed as µmoles /min/mg of protein.

Catalase (CAT)

The activity of CAT was measured according to the method of Sinha (1972). The mixture was cooled and absorbance was read at 570 nm. The CAT activity was calculated in terms of μ moles/min/mg protein.

Glutathione peroxidase (GPx)

The GPx was measured using the procedure of Rotruck et al., (1973). Absorbance was read at 420 nm. The results were expressed as n moles/min/mg protein.

Glutathione S-transferase (GST)

GST was determined spectrophotometrically at 25 ◦ C by following the formation of GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm using extinction coefficient of 9.6 × 103 m-1 cm-1 (Habig *et al.*,1974). GST activity was expressed as n mole/min/mg of protein.

Protein estimation

Protein was estimated by colorimetric method and BSA as standard (Lowry*et al.*,1951).

Histopathology

Liver samples were collected for morphological and pathological analysis according to the method described byZhang et al. (2019). Liver tissues were fixed in 4% paraformaldehyde, after then dehydrated with gradient ethanol and embedded in paraffin. Embedded liver tissues were sectioned with 5 μm thick sections. Tissue slices were examine under optical microscope and photographed.

Statistical analysis

The results were expressed as mean and standard error (Mean ± SEM) and determined for all the parameters. The data was analyzed employing the analysis of variance (ANOVA) using statistical software Graph Pad In Stat Software Inc., v. 3.06, San Diego, USA. The Dunnett test for multiple comparisons of groups against the control was performed to determine the significant differences among the groups.

Results

Effect of Cadmium on Oxidative Stress Biomarkers (Enzymatic and Nonenzymatic Antioxidants)

Table 1. Effect of Cadmium Exposure on Liver of *C. punctatus* After 30 Days

Biochemical test	Control	Low dose	High dose
LPO (n moles MDA / hr / mg tissue)	7.695±0.319	8.985±0.334	9.867± 0.113
GSH (nM GSH/mg protein)	5.271±0.115	4.564±0.1395	4.18±0.2486
SOD (µmoles /min/mg of protein)	18.266±0.250	15.299±0.229	12.124±0.282
Catalase (µmoles / min / mg protein)	4.397±0.139	3.201±0.186	1.990±0.061
Protein (mg/ml)	45.92±0.978	43.87± 0.878	40.15± 0.432

Table 2. Effect of Cadmium Exposure on Liver of *C. punctatus* After 60 Days

Biochemical test	Control	Low dose	High dose
LPO (n moles MDA / hr / mg tissue)	7.602±0.269	9.356±0.197*	9.881±0.588**
GSH (nM GSH/mg protein)	5.528±0.105	5.15±0.321	4.684±0.327
SOD (µmoles /min/mg of protein)	47.06±0.8390	42.47±0.692	40.23±1.558**
Catalase (µmoles / min / mg protein)	3.561±0.166	2.705±0.0927**	1.816±0.063**
Protein (mg/ml)	44.701±1.39	39.725±.1840*	38.736±.8121

Lipid Peroxidation (LPO)

The level of LPO was found to be increased significantly in low concentration (P<0.05) and in high concentration (P<0.01) as compared with control in both experimental group (Table1 and 2, Fig.1).

