



## Physio-biochemical responses of Indian major carp *Catla catla* upon sub-chronic exposure to tin oxide nanoparticles

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### ABSTRACT

Nanostructure metal oxides such as tin oxide nanoparticles due to its unique properties have been used as a photocatalyst in semiconductor industries, building optoelectronic devices and numerous other industries. However, the toxicological impact of these SnO<sub>2</sub> nanoparticles is largely yet to be studied, particularly in the aquatic ecosystem. In this direction to evaluate the ecological impact of the SnO<sub>2</sub> nanoparticles on aquatic organisms, selected *Catla catla*, freshwater fish was selected to examine the hematological and biochemical variations upon treatment with SnO<sub>2</sub> nanoparticles. In the present study LC<sub>50</sub> of SnO<sub>2</sub> NPS was found to be 20 mg l<sup>-1</sup> observed for 24 h. Further 1/10<sup>th</sup> of the LC<sub>50</sub> concentration of SnO<sub>2</sub>nps (2 mg l<sup>-1</sup>) was selected for sublethal investigation for 4-20 days with an interval of 4 days. The results showed alterations in hematological parameters with a decrease in Hemoglobin (Hb), Hematocrit test (Hct %), red blood cells (RBC) and white blood cells (WBC), while there was an increase in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values on all days. Further evaluation demonstrated an increase in serum lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) levels compared to control groups. Studies revealed alterations in oxidative stress markers with a significant reduction in the serum superoxide dismutase (SOD) and catalase (CAT) levels, and an increase in malondialdehyde (MDA) and decrease in gill Na<sup>+</sup>K<sup>+</sup>ATPase concentration. The studies also found increased brain glutamate concentrations indicating possible brain tissue damage. The study highlights the toxicological impact of SnO<sub>2</sub>nps and the role of certain potential biomarkers which reflect the impact of the toxicants in the aquatic environment.

### INTRODUCTION

Nanoparticles and nanostructure metal oxides exhibit properties like decreased particle size resulting in reduction of semiconductor band size, which is not seen in bulk materials, their application is more than ever expanding in science (Rashmi *et al.*, 2019). Tin oxide nanoparticles (SnO<sub>2</sub>) NPs have been used in many industries including

transistors, photovoltaic devices, optics, liquid crystal displays, photo sensors, magnetic data storage, magnetic resonance imaging and so on. The above applications of the SnO<sub>2</sub> NPs are predominantly processed because of its structural characteristics (Jin et al., 2015). In this regard array of SnO<sub>2</sub> NPs are synthesized by different methods to replenish the need of technological advancements. Tin is found in two different oxidation states +2 and +4, which results in the derivation of as many different oxides, namely stannic oxide (SnO<sub>2</sub>) and stannous oxide (SnO). However, SnO<sub>2</sub> is more stable than SnO, which provides the reason for its extensive application. There are various reports (Li et al., 2013) attributing to the structural uniqueness of SnO<sub>2</sub> NPs, thus resulting in their wider applications like in electrodes, gas sensors, liquid crystal displays, optoelectronic devices, anti-reflecting coatings in solar cells and others. The increased use of these SnO<sub>2</sub>Nps intensifies the danger of depositing the e-wastes in the environment, which provides for the malevolent interaction of these NPs with diverse ecosystems.

Many studies have reported toxicity due to metal oxide nanoparticles, which include Cobalt oxide nanoparticles, CuO NPs and ZnO NPs to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*(Heinlaan et al., 2008). Earlier studies have reported genotoxicity and alterations in cellular morphology when exposed to SnO<sub>2</sub> NPs (Roopan et al., 2014). Studies have also revealed alterations in metabolic quotient, signifying microbial stress in soil bacteria when exposed to SnO<sub>2</sub> NPs (Vittori et al., 2013). The toxicological impact of NPs is attributed to its size and structure, for instance ZnO NPs with comparatively lesser size to the bigger NPs were shown to exhibit higher toxicity in biological models. The exposure of larvae of *Artemia salina* to SnO<sub>2</sub> NPs, CeO NPs and Iron oxide (III) NPs have shown variations in mortality, behavior and biochemistry in the development phase (Gambardella et al., 2014). Although many reports are available regarding the toxicological impact of metal oxide nanoparticles, very limited data is available related to effect of ecotoxicity of SnO<sub>2</sub> NPs, particularly in aquatic environment. In this regard, with escalating use of nanoparticles due to their properties and their introduction to industrial and non-industrial waste water, which further might enhance the chance of entering the natural aquatic ecosystem, make it essential to understand the possible toxic effects of these NPs.

In this study fish model was adopted as the tool to evaluate the toxic effect of SnO<sub>2</sub> NPs. The study is further supported by adoption of such models in evaluating the early phases in drug development and their toxicity impact. In the present study we have used *Catla catla*, an Indian major carp, a common inhabitant and widely used for consumption as the experimental animal. Due to their abundance in the aquatic ecosystem, carps are identified as possibly the most appropriate models to measure the changes in potential biomarkers such as antioxidant enzymes and physiological indices. Haematological parameters such as Hb, RBC, WBC and other indices were evaluated to determine the effect of SnO<sub>2</sub> on blood. Transaminases such as alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) specifying tissue damage were also measured in the current study. Antioxidant enzyme Superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation by measuring malondialdehyde (MDA) levels were reported to indicate the extent of reactive oxygen species (ROS) generation and degree of the cellular damage. Since the gills are the first line of contact to external substances, gill Na<sup>+</sup>K<sup>+</sup>ATPase were also measured to identify the extent of toxicity. Glutamate levels were also evaluated to demonstrate possible brain tissue damage due to neurotoxicity. On

the whole we designed to evaluate the physio-biochemical and neurochemical responses of *Catla catla* when exposed to tin oxide nanoparticles during short term exposure.

