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Toxicity Effect on Biochemical and Stress Markers Alterations in Liver and Kidney from Cadmium Nanoparticles Exposed Fish, *Catla catla*

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Abstract

Nanometal contamination in the ecosystem is proven by previous research studies. Nanometal persistence and toxicity is differed from heavy metal toxicity. In the present study, cadmium nanoparticles (CdNP) were exposed to *Catla catla* and subsequently the biochemical changes in the fish were recorded. Acute lethal toxicity (LC_{50}) of cadmium nanoparticle to *Catla catla* fish was determined as 50ppm. The fishes were exposed to sublethal concentration of 20ppm of cadmium nanoparticles for 15 days continuously and the biochemical parameters such as protein, carbohydrate and lipids were estimated. The total carbohydrate, protein and lipids in liver and kidney were decreased in their level after exposing the cadmium nanoparticles. Moreover, the level of antioxidant enzymes such as catalase and superoxide dismutase in liver and kidney were increased at beginning days of CdNP exposure and significantly reduced after 10 days of cadmiuim nanoparticle exposure. Metabolic enzymes aspertate aminotransferase, alanine aminotransferase, lactate dehydrogenase and malate dehydrogenase were significantly decreased when compared to control. The present study clearly demonstrated the biochemical alteration upon cadmium nanoparticles exposure in *Catla catla*.

Keywords
Biochemical changes, Cadmiun, *Catla catla*, Nanoparticle toxicity, Stress markers.

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INTRODUCTION

Nanotechnology is an emerging field in different branches of science. The size range and high surface to volume ratio are the main reason for wide application of nanoparticles [1]. Different nanoparticles majorly used in the medical science due to the excellent physical and chemical properties such as stability, electrical conductivity, catalytic and

antibacterial activity [2]. Various forms of nanomaterials such as carbon sphere (C60), metal nanoparticles (AgNPs), metal oxides (SiO_2) and composite made of several metals (quantum dots) are being used in the different field, clinical, electronics, diagnosis, etc. Since usage of nanoparticles is increased, hence the production of nanoparticles is also booming. Previous studies have been reported that products containing nanometal [3] and its presence in the environment [4, 5]. Recent techniques clearly exemplified the nanometal contamination in the environment [6]. Hence the accumulation nanomaterial in the eco-system should be checked for their role in environmental toxicity. In the case of nanometals, the toxicity mechanism described based on their physio-chemical properties such as size and surface charge [7]. These nanoparticles have a tendency to aggregate with one another. The aggregation property of nanoparticles might helpful for predicting the effective interaction with the organism [8]. The toxicity of nanoparticles in the aquatic eco-system and living organism has been reported earlier [9].

The entry of nanoparticles in fishes through the use of ion transporters or by endocytosis. The nanoparticles are deposited in the liver of fishes. Aquatic metal exposure may cause the direct effects on gill functions such as osmo-regulatory or respiratory dysfunction of the gill. Metllothionin accumulation in the gill, ion-channel disturbance followed by loss of bronchial ion-regulatory control, efflux of electrolytes from the organs subsequently heart arrest and death [10]. The gills may excrete the metal ions from the body circulation by active efflux ion transport pathways [11]. Copper nanoparticles inhibits the Na^+/K^+ ATPase and the following the disturbance of ion balance in zebrafish juveniles and adults [12]. Selenium nanoparticles induce the stress in fish and lead to changes in biochemical marker level and causes the damages in liver tissues [13]. Generally, the metal accumulation in fish causes the stress condition followed by free radical generation further damaging the DNA. The anti-oxidant enzymes such as catalase and superoxide dismutase acting on hydrogen peroxide and oxygen radicals respectively [14, 15]. Moreover, other enzymes Lactate Dehydrogenase (LDH) and Malate Dehydrogenase (MDH) show the biochemical changes during the stress condition in the fish upon nanoparticles exposure [13]. Hence the examination of these enzymes in the specific tissues of fish may help to exemplify the stress related homeostatic condition during the exposure of nanoparticles.