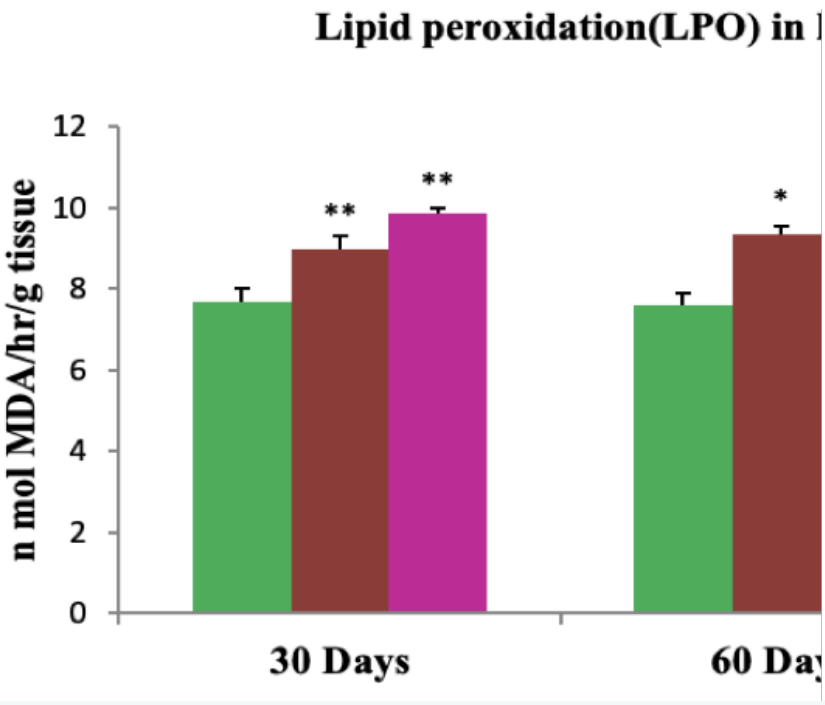

Fig.1. Effect of Cadmium on LPO level in liver of *C. punctatus* after different durations. Values mean ± SEM N=6. P<0.01**, P<0.05*, P>0.05 ns, as compared with Control.

Reduced glutathione (GSH)

The GSH status in liver of control and experimental fish was observed. GSH was decreased significantly in the low dose (P<0.05) and higher dose (P<0.01) as compared to control after 30 days exposure whereas after 60 days exposure, the level was found decreased insignificantly both in low dose (P>0.05) and in high dose as compared with control (Table 1 and 2, Fig.2).

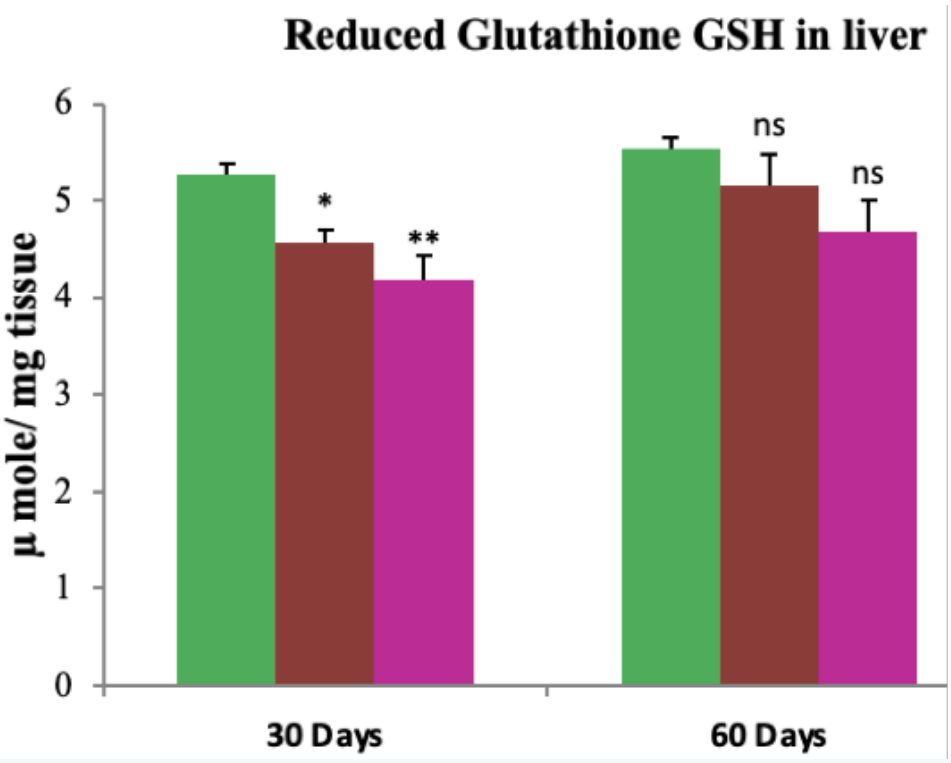

Fig.2. Effect of Cadmium on GSH level in Liver of *C. punctatus* after different durations. Values mean ± SEM, N=6. P<0.01**, P<0.05*, P>0.05 ns, as compared with Control.

