Research Article

Cadmium Toxicity-Induced Changes in Antioxidative Enzyme Levels in Freshwater Catfish Channa Punctatus (Bloch)

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Present study has been planned to observe the effect of Cadmium chloride on the locally available economically important edible fish *Channa punctatus* (body weight 27.04±0.19 g and length 16.7±0.20 cm). To assess the effect of cadmium in terms of enzymatic and non-enzymatic assays, laboratory studies have been done. Fish were acclimatized and treated separately with concentrations of 35 mg/L and 70 mg/L of $CdCl_2$. The livers were dissected out and homogenized in 0.1 M sodium phosphate buffer (pH 7.4). Biochemical analyses were done for lipid peroxidation, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and protein estimations. The results were shown as mean and standard error (Mean ± SEM). The analyses of variance (ANOVAs) were analysed and significantly applied for the Dunnett test for multiple comparisons of groups against the control, which determined the significant differences among the groups. This study is of academic importance and will provide valuable information regarding cadmium toxicity. At the same time, it will address the public health issue as far as consumption of fishes from contaminated water is concerned.

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Introduction

Cadmium is a biologically nonessential heavy metal that has been given great importance from the toxicological and ecotoxicological points of view (Genchi, 2020; Gupta *et al.*, 2022). From its natural

reservoir, this metal is continuously decreased by anthropogenic processes and natural processes and distributed among different environmental compartments, with aquatic ecosystems being important sites for the final disposal of its soluble forms. Cadmium has been listed in the “Black list” of the European Community (Ali *et al*., 2018). Cadmium plays no important role in the biological function of an organism and has a particularly more bioaccumulative 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). It accumulates in the liver and kidneys and has a long biological half-life of 17-30 years in humans (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 (Del et al., 2003). Production of reactive oxygen species and oxidative tissue damage due to Cd have been associated with hepatotoxicity. 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. However, with high-level acute Cd exposure, significant glutathione depletion occurs (Yusuf *et al.,* 2008).

Cadmium occurs naturally in ores together with zinc, lead, and copper. Metallic cadmium has mostly been used as an anticorrosion agent (cadmiation). 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 (Yusuf et al., 2006), producing deleterious effects on flora and fauna by affecting various physiological, biochemical, and cellular processes (Gill *et al.,* 1988; Venugopal and Reddy, 1992; Faverney *et al.,* 2001; Drastichova *et al.,* 2004; Patro, 2006). The major sources of contamination include electroplating, paper, PVC plastic, pigment and ceramic industries, battery, mining, and smoldering units, and many other modern industries (Gupta *et al.,* 2003).

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 conditions, their physiological, biochemical, and behavioral parameters, among others, may become changed (Christopher et al., 2010, Di Giulio and Hinton, 2008; Doving, 1991; Espina *et al.,* 1995, 2000; Shedd *et al.,* 2001), and these alterations can be used as early biomarkers of toxicity. In biological organisms, cadmium binds with enzymes having

sulfhydryl groups, ruptures cell membrane permeability, accumulates in cell organelles, binds with nucleic acids, and also induces endocrine disruption at the hormone level (Anurag et al., 2012, Morsey and Protasowicki, 1990; Jones *et al.,* 2001; Abou El Naga *et al.,* 2005). At high concentrations, it causes adverse changes in the soft tissues of the liver, leading to adverse effects on neurological, behavioural, and physiological functions. 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 the local market of Lucknow city were used for biochemical and enzyme 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 $CdCl_2$ concentrations were taken as 35 mg/L and 70 mg/L. The livers were dissected out and homogenized in 10% (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. Supernatants were taken for analysis of SOD, CAT, GPx, GST, and protein estimations.

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 the 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). The colour intensity of the chromogen was measured at 560 nm. The result was 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 the 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 the GSH conjugate with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm using an extinction coefficient of 9.6 × 103 m-1 cm-1 (Habig et al. 1974). GST activity was expressed as n moles /min/mg of protein.