Commonly occurring heavy metal contamination in water at Agniar estuary revealed that in terms of increasing concentration of Ar > Pb > Cd > Cr > Cu > Zn > Fe > Mn [16]. The order of heavy metals Cadmium (Cd), is classified as one of the most toxic heavy metals and a common environmental contaminant [17, 18], it has also been reported to contaminate the freshwater environment and accumulation in fishes [19] Cadmium nanoparticles are used in electronics, paints, drug delivery, diagnosis, etc. There was no *in-vivo* studies in biochemical changes in fishes upon exposure of cadmium nanoparticles. Thus, the present study was undertaken to investigate the toxicity of cadmium nanopartcles (CdNP), in the freshwater fish *Catla catla* which has a wide distribution in the freshwater environment, abundance of the fish throughout the year, can be easily acclimatize under laboratory conditions and importantly because of its economic importance it is inevitable to study the toxicity and biochemical studies of this species.

MATERIAL AND METHODS

1.Animal maintenance

The live specimens of *Catla catla* (10.0 ± 0.50cm in length and 11.0 ± 1.2g in weight) were purchased from aqua farmers in Poondi, Tamilnadu, India. Fishes were introduced invariable of their sex in cement aquaria of 100 liter's capacity. All the fishes were fed with pellets and acclimatized in a laboratory conditions at 28 ± 2^oC under the 12h:12h (light: dark) conditions. The tank water was top upped with fresh water by removing the old water on a daily basis.

2.Preparation of stock solution and determination of LC_{50} value of CdNPs

The acute semistatic toxicity test was followed by *Food and Agriculture Organization* (FAO) and the American Public Health Association (PUFA). Different concentration (20, 40, 60, 80 and 100ppm) of cadmium nanoparticles (100nm) was mixed intraperitoneal per kg of fish weight. Major Indian carps *Catla catla* was acclimatized in water tanks and the temperature was maintained at 27°C. Water was changed daily and aquaria were cleaned thoroughly and fish were fed with commercial fish feed. After acclimatization, healthy fishes with a homogeneous size (width 14-16cm, weight 200-300g) were selected. A 12 fishes were kept for each concentration nanoparticle and test was replicated. Suitable control also maintained. Mortality of fishes was recorded at the end of 24 hrs. The median lethal concentration was noted as 50ppm at which concentration of CdNP, 50% of fishes were dead. For

sub-lethal studies, 20ppm was taken as the sublethal concentration.

3.Cadmium nanoparticle treatment

The fishes were divided into four groups (one control and three experiment) with 15 fishes in each tank. A 20ppm of CdNP was added into experimental groups. Experiment was conducted for 15 days and the bio-chemical analysis were done for 5 day's intervals. The nanoparticle was exposed daily in order to maintain the constant concentration of toxicant after removal of same volume of water. At the end of 1, 5, 10 and 15th day the liver and kidney were excised from randomly selected experimental fishes.

4.Protein, lipid and carbohydrate estimation

The total amount of protein in liver and kidney was determined followed by Bradford [20] with Bovine Serum Albumin (Sigma Chemicals Co., USA) as a standard. The amount of lipid in liver and kidney was estimated by gravimetric method [21]. The total carbohydrate was determined in liver and kidney followed by Roe [22]. A 10% homogenate of tissue was prepared using 5% TCA and the results were expressed as mg/g of wet tissue.

5.Bio-marker status

Catalase activity was determined followed by Takahara et al [23]. Superoxide dismutase activity was measured based on the oxidation of epinephrine-adrenochrometer transition by the enzyme [24]. The reaction mixture was incubated at 37°C for 60 min to measure the Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities by the estimation of oxaloacetate and pyruvate released, respectively [25]. Lactate dehydrogenase was assayed using 0.1 M phosphate buffer (pH 7.5) and 0.2 mM NADH solution in 0.1 M phosphate buffer. The reaction was initiated by the addition of substrate 0.2 mM sodium pyruvate and absorbance was recorded at 340 nm [26]. A similar reaction mixture was used for the estimation of malate dehydrogenase (MDH; L-malate: NAD + oxidoreductase) [27].