Superoxide dismutase (SOD)

After 30 days exposure, level of SOD was found to be increased significantly in low dose concentration (P<0.01) and in higher concentration (P<0.01) as compared with control. After 60 days exposure, also the level was found to be increased significantly in the low dose (P<0.05) and in high dose (P<0.01) (Table 1 and 2, Fig.3).

Catalase (CAT)

After 30 days exposure, the effect of cadmium on catalase was observed. The level of CAT was found to be increased significantly in the lower concentration (P<0.05) and in the higher concentration (P<0.01) as compared with control. After 60 days of exposure, the level of CAT was found to be increased significantly in low dose (P<0.01) and in high dose respectively (Table 1 and 2, Fig.4).

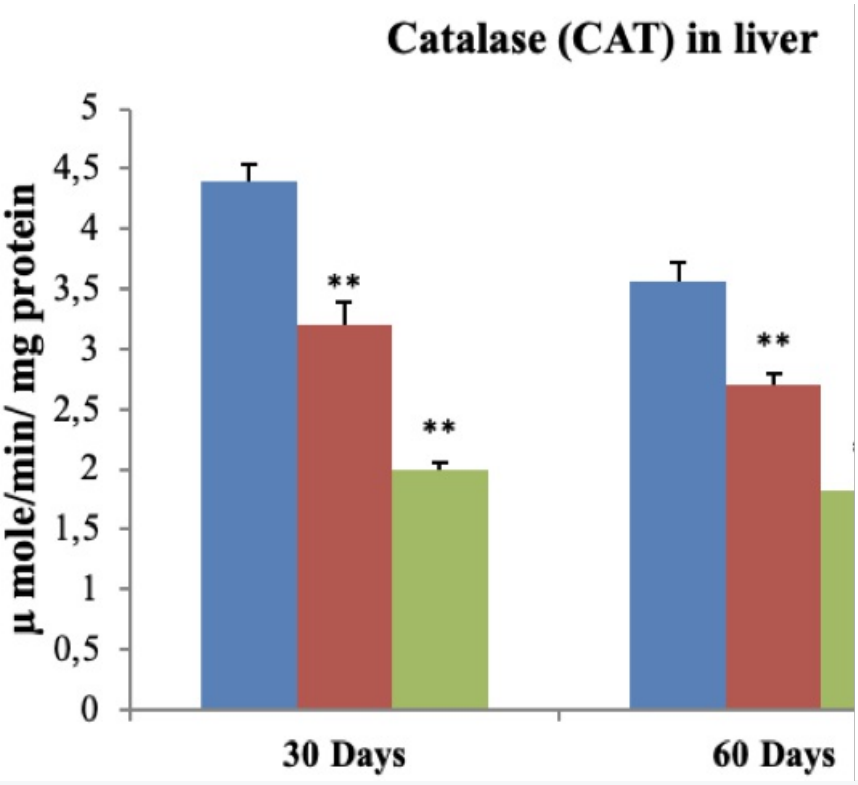

Fig.4. Effect of Cadmium on Catalase level in *Liver of C. punctatus* after different durations. Values mean ± SEM N=6. P<0.01 **, P<0.05*, P>0.05 ns as compared with Control.

Protein

After 30 days cadmium exposure, the level of protein was found to be decreased insignificantly in low dose (P>0.05) and also decreased in high dose (P<0.01) as compared with the control. After 60 days exposure, the level of protein was found to be decreased significantly in low dose (P<0.05) and in high dose (P<0.01) respectively as compared with the control (Table 1 and 2, Fig.5).