Protein estimation

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

Statistical analysis

The results were expressed as mean and standard error (Mean ± SEM) and determined for all the parameters. The data were analyzed employing the analysis of variance (ANOVA) using statistical software GraphPad InStat 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)

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 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)	7.602±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

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

Lipid Peroxidation (LPO)

The level of LPO was found to be increased significantly in the low concentration ($P<0.05$) and in the high concentration ($P<0.01$) as compared with the control in both experimental groups (Tables 1 and 2; Fig. 1).

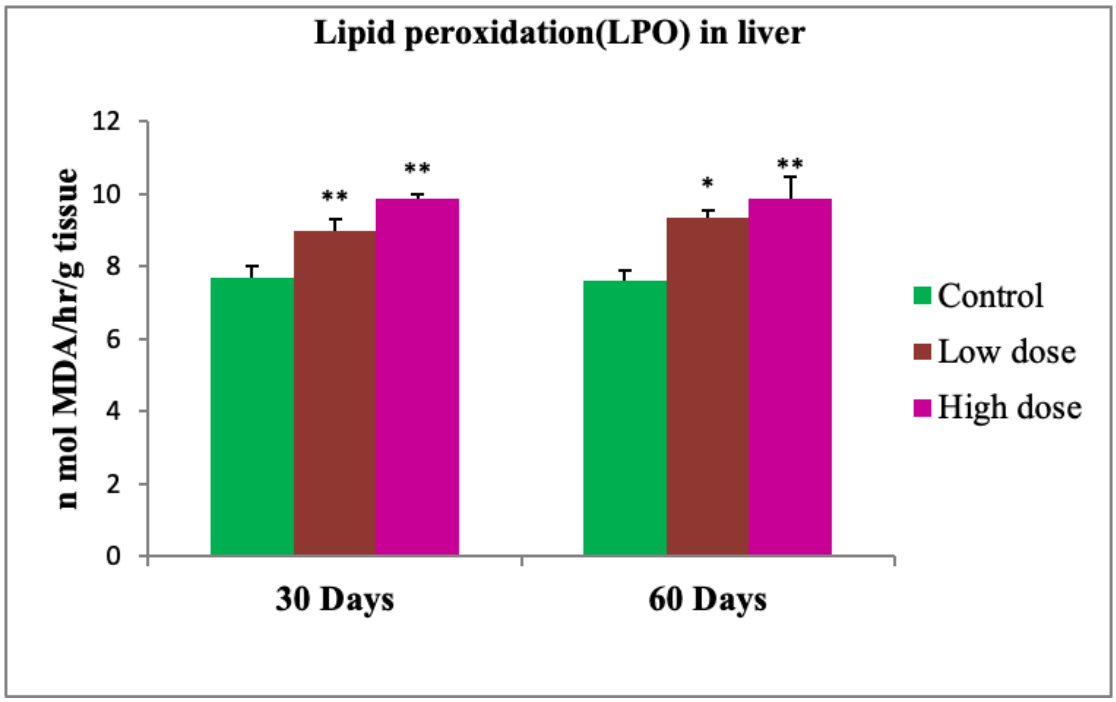

Fig.1. Effect of Cadmium on LPO level in liver of *C. punctatus* after different durations.Values mean ± SEM N=6. P<0.01 ** , P<0.05 * , P>0.05 ns, as compared with Control

Reduced glutathione (GSH)