6.Statistics

The data were statistically analyzed by Statistical Package for the Social Sciences version 16 (SPSS). The one-way ANOVA (Analysis of Variance) was used to observe the treatment effect.

RESULTS AND DISCUSSION

In the present research work carried out on the effect of cadmium nanoparticles on biochemical parameters in *Catla catla*. Lethal concentration of CdNP was determined by acute toxicity assay. It was revealed that the LC_{50} value of CdNP was 50ppm in *C. Catla*. The acute toxicity and lethal concentration (LC_{50}) is widely accepted procedure in worldwide to study the toxicology and ecotoxicology of substances. Lethal concentration defined as the substance to produce the specific effect at a particular dose to cause the 50% mortality of the experimental animals in a fixed duration. Some other nanoparticle lethal concentration also reported in earlier studies. LC_{50} value of Ag-NPs for Japanese medaka (*Oryzias latipes*) as 34.6 ppm [28]. Commercially available Ag-NPs causes the mortality at 96-h with concentration of 1.25 ppm for fathead minnow (*Pimephales promelas*) [29]. Selenium nanoparticles causes the 50% mortality at 3.9ppm for 96 hrs. of exposure in *Pangasius hypophthalmus*. [13].

The biochemical changes occurrence in the body of the organisms gives first indication of stress in aquatic organisms due to heavy metals. The biochemical parameters such as protein, carbohydrates and lipids were evaluated upon the CdNP exposure in *C. catla*. The protein, lipid and carbohydrate concentration in liver and kidney were analyzed. The carbohydrate concentration in liver was declined at the end of the 1st day of exposure (64.3mg/g of wet issue) when compared to control group (68.6mg/g of wet tissue). On the 10th &15th day of CdNP exposure, the carbohydrate level was significantly decreased (63.1mg and 56.9 mg/g of wet tissue respectively) (Fig.1a). Similarly, the carbohydrate level in kidney was declined from the first day of CdNP exposure (Fig. 2a). At the 15th day of toxicity studies the concentration of carbohydrates in kidney was significantly (p<0.05) reduced (9.6mg/g of wet tissue) when compared to control (16.9 mg/g of wet tissue). Carbohydrates are storage molecules in the liver. Depletion of carbohydrate in the tissues is an indication of typical stress response in fish challenged with metal toxicity [30]. Exposure to chronic toxicity of heavy metal cadmium in the fingerlings of the fish *C. catla* caused changes in the carbohydrate level, which may be attributed to toxic stress, resulting in the disruption of enzyme associate with carbohydrate metabolism.

Interestingly, the protein concentration in liver was increased at the end of 1st day CdNP exposure (144.8mg/g of wet tissue) when compared to control (130.2 mg/g of wet tissue). But in final days of exposure the protein level was diminished in liver (Fig. 1b). On the 10th and 15th day of exposure, the protein level was significantly decreased (119.9mg and 104.7mg/ g of wet tissue). Comparative results were obtained in kidney protein quantity analysis (Fig. 2b). At the end days of CdNP exposure the total protein was significantly decreased in their level

(101.23mg/g of wet tissue) when compared to control (124.6mg/g of wet tissue). The higher expression of stress enzymes might be the reason for sudden increment level of protein. Protein being the essential substance is desirable for enlargement, growth and also serves as an energy source during the stress condition. One of previous study reported that silver nanoparticles decrease the total protein the fish *Mystus gulio* [30]. It might because of the higher dosage and toxicity of silver nanoparticles

Figure 1c showed the lipid concentration in liver significantly decreased (42.1mg/g of wet tissue) at the 15th day of CdNP exposure when compared to control (60.3mg/g of wet tissue). In the case of kidney lipid content was decreased from the 1st day of CdNP exposure. At the 15th day of exposure the lipid (13.2mg/g of wet tissue) level was decreased when compared to control (21.8 mg/g of wet tissue) (Fig. 2c). Lipids are in general triglycerides that can dish up as metabolic reserves. Phospholipids show a quick diminish given that it might aggressively degrade due to the CdNP stress [31].