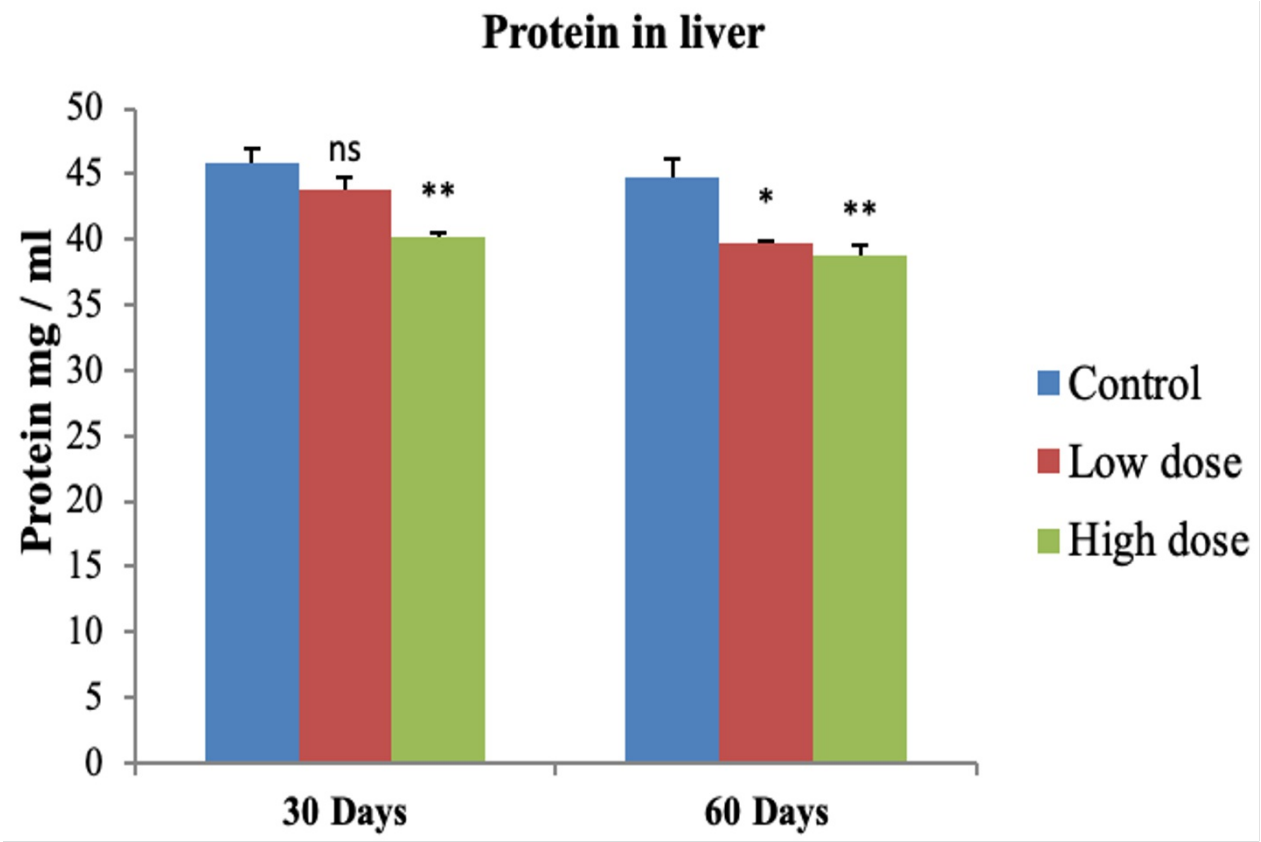

Fig.5. Effect of Cadmium on Protein level in Liver of *C. punctatus* after different durations. Values mean ± SEM, N=6. P<0.01**, P<0.05 *, P>0.05 ns as compared with Control.

The liver of exposed group at lower concentration of cadmium showed a number of degenerative changes in comparison to control. After 30 days of exposure, hepatocytes showed mild focal necrosis, vacuolization and hypertrophy in hepatocytes with pyknotic nuclei were observed. After 60 days of exposure, changes in hepatocytes were more apparent and detrimental. Degenerative changes in the hepatic cells with vacuolisation and nuclear damage were severe. Loss of matrix, nuclear atrophy, focal necrosis, congestion of blood vessel and computer architecture were more prominent. Due to severe degenerative and necrotic changes liver lost its normal cellular architecture.

The liver of exposed fishes at higher concentration showed a number of degenerative changes in comparison to control group fishes. After 30days exposure, vacuolisation (V) and necrosis (NC) were found more prominent. Hepatocytes showed indistinct cell boundaries with pyknotic nuclei (P) and disorganisation of hepatic cord and intravascular haemolysis in hepatoportal blood vessel were observed. After 60days exposure, cellular architecture was almost lost due to degenerative change (Fig.6).