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

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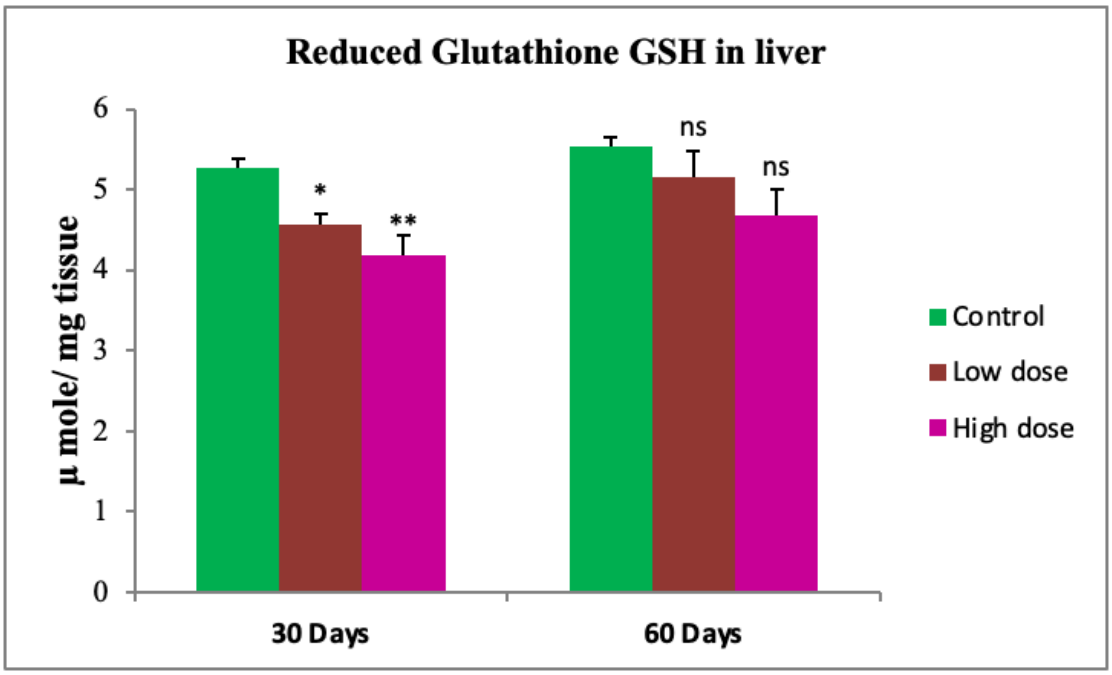

Fig.2. Effect of Cadmium on GSH level in liver of *C. punctatus* after different durations. Values mean ± SEM, N=6. P<0.01 **, P<0.05 *, P>0.05 ns, as compared with control.

Superoxide dismutase (SOD)

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

Catalase (CAT)

After 30 days of 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 the low-dose group (P<0.01) and in the higher-dose group respectively. (Tables 1 and 2; Fig.4).

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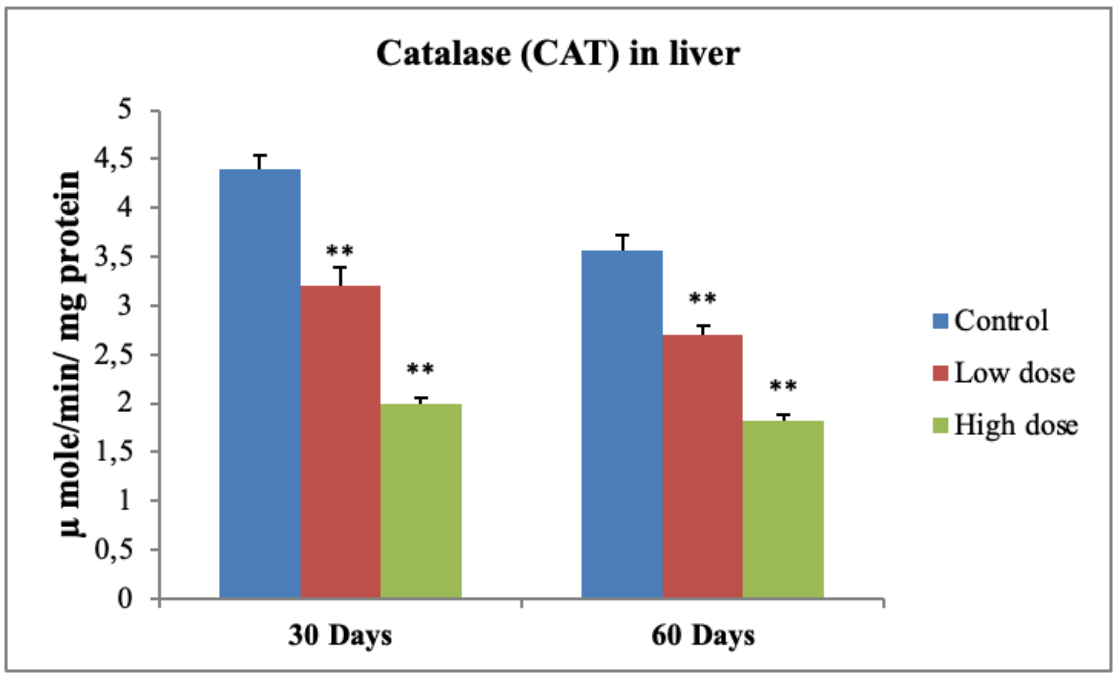