The anti-oxidant status in *C. catla* was examined after exposure of CdNPs. The activity of anti-oxidant markers such as catalase in liver and kidney (18.28 U/ml and 11.12 U/ml respevtively), superoxide dismutase in liver (47.15 U/ml) and kidney (32.01 U/ml) level was increased at the end of first day of CdNP exposure when compared to control (Table 1). At subsequent exposure of CdNP the activity of anti-oxidant was decreased at significant level (p<0.05). Kumar et al. [13] reported that heavy metal contamination causes higher oxidative stress in the form of reduced level of catalase and SOD. Similarly, the activity of other metabolic enzymes such as aspartate aminotransferase in liver (18.9 U/ml) and kidney (9.36 U/ml) while alanine aminotransferase (in liver, 11.25 U/ml; kidney, 9.15 U/ml) also significantly decreased on 10 and 15 days of nanometal exposure (Table 2). Lactate dehydrogenase from liver and kidney showed a reduced level (1.02 U/ml and 1.49 U/ml respectively) when compared to control (Table 3). MDH in liver as 0.25 U/ml and kidney as 0.42U/ml also reduced after cadmium nanoparticles exposure (Table 3). Suppression of this enzyme progressed from day 1 to day 15 in all the organs of the fish exposed to cadmium. Aminotransferases (AST and ALT) are a sensitive marker enzymes of liver and they are good indicators of liver damage. Their serum concentration will increase when the toxicity influences the cytosol subsequent breakdown in membrane integrity of the cells [32]. As dehydrogenases are oxidative enzymes involved in Kreb's cycle, any disturbance in this enzyme activity will affect the Kreb's cycle. Since this cycle represents a central oxidative pathway for carbohydrates, fats and amino acids, if there is any disturbance in this cycle the whole metabolism is likely to be affected [33]. This leads to impairment of oxidative metabolism in the mitochondria as a consequence of hypoxic conditions under CdNP exposure, most probably by disrupting the oxygen binding capacity of the respiratory pigment. Histological studies upon CdNP exposure in fish *Catla catla* showed cell damages in previous studies [34]. Thus the results of this study clearly exemplified the biochemical alterations in *Catla catla* fish exposed to cadmiun nanoparticles.

Table 1 The effect of CdNP on the activity of anti-oxidant markers catalase and superoxide dismutase extracted from the liver and kidney at different days of exposure in *C. catla*

Days of CdNP exposure	Catalase				Superoxide dismutase (SOD)			
	Liver		Kidney		Liver		Kidney	
	Control	Test	Control	Test	Control	Test	Control	Test
1	15.96±0.56	18.28±0.32	9.86± 0.22	11.12±0.14	45.32±0.89	47.15±0.96	31.6± 0.44	32.01±0.32
5	16.02±0.43	15.83±0.14	9.85±0.71	8.59±0.97	45.62±0.72	44.01±0.51	32.1±0.65	31.62±0.56
10	16.28±0.54	13.6±0.37*	9.86±0.81	6.62±0.87*	45.31±1.05	41.98±1.28*	33.09±0.98	30.15±0.78*
15	17.08±0.65	10.46±0.36*	10.37±0.4	4.36±0.98*	46.87±1.85	38.02±1.47*	33.91±1.07	27.41±094*

Values are mean± SD of 5 individual observations. * values are significant at $p< 0.05$

