



ALUMINIUM OXIDE NANOPARTICLES INDUCED IRREVERSIBLE ALTERATIONS IN THE ANTIOXIDANT DEFENSE SYSTEM OF THE FISH, *OREOCHROMIS MOSSAMBICUS* (PETERS, 1852)

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ABSTRACT

Aluminium oxide nanoparticles ($\text{Al}_2\text{O}_3\text{NPs}$) are the most abundantly produced nanoparticles having widespread applications in the field of physics, chemistry, engineering and biology. Therefore, exposure of $\text{Al}_2\text{O}_3\text{NPs}$ to organisms and environment is definite with great concern on the fate, ecotoxicity and persistence in the environment. The present study was designed to evaluate the sublethal (4 mg/L) effects of $\text{Al}_2\text{O}_3\text{NPs}$ in gill, liver and brain of the fish, *Oreochromis mossambicus* exposed for short-term (24, 72 and 96 h) and long-term (15, 30 and 60 days) durations. Gill tissue showed significant ($P < 0.05$) decrease in the activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase with concomitant increase in the levels of hydrogen peroxide generation and lipid peroxidation. Liver tissue showed decreased activities of antioxidant enzymes in long-term exposure, with simultaneous increase in levels of hydrogen peroxide and lipid peroxidation. Brain tissue also showed significant reduction in the activities of all antioxidant enzymes along with rise in the levels of hydrogen peroxide generation and lipid peroxidation in short-term and long-term exposure groups. The activity of marker enzyme, alkaline phosphatase reduced in gill and increased in liver after 60 days. Brain marker acetylcholinesterase enzyme showed decreased activity in brain tissue after exposure to $\text{Al}_2\text{O}_3\text{NPs}$. Treatment withdrawal for 60 days also showed similar results like the treatment groups. In conclusion, aluminium oxide nanoparticles induced alterations in the antioxidant defense system, which is responsible for the persistent toxic effects in gill, liver and brain tissues of the freshwater fish, *Oreochromis mossambicus*.

KEYWORDS: $\text{Al}_2\text{O}_3\text{NPs}$, *Oreochromis mossambicus*, antioxidant enzymes, lipid peroxidation, oxidative stress, treatment withdrawal.

INTRODUCTION

Nano-sized aluminium oxide ($\text{Al}_2\text{O}_3\text{NPs}$) is widely used metal oxide nanoparticles in certain products including ceramics, pots, watch glasses, and as an abrasive material in sandpaper. The extraordinary properties of $\text{Al}_2\text{O}_3\text{NPs}$ include thermal stability and hardness, which made it suitable for wide variety of applications such as aerospace, plastic, rubber and in polymer products. Along with the unusual properties of aluminium oxide, the reduction of size to nano-scale made $\text{Al}_2\text{O}_3\text{NPs}$ more reactive and its effect on environment and organisms remains mysterious. The stable form of aluminium naturally occurs as crystalline corundum known as rubies and sapphires, thus also extensively used in jewellery and ornaments. Aluminium oxide is also used as fillers in paints, plastic and cosmetics, formulations of glass, catalyst for reactions, purification and dehydration of gas streams etc. Besides, oral intake of aluminium oxide nanoparticles in the form of tablet is practised to treat the

symptoms of heatburn and is considered as non-toxic to humans. However, long time oral intake of nanoparticles could elevate the level of alumina in blood and cause serious effects on human health.^[1] Aluminium oxide nanoparticles has been shown to contribute in oxidative stress, inflammatory events, breakdown of blood-brain barrier and many neurodegenerative diseases particularly in microvascular endothelial cells of human brain.^[2,3] It is also suspected to cause disorders in kidney, liver, bones and haematopoietic system in human.^[4,5] Dose-dependent cytotoxicity, decreased mitochondrial membrane potential and upregulation of oxidative stress responsive enzyme genes has been observed in human mesenchymal stem cells, which suggests that $\text{Al}_2\text{O}_3\text{NPs}$ induce toxicity mainly by oxidative stress.^[6] Genotoxic potential of $\text{Al}_2\text{O}_3\text{NPs}$ has been reported along with the induction of micronuclei in CHO-K1 cell lines^[7] and in peripheral blood of rat model.^[8] Neurotoxicity induced

by glia activation has been also observed when $\text{Al}_2\text{O}_3\text{NPs}$ was exposed peripherally to rat brain.^[9]