## MATERIALS AND METHODS

### *Animals and Treatments*

Indian fresh water major carp, *Catla catla* ( $6.6 \pm 0.5$  cm of body length and  $5.7 \pm 1.5$  g of weight) were procured from local fish farm and were stored in flow through tank in dechlorinated water for 15 days. Water quality was determined and found to be  $30.6 \pm 2.3$   $\text{CaCO}_3 \text{ mg l}^{-1}$  of hardness and a pH of  $7.2 \pm 0.7$ , at a constant temperature of  $22 \pm 1^\circ\text{C}$  with a light dark cycle of 12:12 h. Throughout this phase the fishes were provided with commercial fish food. Debris and residues were cleaned once every 24 h. The fishes to be treated were divided into three groups of 80 each which were placed in 100L aquarium with continuous aeration along with separate tank for control group.

### *SnO<sub>2</sub>NPs sample preparation and acute toxicity evaluation*

$1 \text{ g l}^{-1}$  of test solution was prepared by adding SnO<sub>2</sub>NPs in double distilled water and was kept for dissolving in a sonicator for 10 hr (Biotechnics). The test samples were further sonicated for 90 min before inoculation each day. Sedimentation of NPs was reduced by allowing aeration to the sample, which held them in suspension form. However, a small fraction of SnO<sub>2</sub>NPs were found to form aggregates. The groups of fishes were exposed initially to different concentrations of SnO<sub>2</sub>NPs for short period of 96 hr (acute toxicity). We observed the mortality under dose dependent concentrations (100, 80, 60, 40 and 20  $\text{mg l}^{-1}$ ). The LC<sub>50</sub> of SnO<sub>2</sub>NPs was considered at 20  $\text{mg l}^{-1}$  concentration at the end of 96 h.

### *Sample collection*

Cardiac puncture technique was used to draw blood from the fishes. The syringes were pre-coated with heparin, an anticoagulant. The brain tissue samples for evaluation of glutamate, a neurotransmitter, was quickly dissected and placed in ice for analysis.

### *Haematological measurements*

RBC and WBC were measured using Neubauerhaemocytometer. Cyanomethemoglobin method (**Drabkin, 1946**) was used to measure the amount of Hb content in blood. Hct percentage was derived by using the microhematocrit methodology (**Nelson and Morris, 1989**). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were calculated.

### *Biochemical parameters measurement*

Blood glucose level was measured by preparing a reaction mixture of 0.1 ml of plasma, 4 ml of o-toluidine (**Cooper and McDaniel, 1970**), which was then mixed and incubated for 10min on a boiling water bath. The samples were then cooled and the absorbance was read at 630 nm in a UV spectrophotometer. The glucose concentration was expressed in  $\text{mg l}^{-1}$ . Plasma protein concentration was assessed by lowry's method (**Lowry et al, 1951**). 100 $\mu\text{l}$  of plasma, 900 $\mu\text{l}$  of double distilled and 4ml of Lowry reagent was mixed well and incubated for 10min at room temperature, to this reaction mixture 0.5ml of FC reagent was added again incubated for 20 in RT. The sample was then measured at 720nm and expressed in  $\mu\text{g/ml}$

### *Analysis of serum transaminases and gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity*

Serum L aspartate aminotransferase (L-AST) and L-alanine aminotransferase (L-ALT) activities were determined at  $37^\circ\text{C}$  by colorimetric method of Reitman and Frankel (1957) and enzyme activity was expressed as IU/L. Lactate dehydrogenase (LDH) was

determined by the method developed by Anon (1984) and obtained activity was expressed in  $\text{IUL}^{-1}$ . Gills  $\text{Na}^+/\text{K}^+$ -ATPase activity was performed by Shiosaka *et al.*, 1971. by removing gills and weighed 100mg of the tissue was homogenized using Teflon glass hand homogenizer by adding 1.5mL of 0.1M tris-HCl buffer of pH 7.2. The homogenate was further centrifuged at 93.9g at 4°C for 15min and the supernatant was used for estimation and expressed as  $\mu\text{g/h/g}$ .