Fig.4. Effect of Cadmium on Catalase level in *liver of C. punctatus* after different durations. Values mean ± SEM N=6. P<0.01 ** , P<0.05 *, P>0.05 ns, as compared with control.

Protein

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

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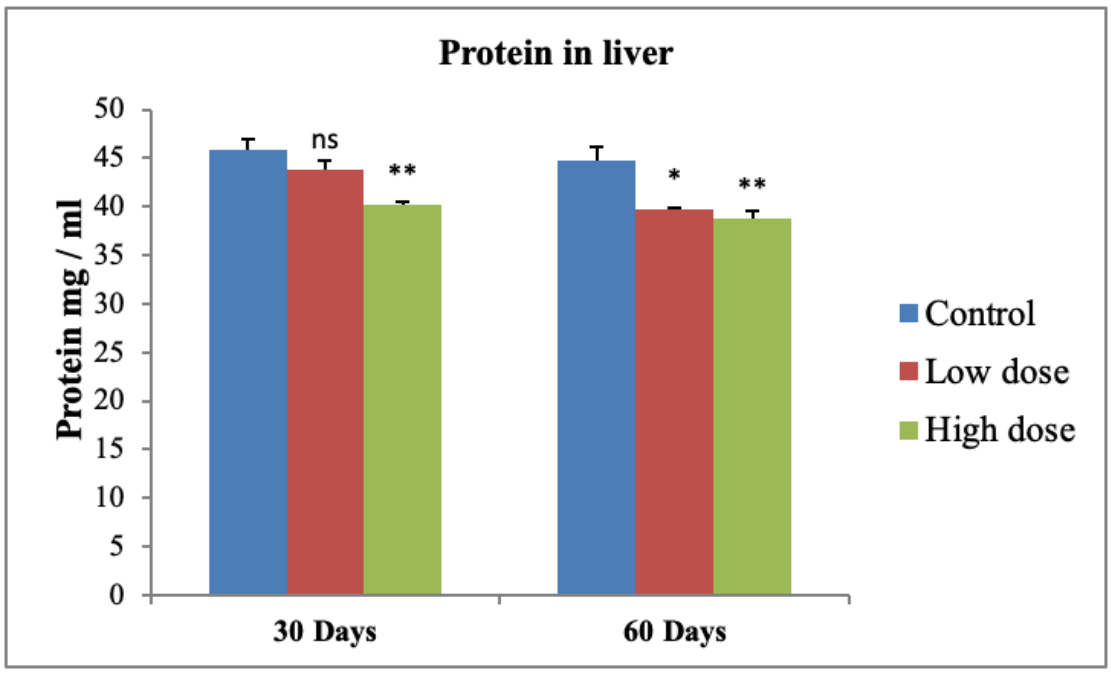

Fig.5. Effect of Cadmium on Protein Level in the Liver of *C. punctatus* after Different Durations. Values: mean ± SEM, N=6. P<0.01 **, P<0.05 *, P>0.05 ns, as compared with control.

The livers of the exposed groups at lower concentrations of cadmium showed a number of degenerative changes in comparison to the control group. 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 vessels, and computer architecture were more prominent. Due to severe degenerative and necrotic changes, the liver lost its normal cellular architecture.

The livers of the exposed group of fishes at higher concentrations showed a number of degenerative changes in comparison to the control group of fishes. After 30 days of exposure, vacuolisation and necrosis were found to be more prominent. Hepatocytes showed indistinct cell boundaries with pyknotic nuclei, and disorganisation of hepatic cord and intravascular haemolysis in the hepatoportal blood vessels were observed. After 60 days of exposure, the cellular architecture was almost lost due to degenerative change.