Owing to the wide rise in industrialization, there are possibilities of nanoparticles to enter into the aquatic ecosystem. The most important processes affecting the fate of nanoparticles in aquatic ecosystems are agglomeration, aggregation, dissolution, redox reactions and transformation into new solid phases.^[10] The uptake of nanoparticles by the aquatic organisms is primarily by the impairment of environment-organisms barrier and secondly, by the translocation from the barrier tissue into organisms by means of internal distribution. Bioaccumulation of $\text{Al}_2\text{O}_3\text{NPs}$ in the environment enable the trophic transfer and biomagnification in different organisms from primary producers to consumers.^[11] In ecotoxicological studies, fish is the first choice of test organisms as it is the immediate consumer of human through the food chain and also function as an indicator of water quality and health of aquatic ecosystem. One of the mechanisms of nanoparticles toxicity is the generation of reactive oxygen species, which could induce DNA damage, oxidative protein damage and lipid peroxidation.^[12] In recent years, the impact of $\text{Al}_2\text{O}_3\text{NPs}$ on fish model has attained much attention. One of our previous studies have reported the median lethal concentration (LC_{50}) of $\text{Al}_2\text{O}_3\text{NPs}$ with 16.7nm size for 96 h in the freshwater fish, *Oreochromis mossambicus* as 40 mg/L.^[13] On the other hand, $\text{Al}_2\text{O}_3\text{NPs}$ of 40nm size showed LC_{50} -96 h value between 235 and 245 ppm in *Oreochromis mossambicus*.^[14] and this disparity could be due to the change in the size of the nanoparticles. Sublethal exposure of $\text{Al}_2\text{O}_3\text{NPs}$ has been shown to accumulate the nanoparticles in fish liver causing alteration in hepatosomatic index and histological changes.^[14] Single and combined exposure of aluminium and zinc oxide nanoparticles has been shown to cause sublethal effects in the freshwater fish, *Carassius auratus*, which is evident by the alteration of antioxidant defense system in gill and liver tissues.^[15] In another study, $\text{Al}_2\text{O}_3\text{NPs}$ has been demonstrated to induce cytotoxicity and oxidative stress in dose-dependent manner in CHSE-214 cell lines of Chinnok salmon.^[16] The available literatures updated on the adverse toxic effects of $\text{Al}_2\text{O}_3\text{NPs}$ in various fish models. However, the question of whether the sublethal toxic effects of $\text{Al}_2\text{O}_3\text{NPs}$ could remain stable even after the treatment withdrawal for certain period of time is still open to investigation. Therefore, the present study was designed to explore the sublethal effects of $\text{Al}_2\text{O}_3\text{NPs}$ by means of alteration in the antioxidant defense system as the mechanism of toxicity in various tissues such as gill, liver and brain. In addition, the experiment emphasised on the stability of $\text{Al}_2\text{O}_3\text{NPs}$ toxicity by evaluating treatment withdrawal for a period of 60 days in order to prove irreversible tissue damage and toxicity of nanoparticles in the fish, *Oreochromis mossambicus*.

MATERIALS AND METHODS

Test animal

Oreochromis mossambicus weighing $6\pm 1.5\text{g}$ and length $6.5\pm 1\text{cm}$ were collected from local fish farm, Safa Aquarium, Kozhikode, Kerala ($11^\circ 22'\text{N}$, $75^\circ 85'\text{E}$). Fish were acclimatized to the laboratory conditions in a glass tank of 40 L capacity prior to the experiment. Fish were properly maintained in good condition by providing aeration, light and dechlorinated water. The physico-chemical features of the tap water were estimated as per APHA guidelines.^[17] Standard water temperature ($28 \pm 2^\circ\text{C}$), oxygen saturation of water (70 to 100%), pH (6.5 to 7.5) was maintained throughout the experiment in both control and treatment groups.