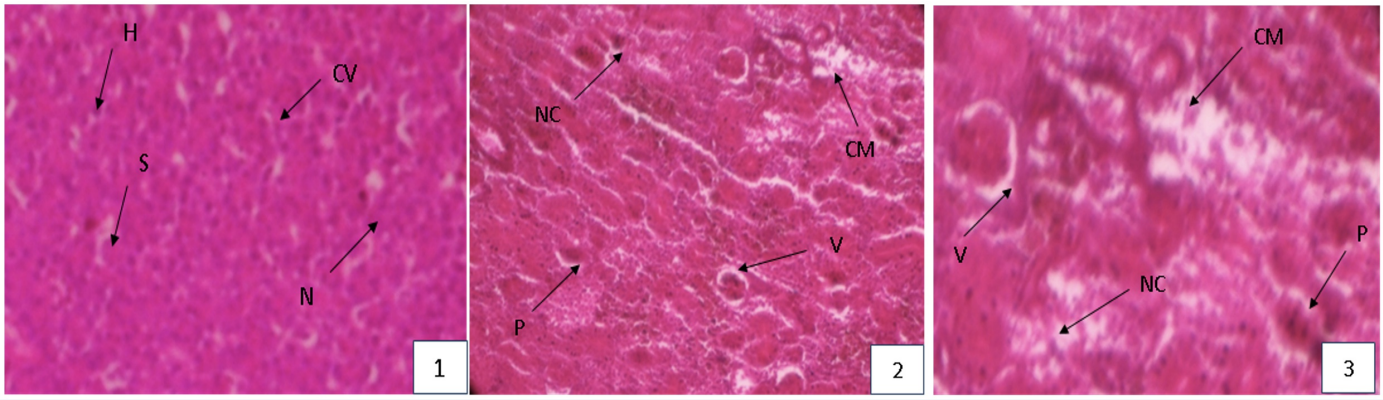

Fig.6. Shows degenerative changes in C. *punctatus* liver after 30 days (2) and 60 days (3) exposure of cadmium with comparison to Control (1).

Discussion

The values of LPO measured in *C. punctatus* are in agreement with those reported for various fish species (Pandey*et al.*,2008). Cd produces inhibitory effect as a response the respiratory chain becomes highly reduced and the electrons are transferred directly to available oxygen that induce ROS formation which causes oxidative damage in the liver. Usually, it works as a coordinated manner in order to ensure the optimal protection against oxidative stress by scavenging H_2O_2.

Lipid peroxidation is a free radical mediated process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, low concentrations of lipid peroxide are found in plasma and tissues. Oxygen derived free radicals generated in excess in response to various stimuli could be cytotoxic to several tissues. Most of the damage is considered to be mediated by these free radicals by attacking membranes through peroxidation of polyunsaturated fatty acids. Reactive oxygen species (ROS), including the hydroxyl radical (·OH), superoxide anion (O^{2-}), Hydrogen peroxide (H_2O_2) and nitric oxide (NO), mediated lipid peroxidation can cause oxidation and lead to damage of biomolecules like nucleic acid, protein and carbohydrate. Peroxidation involves the direct reaction of oxygen and lipid to form radical intermediates and to produce semi stable peroxides which in turn damage enzymes, nucleic acids, membranes, and proteins. Lipid peroxidation has long been known and has been suggested to be responsible for numerous deleterious effects observed in biological systems **(**Recknagel *et al.*, 2020**)**. After initiation, it concurrently proceeds by a free radical reaction mechanism and is regarded as one of the basic mechanism of cellular damage caused by free radicals (Recknagel *et al.*, 2020). It is the presumptive markers for free radical generation and increase of oxidative damage. Free radicals are short lived reactive chemical species involved in a variety of functions like the oxidation of polyunsaturated fatty acids in cell membrane.

In present study, elevated level of LPO was observed in liver and kidney of*C. punctatus* exposed to cadmium. The rise in LPO may be due to the increase in the generation of free radicals. These free radicals attack cell structures within the body, causing damage to cell membrane and enzyme systems.