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Discussion

The values of LPO measured in *C. punctatus* are in agreement with those reported for various fish species (Flohe et al. 1997; Almeida et al. 2004; Pandey et al. 2008). Cd produces an inhibitory effect, and as a response, the respiratory chain becomes highly reduced and the electrons are transferred directly to available oxygen, which induces ROS formation, causing oxidative damage in the liver (Stohs et al. 2000; Miyamoto et al. 2003). Michiels et al. 1994 found that although the glutathione levels in *C. punctatus* were elevated due to Cd exposure, the levels of free radicals increased. Usually, it works in a coordinated manner in order to ensure the optimal protection against oxidative stress (Hermes-Lima, 2004) by scavenging H_2O_2.

Lipid peroxidation is a free radical-mediated process leading to the oxidative deterioration of polyunsaturated lipids. Under normal physiological conditions, low concentrations of lipid peroxides 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, which attack 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 acids, proteins, and carbohydrates (Zhang *et al.,* 2008; Ivon et al., 2015). Peroxidations involve the direct reaction of oxygen and lipid to form radical intermediates and to produce semistable 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 (Banerjee *et al.,* 1999). After initiation, it concurrently proceeds by a free radical reaction mechanism and is regarded as one of the basic mechanisms of cellular damage caused by free radicals (Hfaiedh *et al.,* 2012). It is the presumptive marker for free radicals 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 membranes. The generation of free radicals can be measured by the rate of lipid peroxidation (Hoffmeyer *et al.,* 2009). It is initiated by the obstruction of a hydrogen atom from the side chain of polyunsaturated fatty acids (PUFAs) in membrane lipids. Increased lipid peroxidation alters membrane fluidity and membrane potential and thereby leads to loss of cellular function and cell death (Fong *et al.,* 1973).

In the present study, an elevated level of LPO was observed in the liver and kidneys 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 membranes and enzyme systems. A similar observation has been reported by various workers (Lushchak *et al.,* 2009; Kubrak *et al.,* 2010; Vasylkiv *et al.,* 2010; Velma and Tchounwou, 2010) after chromium-induced oxidative stress in fish. Berntssen *et al.* (2003) and Romeo *et al.* (2000) reported similar results with increased LPO levels in hepatic and ovarian tissues, which affected growth.

In this study, increased LPO levels were observed after cadmium exposure. Such increased LPO levels were also found by previous investigators (Shivarajashankara *et al.,* 2002) in the liver (Chinoy, 2004) and kidney (Birkner *et al.,* 2006). Guney *et al.* (2007) 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 (Niki, 2008). The lipid peroxidation end product, LPO, was significantly increased in the 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 (Wang *et al.,* 2002; Farombi *et al.,* 2007; Hashish *et al.,* 2015). In the present study, lipid peroxidation was increased after cadmium exposure in the kidney. Increased LPO levels in the kidney caused by cadmium have been reported in rats (Guan *et al.,* 2000; Karaoz *et al.,* 2004; Birkner *et al.,* 2006; Blaszczyk *et al.,* 2008; Rao *et al.,* 2009). In the present study, increased LPO levels and modulation in the antioxidant defense system are similarly reported by Anurag *et al.* (2012) in *C. punctatus,* exposed to cadmium.

GSH is an important non-protein cellular thiol that, in conjunction with GPx, plays a regulatory role in cell proliferation (Bhuvaneswari *et al.,* 2001). GSH and GSH-dependent enzymes are involved in scavenging the electrophilic moieties (Michiels *et al.,* 1994). It is known that GSH is one of the most powerful antioxidants in mammals and is essential for normal cell functioning, replication, and the cell signalling system (Exner *et al.,* 2000). It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (Winterbourn, 1995). GSH contains an easily oxidizable sulphydryl residue and behaves as an efficient sink for the ROS (Amores-Sanches *et al.,* 1999). Glutathione (GSH) is a tripeptide that contains an unusual peptide linkage between the amine group of cysteine (which is attached by a normal peptide linkage to a glycine) and the carboxyl group of the

glutamate side chain. It is an important antioxidant, preventing damage to cellular components caused by reactive oxygen species such as free radicals and peroxides (Pompella *et al.*, 2003).