Test chemical and toxicity testing

$\text{Al}_2\text{O}_3\text{NPs}$ (Cat. No: 0140408) was obtained from SISCO Research Laboratory (SRL), India. The characterization, purity and size of the nanoparticles were previously performed in our laboratory. The characterization data showed that $\text{Al}_2\text{O}_3\text{NPs}$ are pure, crystalline and size derived by Scherrer's formula is 16.7nm.^[13] The nanodispersion was prepared just before exposure by ultra-sonication at 100 kHz for 30 min using double distilled water and maintained as stock. The median lethal concentration or LC_{50} value for $\text{Al}_2\text{O}_3\text{NPs}$ in *Oreochromis mossambicus* determined by probit analysis was 40 mg/L.^[13] Sublethal concentration, i.e., 4 mg/L of $\text{Al}_2\text{O}_3\text{NPs}$ ($1/10^{\text{th}}$ of LC_{50}) was used as the test concentration and exposed for short-term (24, 72 and 96 h) and long-term (15, 30 and 60 days) durations maintaining control group. After the long-term treatment duration, withdrawal of treatment for 60 days was also performed by maintaining the animal without toxicant. In each treatment groups, ten fish were maintained and the health conditions were continuously monitored throughout the experiment. At the end of every treatment, the fishes were captured using small dip nets with minimum disturbances in order to avoid stress. Fishes were killed by decapitation and the tissues such as gill, liver and brain were dissected out, weighed and transferred to cold normal saline to clean from debris, blood and mucous. Crude tissue homogenates (1% w/v) were prepared in ice-cold normal saline using a motor driven tissue homogenizer and centrifuged at 800g for 15 min at 4°C . The supernatants collected were then used for various biochemical analyses.

Biochemical analysis

All biochemical analyses were performed in duplicate in order to avoid statistical errors. Total protein was estimated by the method of Lowry et al^[18] with BSA as the standard. Activities of antioxidant enzymes such as, superoxide dismutase,^[19] catalase,^[20] glutathione reductase^[21] and glutathione peroxidase,^[22] were analysed in the supernatants of crude homogenates. Levels of hydrogen peroxide generation^[23] and lipid peroxidation^[24] were also evaluated. The activity of alkaline phosphatase^[25] was analysed in the gill and liver

tissue whereas the activity of acetylcholinesterase^[26] was measured in the brain tissue of the fish.

Data presentation and statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p < 0.05$ against the control group. Data are presented as mean $\pm$ SD for ten animals per group.

RESULTS

Sublethal effects of Al_2O_3 NPs on the body weight and tissue weights of the fish, *Oreochromis mossambicus*

Body weight of the fish remained unaltered after Al_2O_3 NPs exposure throughout the treatment period

(Table 1). The weight of the gill showed significant ($P < 0.05$) increase from 96 h of Al_2O_3 NPs treatment in time-dependent manner (Table 1). A significant ($P < 0.05$) increase in the weight of brain was noted after 96 h and continued up to 30 days while the weight was found to decrease significantly ($P < 0.05$) after 60 days (Table 1). In treatment withdrawal group, the weights of gill and brain showed significant ($P < 0.05$) increase when compared to the control group (Table 1). Hepatosomatic index (HSI) showed significant decrease only after 60 days of treatment whereas HSI remained similar to the control group after treatment withdrawal (Table 1).