#### ***Oxidative stress markers and glutamate analysis***

Superoxide dismutase (SOD) was determined (Das *et al.*, 2000) by the inhibition of superoxide led nitrite formation from hydroxylamine hydrochloride and the absorbance was measured at 540 nm and activity was expressed as U/mg of protein. Catalase activity was measured depending on the formation of stable complex of hydrogen peroxide with ammonium molybdate and absorbance was recorded at 405 nm and expressed as  $\text{U mg}^{-1}$  (Goth, 1991). Lipid peroxidation was determined by using malondialdehyde (MDA) as standard and in terms of thiobarbituric acid (TBARS). The reaction mixture of TCA-TBA HCl reagent and the sample were boiled for 10min, cooled and latter centrifuged at 10000g and the supernatant was used for measurement at 535nm and expressed as  $\text{nmol/mg}$  (Buegeand Aust, 1978). Glutamate levels were estimated by multiple development of paper chromatography. The supernatant obtained as mentioned earlier was evaporated at 70°C and mixed with 100ml double distilled water. 2mM glutamate standard solution along with the sample is spotted on Whatman no.1 chromatography paper which is allowed to develop on the mobile phase (Butanol: acetic acid: water 12:3:5 v/v). The chromatogram is developed again, subsequently the papers are dried and sprayed with ninhydrin and incubated at 100°C for 4min. The bands which exhibit glutamate corresponding to the standard is cut and eluted in 75% ethanol with 0.005%  $\text{CuSO}_4$ . The absorbance is measured against the blank at 515nm in a spectrophotometer (Raju *et al.*, 2004). The concentration is expressed as  $\mu\text{mol/g}$  of glutamate.

#### ***Statistical analysis***

All data were statistically analyzed and represented as Mean  $\pm$  SE. In all experiments, the level of statistical significance was set for  $P < 0.05$  and  $P < 0.01$ . The significance was calculated by Student's *t*-test using MS-Excel.

## **RESULTS AND DISCUSSION**

The present study reports the impact of  $\text{SnO}_2$  NPs exposure in the biochemistry and physiology of fresh water carp *C catla*, by examining the haematological and biochemical variables. Initially the mortality (Table 1) was recorded under dose dependent concentrations (100, 80, 60, 40 and 20  $\text{mg l}^{-1}$ ). Since only 90% mortality was observed below 20  $\text{mg l}^{-1}$  dosages, the  $\text{LC}_{50}$  of  $\text{SnO}_2$  NPs was considered at 20  $\text{mg l}^{-1}$  concentrations at the end of 96 hr.

Hb ( $\text{g dl}^{-1}$ ) levels was seen to increase from 4<sup>th</sup> day ( $6.01 \pm 0.04$ ) when compared to the control ( $5.82 \pm 0.12$ ). However, the Hb levels reduced on all the other days of sacrifice when compared to the control with maximum difference ( $5.54 \pm 0.12/3.13 \pm 0.05$ ) on the last day (20<sup>th</sup> day) of the sacrifice. Haematological manifestations reveal an increase in Hb levels on the 1<sup>st</sup> day (day 4) of sacrifice, which may be attributed to the feedback response of the system to compensate the immediate loss of RBC (Shah and Altindag, 2004). However, there is a gradual and significant decrease in the Hb levels in the treated

groups compared to the control groups on all the other days of experiment. This decrease in the level of Hb may be attributed to the heavy metal induced alterations in the respiratory mechanism of the fish. Substantial expulsion of mucus and swelling along with necrosis in the gills of the common carps has been reported leading to the impairment of respiratory mechanism. The stressed induced by heavy metals on the gills have been associated with decrease in the Hb levels, also resulting in reduced affinity for oxygen binding. The decrease in the Hb levels due to exposure of fish to metal oxides nanoparticles in the present study is well supported by other research groups (**Shaluei et al., 2018**). Hct (%) saw a reduction in values when compared to the control group on all the days of sacrifice, with 12<sup>th</sup> day reporting the highest difference ( $14.71 \pm 0.002/9.98 \pm 0.03$ ). The studies revealed decreased levels of Hct (%) throughout the experimental period of 4, 8, 12, 16 and 20 days (Table 2). Earlier studies have reported lowering of Hct levels upon exposure to metal oxides. This observation can be attributed to defense mechanism against SnO<sub>2</sub> NPs toxicity by inducing erythropoiesis. RBC values expressed in 10<sup>6</sup>cm/mm were also found to decrease on all the days of sacrifice with least value ( $2.58 \pm 0.42/0.82 \pm 0.04$ ) found on the 20<sup>th</sup> day of sacrifice. The decrease in RBC count may be attributed to the onset of acute anaemia, furthered by aberrations in erythropoiesis and formation of RBCs. The studies have related the decrease in RBC to toxicant induced stress and modification in the selective permeability of the RBC membrane (**Affonso et al., 2002**). WBC (1000/cumm) levels were initially (4<sup>th</sup> day) found to decrease with a minor difference ( $74.32 \pm 0.45/72.41 \pm 0.75$ ), however significant difference could be observed between the control and test groups on all other days of sacrifice. Similar observations are made by other researchers (**Luo et al., 2002**), attributing the alterations to the possible onset of leucopenia. MCV (fl) and MCH (pg) values in test animals were found to increase gradually from first day of sacrifice to the last day of sacrifice. MCHC (gdl<sup>-1</sup>) values in treated fish were seen increasing on the first three days of sacrifice (4, 8, 12 days), but values of test groups were comparatively less to control fishes on 16<sup>th</sup> and 20<sup>th</sup> day (Table 2). As MCV, MCH and MCHC levels are calculated based on the values of Hb, Hct and RBC values, alterations were gradually observed. Reports have been recorded indicating significant increase in MCV and MCH values, and reduction in RBC count upon exposure to metal oxides, due to feedback responses of morphological damages in RBC membrane.