In this study, increased LPO levels were observed after cadmium exposure. Such increased LPO levels were also reported in Rat liver and kidney (Andjelkovic *et al.*,2019). Amr *et al.* (2022) also found that cadmium administration to

female Wistar rats triggered apoptosis in endometrial tissues and significantly increased endometrial LPO content. Increased LPO production has been considered as a reliable biomarker for oxidative stress in animals exposed to environmental contaminants (Poli *et al.*,2022). Lipid peroxidation end product, LPO, was significantly increased in kidney tissue of lead treated *O. niloticus*. The reactive oxygen species (ROS) are involved in the initiation of lipid peroxidation and oxidative stress in different tissues. The toxic action produced by lead might be attributed to its ability to generate ROS which induce oxidative damage in several tissues by enhancing lipid peroxidation (Hashish *et al.,* 2015). In the present study, lipid peroxidation was increased after cadmium exposure in kidney.

GSH is an important non protein cellular thiol that, in conjunction with GPx, plays a regulatory role in cell proliferation (Scire *et al.*,2019, Averill, 2023). GSH and GSH dependent enzymes are involved in scavenging the electrophilic moieties (Georgiou-Siafis and Tsiftsoglou, 2023). It is known that GSH is one of the most powerful antioxidants in mammalian and is essential for normal cell functioning, replication and cell signalling system (Sadiq,2023). It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (Raj *et al.*,2021).

The present study showed decreased level of GSH in liver and kidney of*Channa punctatus* after exposure to cadmium. The reduction in the GSH level may be due to direct conjugation of GSH with electrophiles species which are produced increasingly by fluoride exposure, or due to inhibition of enzymes such as glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase etc which are involved in GSH synthesis and regeneration. Cadmium exposure in rats caused reduction in GSH contents in liver and kidney, GSH being a multifunctional nonenzymatic antioxidant.

Increase in ROS and LPO resulted in the depletion of GSH in the liver and ovary of exposed fish. In this study, GSH is an endogenous, peptidal antioxidant which prevents damage to cellular components by ROS and peroxides.

Superoxide dismutase is a class of enzymes that catalyze the binding of ROS with water to dismutation of superoxide anion into molecular oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. Superoxide dismutase is the main enzymatic defense against the superoxide anion. This enzyme detoxifies the superoxide anion, thus converting it into H_2O_2 and water. Superoxide dismutase is a ubiquitous chain-breaking antioxidant found in all aerobic organisms. It is the first enzymatic defense against the superoxide anion. CAT is responsible for the breakdown of H_2O_2 to water and oxygen, protecting the cell from the damaging action of H_2O_2 and the hydroxyl radical. In the present study, SOD, CAT and GST levels increased significantly in the liver and ovary with increased concentration of fluoride and exposure duration. Similar result was found with most investigations after exposure to cadmium (Wang *et al.,*2019, Zhang *et al.*,2023). Antioxidant enzymes (CAT, GST and SOD) of liver and gills revealed a significant increase in their activities in *O. niloticus* expose to a sub lethal concentration of fenitrothion for 30 days (Ibrahim,2019).

In present findings, SOD and CAT levels decreased in the kidney with increased concentration of cadmium. The decreased activity of SOD in the kidney tissue might cause the accumulation of ROS. Cadmium exposure is considered to generate the superoxide anion ($O2^-$). During the period of oxidative stress, cadmium can inhibit the activity of antioxidant enzymes such as SOD, GSH, and CAT which play an important role in antioxidative cell defense and eliminating free radicals, owing to its interactions with enzymes. It involves binding with the active sites on the enzymes

and result in the excessive production of ROS at the mitochondrial level which causes the damage of cellular components (Zhang *et al*.,2016).

Similarly, decrease in SOD in kidney was reported by (Hashish*et al.,*2015) as compared to the control group after lead treatment of fishes. Lead showed inhibitory effects of SOD activities this leads to impairment of cell antioxidant defence mechanisms, which would render cells exposed to oxidative attacks. Significant increase in MDA level and decreased activities of superoxide dismutase and catalase in the kidney suggested that oxidative stress mediated toxic effect in cadmium intoxicated rats (Wang *et al.,*2019).