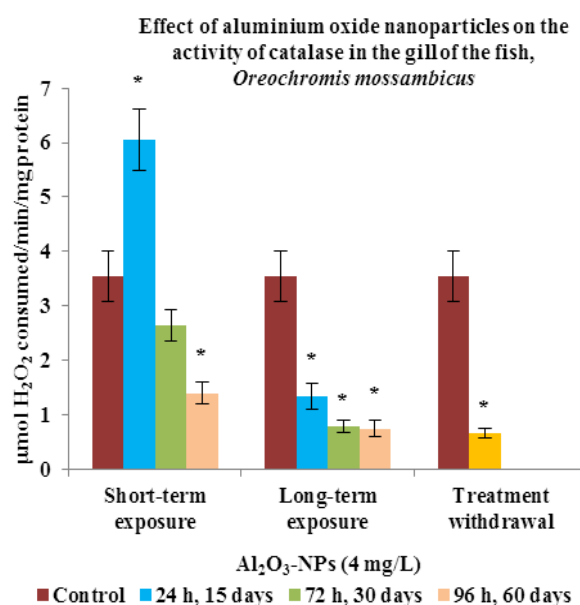
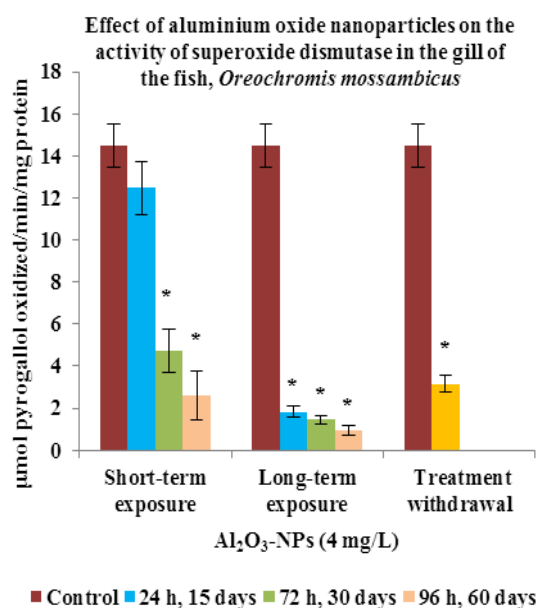
Table 1: Effect of aluminium oxide nanoparticles (Al_2O_3 -NPs) on the body weight and tissue weights of the fish, *Oreochromis mossambicus* (Mean $\pm$ SD; n = 10/ group; * $P < 0.05$).

Parameters	Al ₂ O ₃ -NPs (4 mg/L)							Treatment withdrawal (60 days)
	Short-term exposure				Long-term exposure			
	Control	24 h	72 h	96 h	15 days	30 days	60 days	
Body weight (g)	6.71±0.19	6.54±0.33	6.09±0.21	6.15±0.15	6.58±0.32	6.76±0.25	6.78±0.19	7.03±0.13
Gill weight (mg)	143±11.9	145±2.87	159±3.72	161±4.09*	174±3.16*	175±2.71*	179±2.28*	193±3.23*
Hepatosomatic index (%)	13.53±1.86	12.42±1.61	13.84±2.29	13.39±1.85	12.50±1.12	12.09±0.83	10.84±0.93*	13.19±0.59
Brain weight (mg)	16.5±1.81	15.8±1.31	16.3±3.77	19.5±3.30*	19.6±2.22*	19.7±3.80*	13.9±1.37*	19.9±0.87*

Sublethal effects of Al_2O_3 NPs in the gill tissue of *Oreochromis mossambicus*

Exposure of Al_2O_3 NPs to fish for short-term (24, 72 and 96 h) and long-term (15, 30 and 60 days) durations showed significant ($P < 0.05$) decrease in the activities of antioxidant enzymes as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase when compared to the corresponding control group (Fig. 1).

The levels of hydrogen peroxide generation and lipid peroxidation showed significant ($P < 0.05$) increase after Al_2O_3 NPs treatment (Fig. 1). After treatment withdrawal for a period of 60 days showed the activities of all antioxidant enzymes and the levels of hydrogen peroxide generation and lipid peroxidation similar to that of treatment group (Fig. 1).