Assessment of glucose (Table 3) revealed a significant increase in the glucose concentration in the treated groups when compared to the control fish, with maximum difference in glucose seen on the 12<sup>th</sup> day ( $4.14 \pm 0.78/28.62 \pm 0.15$ ). This observation can be due to induction of stress resulting in hyperglycemia. Similar results were obtained which can be attributed to the alterations in carbohydrate metabolism, due to glycogenolysis in various fresh water fish. Increase in glucose levels due to exposure to metal oxide nanoparticles have also been reported due to synthesis of glucose from extra hepatic tissue amino acids (**Almeida et al., 2001**). Other studies have also related increase in plasma glucose levels to release of glucocorticoids and catecholamines from adrenal tissues of fish when under stress (**Klaper et al., 2010**). The decrease in plasma protein level was significantly ( $P < 0.05$ ) observed in treated groups (2mg/L of SnO<sub>2</sub> NPs) compared to control groups. The estimation of protein (Table 3) demonstrated increase in protein concentration of treated animals on 4<sup>th</sup> day ( $17.95 \pm 1.13/19.29 \pm 1.51$ ), conversely there was a gradual decrease in the protein levels on all the other days of

sacrifice. The low protein levels are observed due to nanoparticles binding to blood and tissue proteins resulting in tissue injury, oxidative stress, changes in physicochemical properties which manifest in protein structure conformational change (Chen et al., 2011), and this might be the cause of decline in protein concentration in the present study. Toxicity of NPs might be the reason for declined protein concentration as it inhibits transcription and translational processes.

Serum AST (IU/L) and ALT (IU/L) levels were increased in treated groups on all the days of sacrifice when compared to the control groups. LDH (IU/L) was found to increase in the test fish when compared to control, however the values almost remained similar on last three days of sacrifice (12, 16, 20 days).

The detection of transaminases like aspartate transaminase (AST/GOT) and alanine transaminase (ALT/GPT) in blood have been clinically employed to assess any tissue damage (Chandra and Sukumaran, 2020). Presence of these enzymes can also be attributed to the enzyme inhibition in metabolic pathways upon exertion of stress. In the current investigation there was a marked increase in both AST and ALT levels in treated groups compared to the control groups. This can be related to the tissue and organ damage caused upon exposure to SnO<sub>2</sub> NPs and other toxicants (Abhijith et al., 2016). Hence, indicates the strong role of transaminases as potential biomarkers during exposure to toxicants thus resulting in metabolic stress. AST and ALT are enzymes mainly used an indicator in liver function tests, as AST is known to be synthesized by liver hepatocyte and mainly found in liver and heart, while ALT predominantly present in liver and kidney. Subsequently increased activity of these enzymes has been reported in fish exposed to pesticides (Rao, 2006). Lactate dehydrogenase (LDH) an isoenzyme playing a significant role in glycolysis is also considered as important biomarkers of organ and tissue damage. Table 2 Indicates increased activity of LDH upon treatment of catla fish to tin oxide nanoparticles. The increase in LDH can mainly be attributed to increased glycolysis upon metabolic stress. Furthermore, onset of anoxia is also considered to be a significant reason for increased LDH activity. Prevalence of anaerobic condition due to metal toxicity is also related to rise in LDH activity (Min and Ju-Chan, 2008).

Assessment of gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity ( $\mu\text{g hr}^{-1}\text{g}^{-1}$ ) was evaluated in the gill homogenate of both control and treated fish in triplicates. Based on the evaluation the studies revealed significant decrease in gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity as the number of days of incubation extended. The 8th and 12<sup>th</sup> days of sacrifice recorded maximum decrease in the Na<sup>+</sup>/K<sup>+</sup>-ATPase activity when compared to the control group (figure 1). This might be attributed to the fact that toxic substances are known to inhibit the activity following ionic imbalances. Toxic metal oxides absorbed by gill cells through proton coupled Na<sup>+</sup> channels were further blocks the Na<sup>+</sup>K<sup>+</sup>ATPase. The binding of toxic molecules to the amino acid containing sulfhydryl functional groups of Na<sup>+</sup>/K<sup>+</sup>ATPase has been considered as an important factor for the inhibition of Na<sup>+</sup>/K<sup>+</sup>ATPase activity and was measured by downregulation of Na<sup>+</sup>/K<sup>+</sup>ATPase genes in the gills of zebrafish (Foyer and Noctor, 2005).

The glutamate estimation from brain homogenates demonstrated elevation in glutamate levels in treated fish in comparison to the control groups (figure 2). However, the difference in glutamate levels between the control and treated remained unchanged throughout the period of incubation. This might be related with exotoxicity because of excessive production of glutamate, which might cause damage and death of nerve cells.

The increased glutamate concentration may be attributed to changes in brain physicochemical environment which activates the glutamate receptors by permitting high concentration of calcium ions to enter the cell.