Table 2 The effect of CdNP on the activity of aspartate aminotransferase and alanine aminotransferase extracted from the liver and kidney at different d ays of exposure in *C. catla*

Days of CdNP exposure	Aspartate aminotransferase				Alanine aminotransferase			
	Liver		Kidney		Liver		Kidney	
	Control	Test	Control	Test	Control	Test	Control	Test
1	27.74±0.32	26.35±0.98	18.12±0.79	17.98±0.68	26.85±0.59	19.08±0.63	19.08±0.41	18.23±0.37
5	27.35±0.91	24.91±0.36	18.34±0.77	15.49±0.81	27.06±0.42	16.02±0.29	19.24±0.36	15.64±0.51
10	29.34±0.54	18.9±0.35*	19.94±0.62	9.36±0.12*	30.02±0.51	11.25±0.18*	20.65±0.46	9.15±0.13*
15	29.87±0.85	15.24±0.75*	20.48±0.86	6.59±1.08*	32.48±0.48	8.31±1.64*	21.94±0.86	7.39±0.81*

Values are mean± SD of 5 individual observations. * values are significant at $p< 0.05$

Table 3 The effect of CdNP on the activity of metabolic enzymes LDH and MDH extracted from the liver and kidney at different days of exposure in *C. catla*

Days of CdNP exposure	Lactate Dehydrogenase				Malate Dehydrogenase			
	Liver		Kidney		Liver		Kidney	
	Control	Test	Control	Test	Control	Test	Control	Test
1	4.85± 0.78	4.03±0.62	5.48± 0.79	5.40±0.74	0.55± 0.08	0.55±0.16	1.33± 0.44	1.3±0.26
5	4.9±0.88	3.65±0.84	5.51±0.85	4.36±0.51	0.55±0.1	0.43±0.07	1.46±0.83	0.9±0.16
10	4.9±0.54	1.02±0.37*	5.5±0.89	1.49±0.86*	0.55±0.06	0.25±0.49*	1.45±0.23	0.42±0.07*
15	5.26±0.84	0.8±0.21*	6.88±0.82	0.86±0.05*	0.69±0.12	0.17±0.09*	1.58±0.48	0.37±0.09*

Values are mean± SD of 5 individual observations. * values are significant at $p< 0.05$

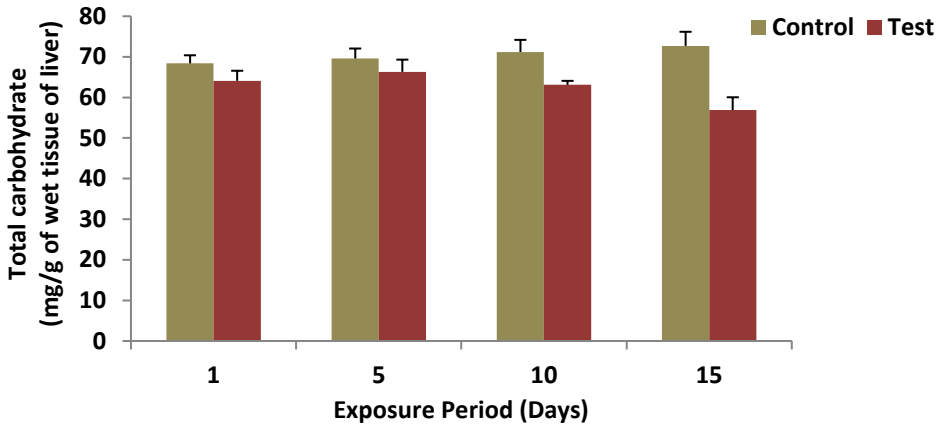

Fig. 1a: Total amount of carbohydrate in the liver after CdNP exposure in fish *C.catla*