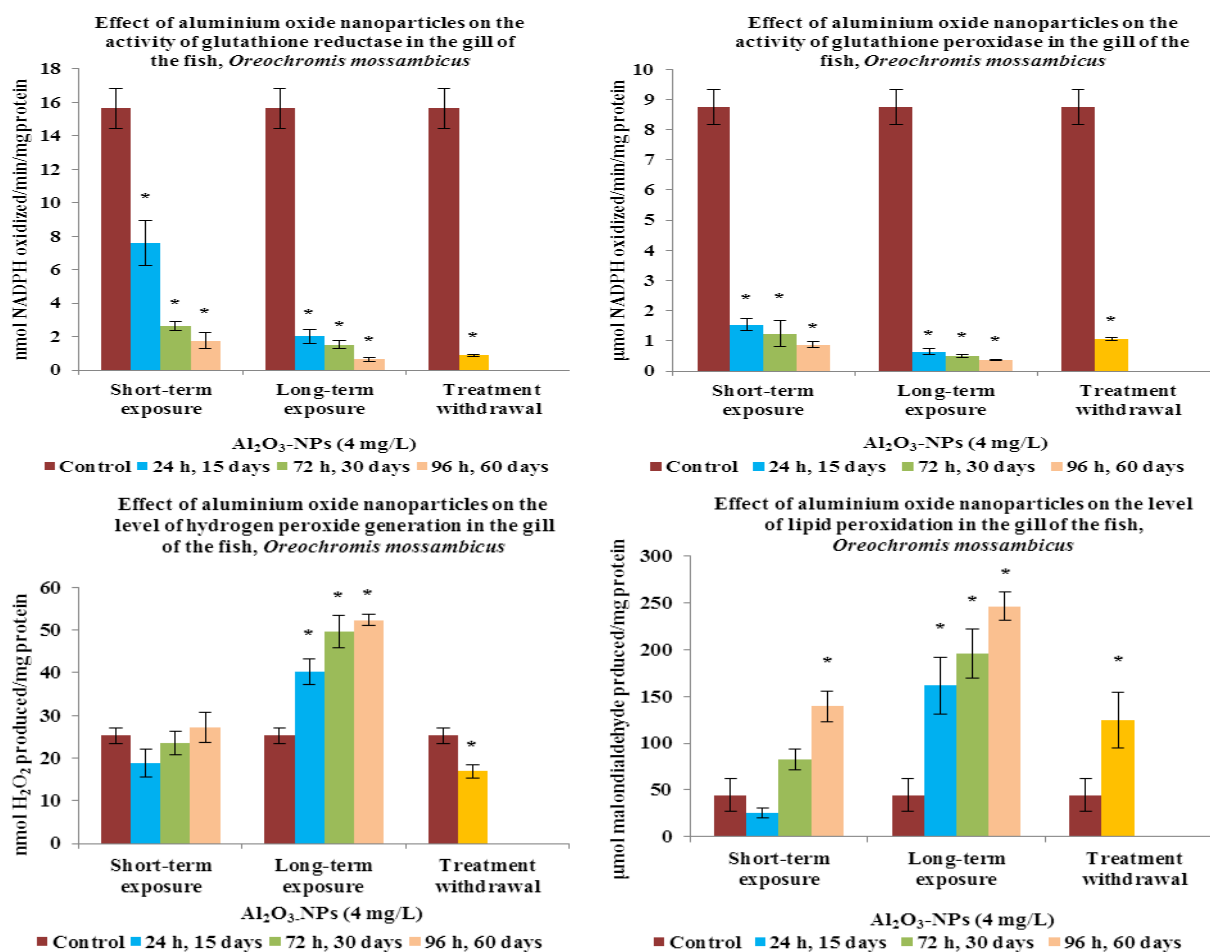
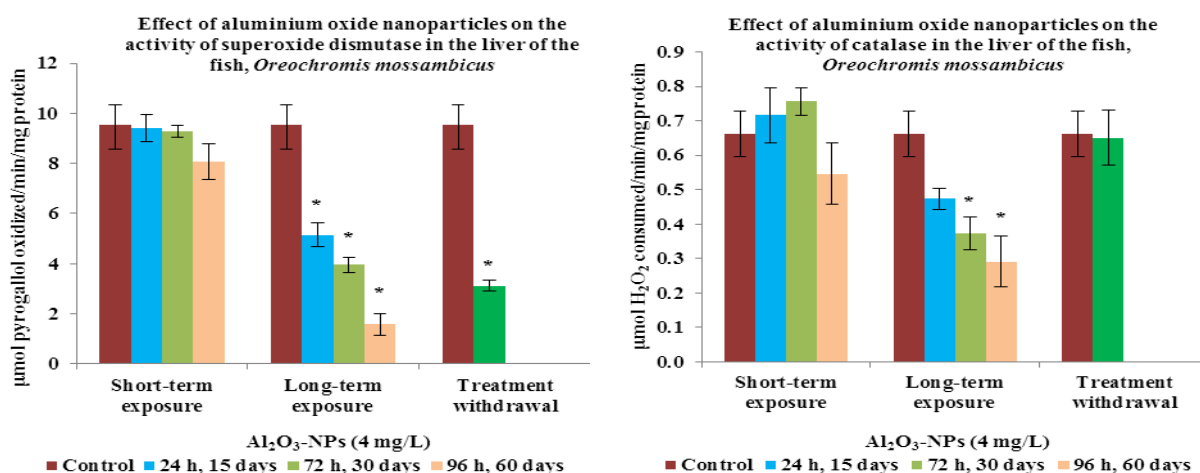


Fig. 1: Effects of $\text{Al}_2\text{O}_3\text{NPs}$ in gill antioxidant defense system of *Oreochromis mossambicus*.