Table 4 reports the antioxidant enzyme serum SOD, CAT and LPO levels during sub chronic analysis of *C. catla* upon treatment with SnO<sub>2</sub> NPs (2mg l<sup>-1</sup>). SOD (u/mL of protein) and CAT (μmol/mL of protein/min) levels decreased throughout the studies in treated fish groups. The SOD levels were almost similar on last three days of sacrifice (12, 16, 20 days). Excess of reactive oxygen species (ROS) causes oxidative stress, which is detrimental to biological mechanisms by unsettling and distorting homeostasis. Subsequently results in disturbance in equilibrium of detoxification mechanism of ROS. To scavenge the excessive generation of ROS, cells provide the system of enzymatic and non-enzymatic responses. Studies have reported the generation of ROS and damages caused by them, upon exposure to metal oxide nanoparticles (**Manke et al., 2013**). The pro-oxidant characteristics of NPs cause ROS generation during mitochondrial respiration and thus activating NADPH-like enzymes (**Chandra et al., 2017**). Enzymes like superoxide dismutase (SOD) and catalase (CAT) play a crucial role in scavenging ROS generated during variations of metabolic and physiological processes caused by toxicants, thus SOD and CAT can be considered the immediate defense mechanism for scavenging reactive oxygen species (**Puneeth and Chandra, 2020**). SOD has been reported to be the main and immediate response to oxidative stress in biological system (**Winston et al., 1991**). In present study the results in Table 3 indicate decrease in SOD and CAT levels of the treated groups when compared to the control groups. This can be attributed to generation O<sub>2</sub> and their conversion to H<sub>2</sub>O<sub>2</sub>, which may further lead to oxidation of cysteine in the antioxidant enzyme, thus affirming the toxicity of NPs. The studies are in accordance with other reports which have also shown decrease in SOD levels upon exposure to heavy metals (**Min and Ju-Chan, 2008**). Decrease in CAT levels was also reported in studies involving biological models upon exposure toxic substances (**Raddam et al., 2017**).

LPO analysis expressed in terms of malondialdehyde (MDA) derived demonstrated the increase in values of treated fish groups in comparison to the control. Lipid peroxidation (LPO) is known as the main culprit in the loss of cell structure and function due to excessive generation of reactive oxygen species. The lipid peroxidation mechanism is generally evaluated by measuring the malondialdehyde levels, which is one of the end products of breakdown of lipids due to peroxidation. The result also suggests that, the existing cellular defense mechanism was not able to avert the oxidative damage.

**Table 1:** Mortality rate of *C catla* at different doses of *C catla* to determine LC<sub>50</sub>

Concentration of SnO <sub>2</sub> NPs	Mortality
100 mg l <sup>-1</sup>	100%
80 mg l <sup>-1</sup>	100%
60 mg l <sup>-1</sup>	100%
40 mg l <sup>-1</sup>	100%
20 mg l <sup>-1</sup>	< 90%

**Table 2:** Alterations in the haematological variables of *C catla* exposed to SnO<sub>2</sub> NPs (2mg l<sup>-1</sup>)

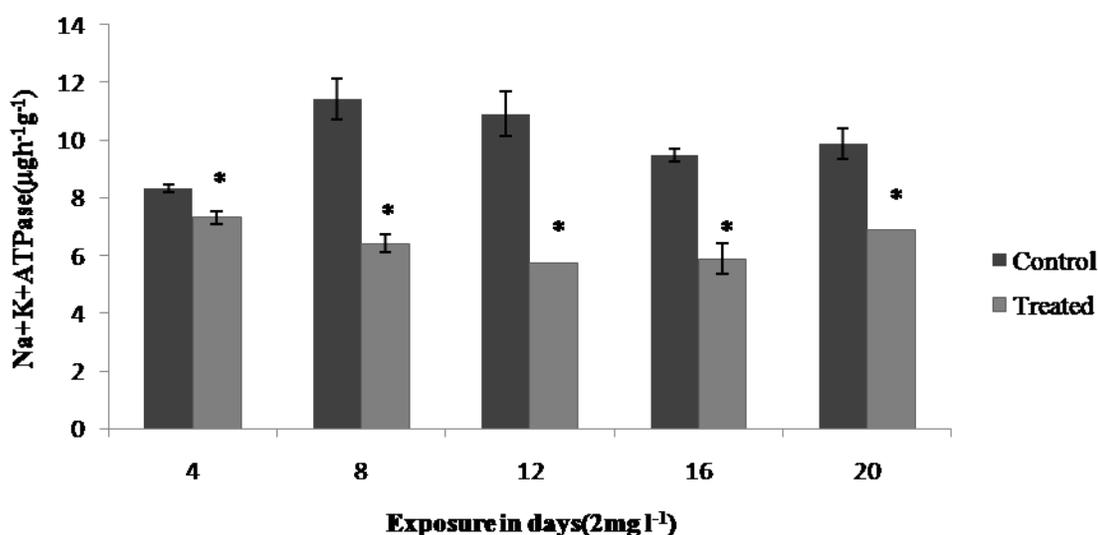
Haematological indices	Exposure duration(in days) 2mg/L									
	4		8		12		16		20	
	C	E	C	E	C	E	C	E	C	E
Hb (gd l <sup>-1</sup> )	5.82 ±0.12	6.01 ±0.04*	5.94 ±0.43	4.96 ±0.21*	5.74 ±0.02	4.11 ±0.19*	5.32 ±0.38	3.32 ±0.06**	5.54±0. 12	3.13 ±0.05*
HCt (%)	13.47 ±1.41	11.42±0 .012*	14.25± 0.12	10.28 ±0.17*	14.71 ±0.002	9.98 ±0.03*	13.81±0 .05	9.32 ±0.44*	13.63±0 .03	8.41 ±0.003*
RBC (10 <sup>6</sup> cm mm <sup>-1</sup> )	2.41 ±0.08	1.88 ±0.63*	2.76 ±0.02	1.22 ±0.92**	2.94 ±0.78	1.03 ±0.73*	2.52 ±0.03	0.87 ±0.03*	2.58 ±0.42	0.82 ±0.04*
WBC (1000 cµmm <sup>-1</sup> )	74.32 ±0.45	72.41 ±0.75*	72.68 ±0.07	52.65 ±0.86*	75.57 ±0.49	44.57 ±0.07*	78.66 ±0.42	48.13 ±0.76**	76.23 ±0.02	51.26 ±0.14*
MCV(Fl)	55.89 ±0.12	60.74 ±0.18*	51.63 ±0.08	84.26 ±0.04*	50.03 ±0.42	96.89 ±0.42*	54.81 ±0.01	107.1 ±0.58*	52.82 ±0.41	102.5 ±0.003*
MCH(Pg)	24.42 ±0.47	31.9 ±0.02*	22.2 ±0.23	40.65 ±0.26**	19.5 ±0.52	39.9 ±0.03*	21.1 ±0.82	38.42 ±0.017*	21.4 ±0.17	38.17 ±0.102*
MCHC(g/dl)	39.19 ±0.08	52.66 ±0.10*	41.68 ±0.72	48.24 ±0.54*	39.02 ±0.48	41.18 ±0.07*	38.53 ±0.15	35.62 ±0.66*	40.69 ±0.002	37.22 ±0.81*