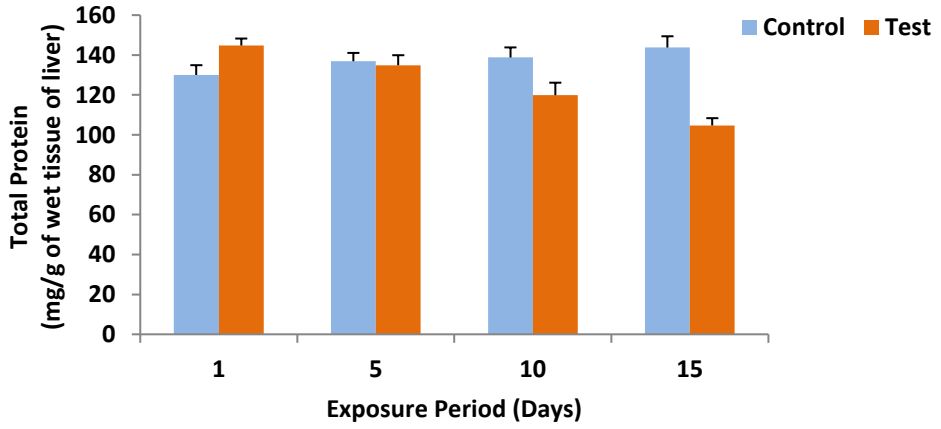

Fig. 1b: Total amount of protein in the liver after CdNP exposure in fish *C.catla*

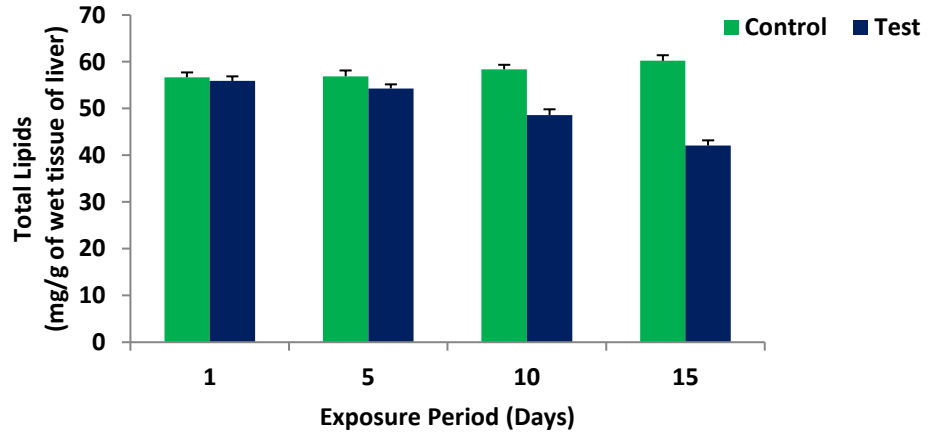

Fig. 1c: Total amount of lipids in the liver after CdNP exposure in fish *C.catla*

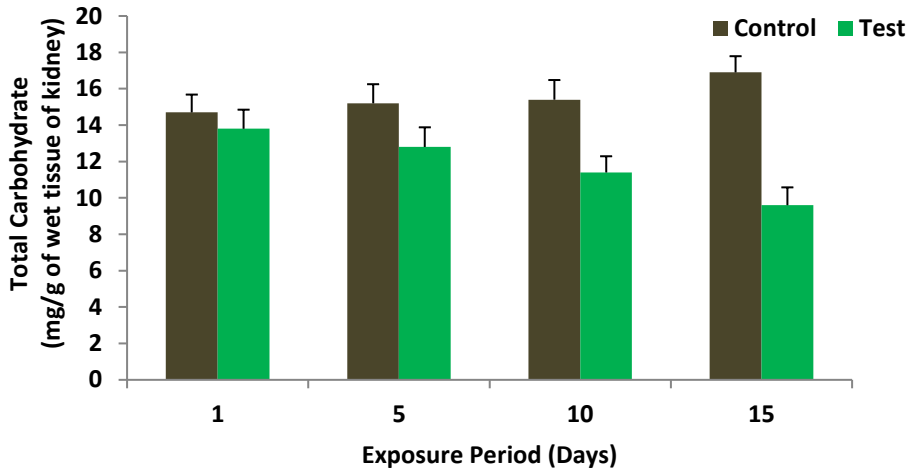

Fig. 2a: Total amount of protein in the kidney after CdNP exposure in fish *C.catla*