After 60 days of exposure, a dose dependent inhibition of SOD activities was found which could be due to its direct toxicity to SOD by its interactions with enzymes, involving its binding with the active sites on the enzymes and its inhibition of the activities of antioxidant enzymes.

Catalase is an enzyme which is present in most of the cells and react to the decomposition of hydrogen peroxide to water and hydrogen using either an iron or manganese as a cofactor (Ighodaro *et al.,*2018). Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide. The level of CAT in kidney decreased due to over production of free radicals. In contrasts, liver and ovary CAT activity in the exposed fish increased as compared to controls both 45 and 90 days of exposure. The alternative explanation for reduction in CAT activity by cadmium exposure may be related to direct binding of fluoride to –SH groups of the enzyme molecule. The reduced CAT activity in the kidney may also be associated with the compensatory high activity of GPx, which acts as a defense against the formation of H_2O_2 or effective antioxidant responses due to a higher renovation of the kidney. Similarly, decreased CAT activity was also observed in the kidney of *C. punctatus* after cadmium exposure (Iwan *et al.,*2015). Superoxide dismutase dismutate O_2^- to generate H_2O_2 which can further react with chromium to form a hydroxyl radical (Engwa *et al*.,2022). Abu-El-Zahab *et al*. (2019) have reported that the activity of antioxidant GPx and SOD in kidney and liver after cadmium treatment was decreased significantly as compared with control.

Glutathione S-transferases (GST) are a family of enzymes which catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates that have electrophilic functional groups. In present study, after cadmium exposure GST level increased in liver and kidney after different duration and doses. GST activity seems to be enhanced in response to the increased free radical production, as the extent of the enzyme activity correspond to extent of LPO recorded in animals exposed to cadmium for different duration. The enzyme glutathione S transferase was found to be increased in the liver during the exposure period.

Exposure on deltamethrin occurred separately as well as combinedly in *C. punctatus*. Shylaja *et al*. (2020) reported that GST consists of a large family of GSH-utilizing enzymes that play an important role in xenobiotic detoxication. Increased GST activity, as observed in the liver and kidney of fish after prolonged exposure to arsenic, can be considered as detoxication process, resulting in increased hepatobiliary excretion of arsenics, as observed in mammalian systems. Proteins are the most important biological material, comprising the nitrogenous constituents of body and performing

different function. Proteins are involved in several physiological functions. Therefore, the assessment of protein content can be considered as diagnostic tool to determine the physiological status of organisms.

In present study, protein level has been found to be decreased significantly in liver after exposure to different concentrations of cadmium. This decreased may be due to inhibition of the metabolism of amino acid and synthesis of protein. Another reason may be depletion of protein for its utilization in conversion to glucose. The loss of protein in different tissues of fish to toxicant exposure is probably due to excessive proteolysis to overcome the metabolic stress. Similar results that is reduction of protein in liver of *C.punctatus* exposed to long term copper sulphate freshwater fish culture has been found (Kumar *et al.*,2023)

Similarly Pichhode *et al.* (2022) showed decreased protein level after sodium arsenite induced stress in catfish*C. batrachus.* Results of the present study revealed significant decline in the level of total protein in the liver which is in agreement with the findings of Lakra *et al.* (2021). Decline in protein level could be related to the possible inhibition of protein synthesis by cadmium because cadmium is reported to act as enzymatic poisons.

Conclusion

Our findings indicate that the antioxidant enzyme assays can be used as a bio indicator for chronic exposure to cadmium in the *C. punctatus.* This could be related to the alterations in antioxidant enzyme activities and other biomarkers of oxidative stress in *C. punctatus* which may cause biochemical dysfunction in this species. In addition, the results provide evidence that enzymatic and non enzymatic biomarkers of oxidative stress can be sensitive indicators of aquatic animals as well as degenerative changes in hepatocytes were shown detrimental effect of cadmium toxicity.

Statements and Declarations

Author Contributions

Conceptualization and Data curation, Rajesh Kumar and **Madhu Tripathi**; Software use and Biostatistical analysis, Sipahee Lal Patel; Writing original draft, Rajesh Kumar; Writing, review and editing, Dinesh Kumar. All authors have read and agreed to the published version of the manuscript.

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