All values are expressed as mean± SE of three individual samples,\*P< 0.05 is significant.

**Table 3: Alterations in the biochemical variables of *C catla* exposed to SnO<sub>2</sub>NPs (2mg l<sup>-1</sup>)**

Biochemical indices	Exposure duration (in days) 2mg/L									
	4		8		12		16		20	
	C	E	C	E	C	E	C	E	C	E
Glucose (mmol l <sup>-1</sup> )	4.24 ±0.34	8.51 ±0.26*	4.29 ±0.28	23.93 ±2.31*	4.14 ±0.78	28.62 ±0.15*	4.43 ±0.06	28.34 ±0.82*	4.52 ±0.18	26.42 ±0.52*
Protein (g l <sup>-1</sup> )	17.95 ±1.13	19.29 ±1.51*	17.12 ±0.06	16.51 ±0.82**	17.52 ±0.53	14.83 ±0.62*	17.62 ±0.02	15.41 ±0.002*	17.81 ±0.10	14.22 ±0.012*
LDH (IU l <sup>-1</sup> )	3.82 ±0.02	4.85 ±0.43*	3.74 ±0.53	4.92 ±0.03*	3.52 ±0.62	5.43 ±0.13*	3.61 ±0.92	5.55 ±0.27**	3.85 ±0.08	5.80 ±0.40*
AST (IU l <sup>-1</sup> )	22.41 ±0.07	28.32 ±0.04*	23.15 ±0.47	31.43 ±0.82*	23.42 ±0.04	35.62 ±1.43*	22.98 ±0.10	36.11 ±2.82*	22.81 ±0.83	35.98 ±0.002*
ALT (IU l <sup>-1</sup> )	28.63 ±0.04	37.40 ±0.41*	28.24 ±0.13	39.81 ±0.53*	28.73 ±0.64	42.61 ±0.08*	28.94± 0.49	44.81 ±0.43**	29.12 ±0.37	42.53 ±0.53*

All values are expressed as mean ± SE of three individual samples, \**P* < 0.05 is significant.

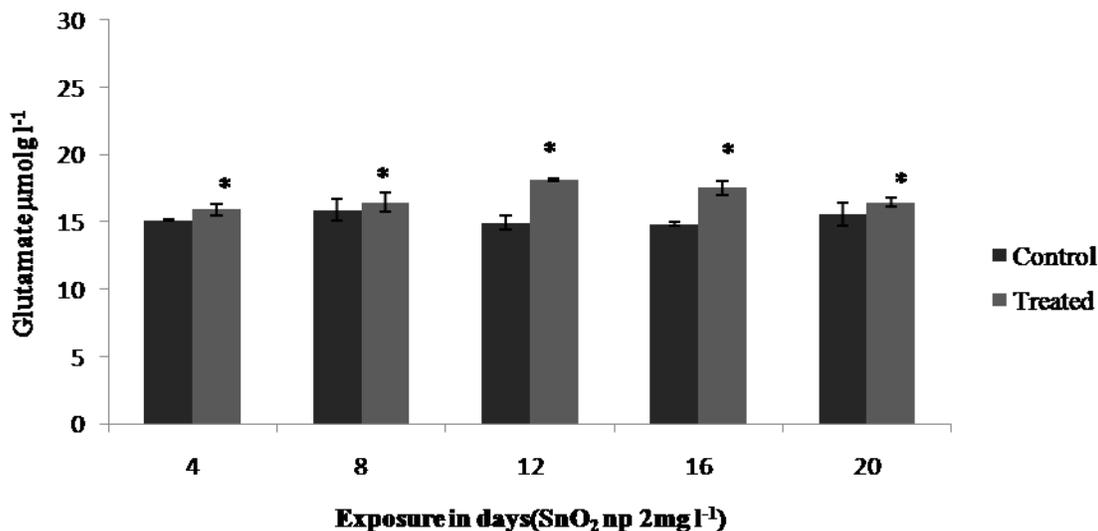
**Fig 1: Alterations in the gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity of *C catla* exposed to SnO<sub>2</sub> NPs (2mg l<sup>-1</sup>)**