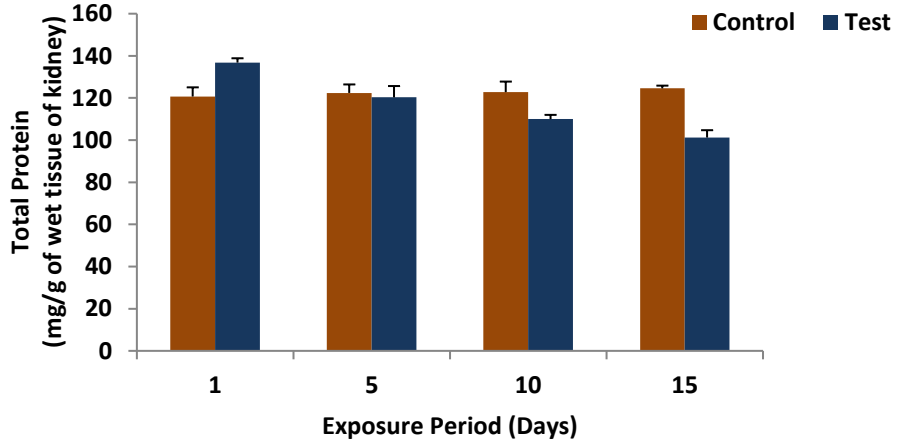

Fig. 2b: Total amount of protein in the kidney after CdNP exposure in fish *C.catla*

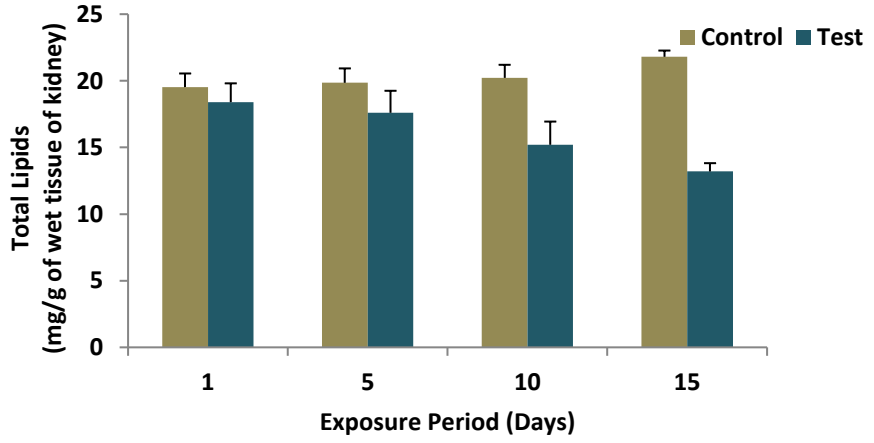

Fig. 2c: Total amount of lipids in the kidney after CdNP exposure in fish *C.catla*

CONCLUSION

As previously said the nanoparticle toxicity was differed from macro heavy metal toxicity. A 20ppm of cadmium nanoparticle made the influence in the biochemical parameters in the *Catla catla* fish. The overall reseearch work concluded that the nanometal contamination in aquatic system had a hazardous effect on the fishes and it might be to other aquatic animals also. The specific effect of nanoparticle may be differing on organism depends on characters of nanoparticles. Aquatic discharge of nanoparticles leads to bio-magnification in other eco-system. The studies to be needed to discharge the nanoparticle in the environment or surface alteration in the nanoparticles to prevent the interaction with living being and longer persistence in the eco-system.

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