The present study showed decreased levels of GSH in the liver and kidneys of *Channa punctatus* after exposure to cadmium. The reduction in the GSH level may be due to direct conjugation of GSH with electrophilic 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 (Ivan et al., 2015). Cadmium exposure in rats caused a reduction in GSH contents in the liver and kidneys, GSH being a multifunctional nonenzymatic antioxidant. Anurag *et al.* (2015) found that cadmium can alter GSH levels. With increased cadmium concentration, a toxic reaction was induced, and the GSH content was decreased.

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 that prevents damage to cellular components by ROS and peroxides (Pompella *et al.,* 2003). In addition to working as a direct free radical scavenger, GSH also functions as a substrate for GPx and GST. A decrease in the GSH level has already been reported after exposure to the organophosphate methyl parathion in freshwater fish (Monteiro *et al.,* 2006). Reduced glutathione (GSH) is a biomarker of oxidative stress (Pacini *et al.,*2012). GSH has been reported to be consumed in the prevention of free radical-mediated lipid peroxidation (Rahman, 2007). The GSH level was significantly decreased in the kidney after lead intoxication. Lead depletes GSH levels, and several enzymes in the antioxidant defense system protect the imbalance between pro-oxidant and antioxidant. Most of the enzymes contain sulfhydryl groups at their active site and become inactive due to the direct binding of lead to the sulfhydryl group (Flora *et al.,* 2008; Quig, 1998; Hashish *et al.,* 2015). Uner *et al.* (2006) have also reported that different concentrations of dizinon decrease the level of GSH in *O. niloticus*. GSH, one of the major non-enzymatic antioxidants *in vivo,* can protect lipids, proteins, and DNA from oxidation by the superoxide radical and maintain normal life activities by clearing the free radicals generated in the body. In addition, when the animal is exposed to pollutants, the GSH content decreases by an adaptive reaction or the toxic reactions induced by pollutants (Giulio *et al.,* 1989). GSH depletion is generally attributed to an imbalance between pro-oxidant and antioxidant activities inside the cell and overproduction of ROS at the mitochondrial level, leading to the damage of cellular components (Gusev and Agalakova, 2012).

Superoxide dismutase is a class of enzymes that catalyze the binding of ROS with water or the dismutation of the 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. A similar result was found in most investigations after exposure to cadmium (Tribow et al., 2015; Bouraoui *et al.,* 2008). Dabas *et al*. (2012) reported a similar result with the effect of cadmium in freshwater fish *C.punctuatus.* Mukhopadhyay and Chattopadhyay (2014) have reported similar results which indicate increased levels of SOD, CAT, and GST due to the oxidative stress of cadmium. Elif and Uner (2000) reported an increase in the hepatic SOD activity in *O. niloticus* exposed to cadmium that could be due to the induction of free radicals and ROS/RNS by the heavy metal. Antioxidant enzymes (CAT, GST, and SOD) of liver and gills revealed a significant increase in their activities in *O. niloticus* exposed to a sub-lethal concentration of fenitrothion for 30 days (Eman *et al.,* 2013).

In the 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 (Barbiera *et al.,* 2010) and results in the excessive production of ROS at the mitochondrial level, which causes the damage of cellular components (Mittal and Flora, 2006; Zhang *et al.,* 2007; Ghosh *et al.,* 2008; Izquierdo-Vega *et al.,* 2008; Garcia-Montalvo *et al.,* 2009;Iwan et al., 2015).

Similarly, a decrease in SOD in the kidney was reported by (Flora *et al.,* 2008; Hashish *et al.,* 2015) as compared to the control group after lead treatment of fishes. Lead showed inhibitory effects on SOD activities; this leads to impairment of cell antioxidant defence mechanisms, which would render cells exposed to oxidative attacks. A significant increase in MDA levels and decreased activities of superoxide dismutase and catalase in the kidney suggested that oxidative stress mediated toxic effects in cadmium-intoxicated rats (Tribow *et al.,* 2014).