All values are expressed as mean ± SE of three individual samples, \**P* < 0.05 is significant

**Table 4:** Alterations in the ROS variables of *C catla* exposed to SnO<sub>2</sub> NPs (2mg l<sup>-1</sup>)

ROS indices	Exposure duration (in days) 2mg l <sup>-1</sup>									
	4		8		12		16		20	
	C	E	C	E	C	E	C	E	C	E
SOD (Uml <sup>-1</sup> protein)	5.34 ±0.02	4.72** ±0.43	5.92 ±0.81	4.12* ±1.31	5.42 ±0.76	3.73* ±2.14	4.91* ±0.42	3.65 ±0.02	5.12* ±0.63	3.52 ±0.14
CAT (µmolml <sup>-1</sup> protein <sup>-1</sup> min <sup>-1</sup> )	1.24 ±0.03	1.01 ±0.51*	1.38 ±0.43	0.92 ±0.42*	1.32 ±0.62	0.88 ±0.36*	1.19 ±0.64	0.97 ±0.42**	1.22 ±0.75	0.83 ±0.32*
LPO (nmol of lDAmg <sup>-1</sup> protein)	1.43 ±0.81	2.78 ±0.33*	1.45 ±0.15	2.81 ±0.04*	1.39 ±0.27	3.85 ±0.39*	1.48 ±0.22	3.97 ±0.54*	1.44 ±0.48	2.72 ±0.37*

All values are expressed as mean± SE of three individual samples, \**P*< 0.05 is significant.

**Fig 2:** Alterations in the brain glutamate level of *C catla* exposed to SnO<sub>2</sub> NPs (2mg l<sup>-1</sup>)

All values are expressed as mean± SE of three individual samples, \**P*< 0.05 is significant

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## REFERENCES

- Abhijith, B.D.; Ramesh, M.; and Poopal, R.K.**(2016). Responses of metabolic and antioxidant enzymatic activities in gill, liver and plasma of *Catla catla* during methyl parathion exposure, *The Journal of Basic & Applied Zoology* 77: 31-40.
- Affonso, E. G., Polez, V. L. P., Corrêa, C. F., Mazon, A. F., Araujo, M. R. R., Moraes, G., & Rantin, F. T.** (2002). Blood parameters and metabolites in the teleost fish *Colossoma macropomum* exposed to sulfide or hypoxia. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 133(3), 375-382.
- Almeida, J. A.; Novelli, E. L. B.; Silva, M. D. P.; and Júnior, R. A.** (2001). Environmental cadmium exposure and metabolic responses of the Nile tilapia, *Oreochromis niloticus*. *Environmental Pollution*, 114(2): 169-175.
- Antisari, L. V.; Carbone, S.; Gatti, A.; Vianello, G.; and Nannipieri, P.** (2013). Toxicity of metal oxide (CeO<sub>2</sub>, Fe<sub>3</sub>O<sub>4</sub>, SnO<sub>2</sub>) engineered nanoparticles on soil microbial biomass and their distribution in soil. *Soil biology and biochemistry*, 60: 87-94.
- Buege, J.A.; and Aust SD.** (1978) Microsomal lipid peroxidation. *Methods in Enzymology*, 31; 52: 302-10.
- Chandra, S. P.; Puneeth, H. R.; Mahadimane, P. V.; and Sharada, A. C.** (2017). Biochemical Evaluation of *Cordia dichotoma* Seed Extracts. *Advanced Science Letters*, 23(3):1823-1825.
- Chandra, S.; and Sukumaran, S.** (2020). Physiological, Biochemical and Neurochemical responses of *Cirrhinus mrigala* upon short term exposure to Cerium oxide. *International Journal of Aquatic Biology*, 7(6):368-373.
- Chen, P. J.; Su, C. H.; Tseng, C. Y.; Tan, S. W.; and Cheng, C. H.** (2011). Toxicity assessments of nanoscale zerovalent iron and its oxidation products in medaka (*Oryzias latipes*) fish. *Marine pollution bulletin*, 63(5-12): 339-346.
- Cooper GR.; and McDaniel** (1970) *Standard methods of clinical chemistry*. Academic, New York, 159.
- Das, K.; Samanta, L.; and Chainy, G.B.N.** (2000) A modified spectrophotometric assay of superoxide dismutase using nitrite formation by superoxide radicals. *Indian Journal of Biochemistry and Biophysics* 37: 201–204.
- Drabkin, D.L.** (1946) Spectrophotometric studies XIV. The crystallographic and optical properties of the hemoglobin of man in comparison with those of other species. *Journal of Biological Chemistry* 1;164(2):703-23.
- Foyer, C. H.; and Noctor, G.** (2005). Oxidant and antioxidant signalling in plants: a reevaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment*, 28(8): 1056-1071.
- Gambardella, CH. T.; T. Mesaric, K.; Milivojevic, L.; Sepcic, S.; Gallus, S.; Carbone, Ferrando.; and M. Faimali.** (2014) Effects of selected metal oxide nanoparticles on *Artemia salina* larvae: evaluation of mortality and behavioral and biochemical responses. *Environmental Monitoring and assessment*: 4249- 4259.
- Goth L.** (1991) A simple method for determination of serum catalase activity and revision of reference range. *ClinChimActa* 15;196(2-3):143-51.
- Heinlaan, M.; Blinova, I.; Dubourguier, H.C.; and Kahru, A.** (2008) Toxicity of nanosized and bulk ZnO, CuO and TiO<sub>2</sub> to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus*. *Chemosphere* 71:1308–1316.
- Jin, W. X.; Ma, S. Y.; Tie, Z. Z.; Li, W. Q.; Luo, J.; Cheng, L.; and Mao, Y. Z.** (2015). Synthesis of hierarchical SnO<sub>2</sub> nanoflowers with enhanced acetic acid gas sensing properties. *Applied Surface Science*, 353: 71-78.

- Klaper, R.; Arndt, D.; Setyowati, K.; Chen, J.; and Goetz, F.** (2010). Functionalization impacts the effects of carbon nanotubes on the immune system of rainbow trout, *Oncorhynchus mykiss*. *Aquatic toxicology*, 100(2): 211-217.
- Li, K. N.; Wang, Y. F.; Xu, Y. F.; Chen, H. Y.; Su, C. Y.; and Kuang, D. B.** (2013). Macroporous SnO<sub>2</sub> synthesized via a template-assisted reflux process for efficient dye-sensitized solar cells. *ACS applied materials & interfaces*, 5(11): 5105-5111.
- Lowry, OH.; Rosebrough, NJ.; Farr, AL.; and Randall, RJ.** (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 1;193(1):265-75.
- Luo, JJ.; Hsieh, LL.;and Chang, MJW.** (2002). Decreased white blood cell counts in semiconductor manufacturing workers in Taiwan. *Occupational and Environmental Medicine*, 59:44-48.
- Manke, A.; Wang, L.; and Rojanasakul, Y.** (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMedical Research International*: 916-942.
- Min, E. Y.; and Kang, J. C.** (2008).Effect of waterborne benomyl on the hematological and antioxidant parameters of the Nile tilapia, *Oreochromis niloticus*. *Pesticide Biochemistry and Physiology*, 92(3): 138-143.
- Nelson, DA.; and Morris, MW.** (1989) Basic methodology. *Hematology and coagulation*, part IV. Clinical diagnosis and management by laboratory methods, 7th edn. Saunder Company, Philadelphia: 578–724.
- Puneeth, H. R.; and Chandra, S. S. P.** (2020).A review on potential therapeutic properties of Pomegranate (*Punica granatum* L.). *Plant Science Today*, 7(1): 9-16.
- Raddam, Q. N.; Zeidan, M. M.; Asaad, N. K.; and Abdulrahman, M. A.** (2017). Smoking effects on blood antioxidants level: lactate dehydrogenase, catalase, superoxide dismutase and glutathione peroxidase in university students. *Journal of Clinical & Experimental Pathology*, 7(6): 2161-0681.
- Raju, TR.; Kutty, BM.; Sathyaprabha, TN.; and Shankarnarayana, Rao BS.** (2004). Brain and behavior.National Institute of Mental Health and neurosciences, Bangalore.134-138.
- Rao, J. V.** (2006). Sublethal effects of an organophosphorus insecticide (RPR-II) on biochemical parameters of tilapia, *Oreochromis mossambicus*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 143(4): 492-498.
- Rashmi, N.; Ranjitha, T.; and Sharath Chandra, SP.** (2019). Chromium and their derivatives causes physiological and biochemical modifications in diverse fish models: A Review. *Biomed Pharmacol J* 12 (4): 2049-2053.
- Roopan, S.M.; Kumar, S.H.S.; Madhumitha, G.; and Suthindhira, K.** (2014) Biogenic-production of SnO<sub>2</sub> nanoparticles and its cytotoxic effect against hepatocellular carcinoma cell line (HepG<sub>2</sub>). *Appl. Biochem. Biotechnol*: 1381-5.
- Rusia, V., and Sood, SK.** (1992) Routine hematological tests. In: Mukerjee KL (ed) *Medical laboratory technology*. Tata McGraw Hill Publishing Company Limited, New Delhi: 252–258.
- Shah, S. L.; and Altindag, A.** (2004).Hematological parameters of tench (*Tincatinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments.*Bulletin of environmental contamination and toxicology*, 73(5): 911-918.
- Shaluei, F.; Hedayati, A.; Jahanbakhshi, A.; Kolangi, H.; and Fotovat M.** (2013) Effect of subacute exposure to silver nanoparticle on some hematological and plasma biochemical indices in silver carp (*Hypophthalmichthys molitrix*). *Human & Experimental Toxicology* 32(12): 1270–1277.
- Winston, G. W.; and Di Giulio, R. T.** (1991).Prooxidant and antioxidant mechanisms in aquatic organisms.*Aquatic toxicology*, 19(2):137-161.