After 60 days of exposure, a dose-dependent inhibition of SOD activities was found, which could be due to its direct toxicity to SOD through its interactions with enzymes, involving its binding with the active sites on the enzymes and its inhibition of the activities of antioxidant enzymes (Garcia-Montalvo *et al.,* 2009; Barbiera *et al.,* 2010).

Catalase is an enzyme that is present in most cells and reacts to the decomposition of hydrogen peroxide to water and hydrogen using either iron or manganese as a cofactor (Chelikani *et al.,* 2004). This portion is localized to peroxisomes in most eukaryotic cells (Del Rio *et al.,* 1992; Buettner, 1993). 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 hydrogen peroxide. The level of CAT in the kidney decreased due to overproduction of free radicals. In contrast, liver and ovary CAT activity in the exposed fish increased as compared to controls both 45 and 90 days after exposure. The alternative explanation for the reduction in CAT activity by cadmium exposure may be related to the 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 H2O2, or effective antioxidant responses due to a higher renovation of the kidney. Similarly, a decreased CAT activity was also observed in the kidney of *C. punctatus* after cadmium exposure (Iwan *et al.,* 2015; Dabas *et al.,* 2012). Similar results with CAT activity have been reported by various workers. Chromium exposure also caused a significant decrease in CAT activities in kidney and gill tissues of *Anguilla anguilla* (Ahmad *et al.,* 2006). Naveed and Janaiah (2011) reported the decrease in CAT activity of the brain and kidney of *C. punctatus* exposed to Triazophos. An indirect antioxidant effect could be responsible for the lower kidney CAT activity; once formed, GSH radicals can further react with other thiol molecules in oxygenated tissues to provide O2. Superoxide dismutase dismutates O2 - to generate H2O2, which can further react with chromium to form a hydroxyl radical (Valko *et al.,* 2006). Lowered CAT activity in the kidney of chromium- and cadmium-treated common carp (Karaytug *et al.,* 2014).

Moreno *et al.* (2014) reported that CAT activity increased in the liver of *C. carpio* after exposure to methomyl. Thus, it is possible that an increase in the activity of these enzymes contributes to the elimination of ROS induced by cells exposed to pesticides (Stara *et al.,* 2012; Ural, 2013). Some other workers also reported increased levels of CAT activity after exposure to pesticides (Ferrari *et al.*, 2007; Nwani *et al.,* 2010; Capkin and Altinok, 2013).Glutathione peroxidase plays a primary role in minimizing oxidative damage.

It is an enzyme with selenium, and glutathione S-transferase works together with glutathione in the decomposition of H_2O_2 or other organic peroxides to nontoxic products at the expense of reduced glutathione (Bruce *et.al.,* 1982). Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme (Hodgson and Fridovich, 1975).

Anurag *et al.* (2012) have reported that the activity of antioxidant GPx and SOD in the kidney and liver after cadmium treatment was decreased significantly as compared with control. Similar findings are reported after exposure to cadmium and chromium by Talas *et al.* (2008). Similarly, the organophosphorus insecticide malathion reduced GPx activity in mice erythrocytes (Yarsan *et al.,* 1999). GPx inhibition was reported after combined treatment with the pesticides 2, 4-D and azinphosmethyl in the brain of *C. carpio* (Oruc *et al.,* 2004) and in the liver of *O. niloticus* (Oruc and Uner, 2000).

Glutathione S-transferases (GST) are a family of enzymes that catalyze the addition of the tripeptide glutathione to endogenous and xenobiotic substrates that have electrophilic functional groups. They play an important role in the detoxification and metabolism of many xenobiotic and endobiotic compounds (Ji *et al.,* 1992). GST plays an important role in cellular detoxification of xenobiotic and toxic products of lipid peroxidation. GST catalyzes the conjugation of reduced glutathione via a sulfhydryl group to electrophilic centres on a wide variety of substrates. This activity detoxifies endogenous compounds such as peroxide lipids, as well as the breakdown of xenobiotics. GST may also bind toxins and function as transport proteins. GST is an abundant cytosolic antioxidant involved in the conjugation of toxic reactive metabolites. The higher tripeptide content is involved in the activation of γ-glutamylcysteine synthetase, one of the enzymes involved in glutathione synthesis (Ding *et al.,* 2000).

In the present study, after cadmium exposure, GST levels increased in the liver and kidney after different durations and doses. GST activity seems to be enhanced in response to the increased free radical production, as the extent of the enzyme activity corresponds to the extent of LPO recorded in animals exposed to cadmium for different durations. The enzyme glutathione S-transferase was found to be increased in the liver during the exposure period. The kidney enzyme activity slowly increased on the successive exposure days (Vinodhini and Narayanan, 2009). Similar results were reported by Atif *et al.,* (2005) in the level of GST in the liver and kidney after cadmium.

Exposure to deltamethrin occurred separately as well as combinedly in *C. punctatus*. Allen and Rana (2004) 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 a detoxication process, resulting in increased

hepatobiliary excretion of arsenic, as observed in mammalian systems (Lee *et al.,* 1989, Gyurasics *et al.,*1991).

Elevation in GST activity in the liver was found in *Lepomis macrochirus* exposed to atrazine (Elia *et al.,* 2002). Pena-Llopis *et al.* (2003) reported that dichlorvos treatment causes an increase in the GST activity in the liver of *Anguilla anguilla.*Increased GST activity in tissues may indicate the development of a defensive mechanism to counteract the effects of methyl parathion and may reflect the possibility of a more efficient protection against pesticide toxicity.

Proteins are among the most important biological materials, comprising the nitrogenous constituents of the body and performing different functions. Proteins are involved in several physiological functions. Therefore, the assessment of protein content can be considered a diagnostic tool to determine the physiological status of organisms.

In the present study, protein levels have been found to be decreased significantly in the liver after exposure to different concentrations of cadmium. This decrease may be due to inhibition of the metabolism of amino acids and synthesis of proteins. Another reason may be depletion of protein for its utilization in the conversion to glucose (Sirvastava *et al.,* 2002). Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms (Singh and Sharma, 1998). The loss of protein in different tissues of fish to toxicant exposure is probably due to excessive proteolysis to overcome the metabolic stress. Almeida *et al.* (2001) reported that there was a decrease in total protein content in the liver of *O. niloticus* after cadmium exposures. He considered that this decrease may be due to protein reserve depletion induced by cadmium treatment, liberating amino acids for gluconeogenesis. Selamoglu *et al.* (2012) reported that total protein was decreased after treatments with carps exposed to arsenic.

Similar results, such as reduction of protein in the liver of *C.punctatus* exposed to long-term copper sulphate (Atif *et al.,*2005; Mastan, 2008), and cadmium chloride in different tissues of the freshwater fish *Ophicephalus striatus* (Bais and Lokhande, 2012), have been found. Tilaks and Rao (2003) reported a decrease in protein content in *C. punctata* exposed to sublethal concentrations of fenvalerate; similarly, total proteins were also reported in the liver. Total protein content declined in different tissues of *C. puntatus* as reported by Atif *et al.,* 2005. Cadmium is known to decrease protein synthesis in the kidneys of mice and rats (Chinoy *et al.,* 2000; Birkener *et al.,* 2006; Rao *et al.,* 2009).

Similarly, Kumar and Banerji (2012) showed decreased protein levels after sodium arsenite-induced stress in the catfish *C. batrachus.*The results of the present study revealed a significant decline in the level of

total protein in the liver, which is in agreement with the findings of others (Shashi *et al.,* 1987, 1994; Kathpalia and Susheela, 1978). Kausal et al. (2011) have reported similar findings in *C.puntatus* administered who have reported their findings after cadmium exposure. The decline in protein levels could be related to the possible inhibition of protein synthesis by cadmium because cadmium is reported to act as enzymatic poison.

Conclusion

Our results indicate that antioxidant enzyme assays can be used as a bioindicator for chronic exposure to cadmium in *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 nonenzymatic biomarkers of oxidative stress can be sensitive indicators of aquatic animals.

Statements and Declarations

Author Contributions

R.K., S.S.Y., and M.T. contributed equally to this work.

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Declarations

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