Sublethal effects of $\text{Al}_2\text{O}_3\text{NPs}$ in the antioxidant defense system of liver tissue in *Oreochromis mossambicus*

In liver tissue, after short-term exposure of $\text{Al}_2\text{O}_3\text{NPs}$ there were no significant changes in the activities of all antioxidant enzymes except glutathione peroxidase when compared to the control group (Fig. 2). The levels of hydrogen peroxide and lipid peroxidation also remained unchanged in the short-term exposure groups (Fig. 2). However, in long-term exposure groups the activities of superoxide dismutase, catalase, glutathione reductase and

glutathione peroxidase decreased significantly in time-dependent manner (Fig. 2). Consequently, the levels of hydrogen peroxide generation and lipid peroxidation also found increased significantly ($p < 0.05$) in time-dependent manner (Fig. 2). The treatment withdrawal showed significant decrease only in the activities of superoxide dismutase and glutathione peroxidase, whereas the activities of other enzymes and the levels of hydrogen peroxide generation and lipid peroxidation remained unchanged when compared to control group (Fig. 2).



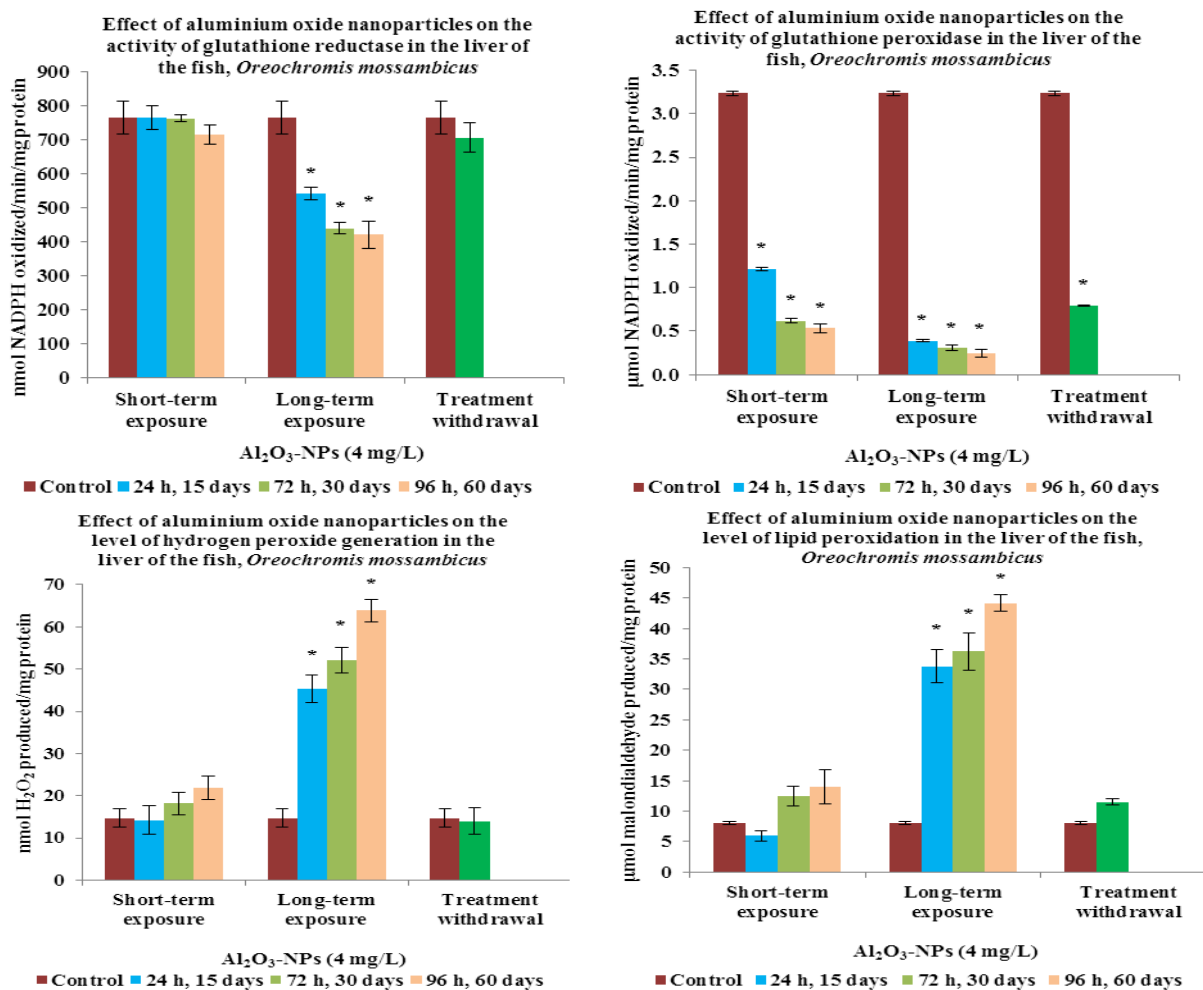
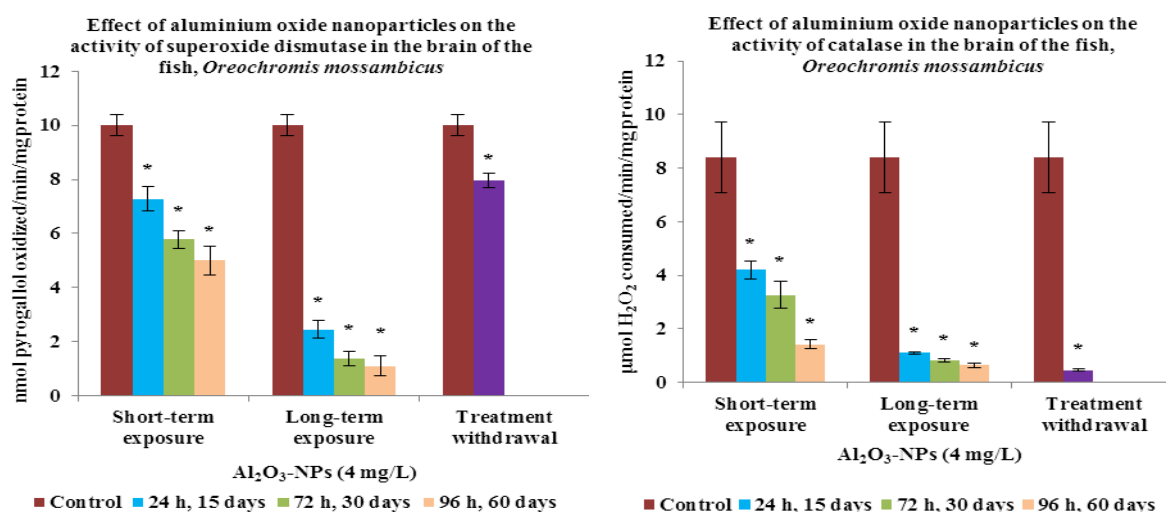


Fig. 2: Effects of Al₂O₃NPs in the antioxidant defense system of liver tissue in *Oreochromis mossambicus*.

Sublethal effects of Al₂O₃NPs in the antioxidant defense system of brain tissue in *Oreochromis mossambicus*

The activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase decreased significantly ($p < 0.05$) in the brain of *Oreochromis mossambicus* exposed to Al₂O₃NPs for

short-term and long-term durations (Fig. 3). The levels of hydrogen peroxide generation and lipid peroxidation increased significantly ($P < 0.05$) only after long-term exposure (Fig. 3). Treatment withdrawal showed significant ($P < 0.05$) decrease in the activities of all antioxidant enzymes with increase in the hydrogen peroxide generation and lipid peroxidation (Fig. 3).



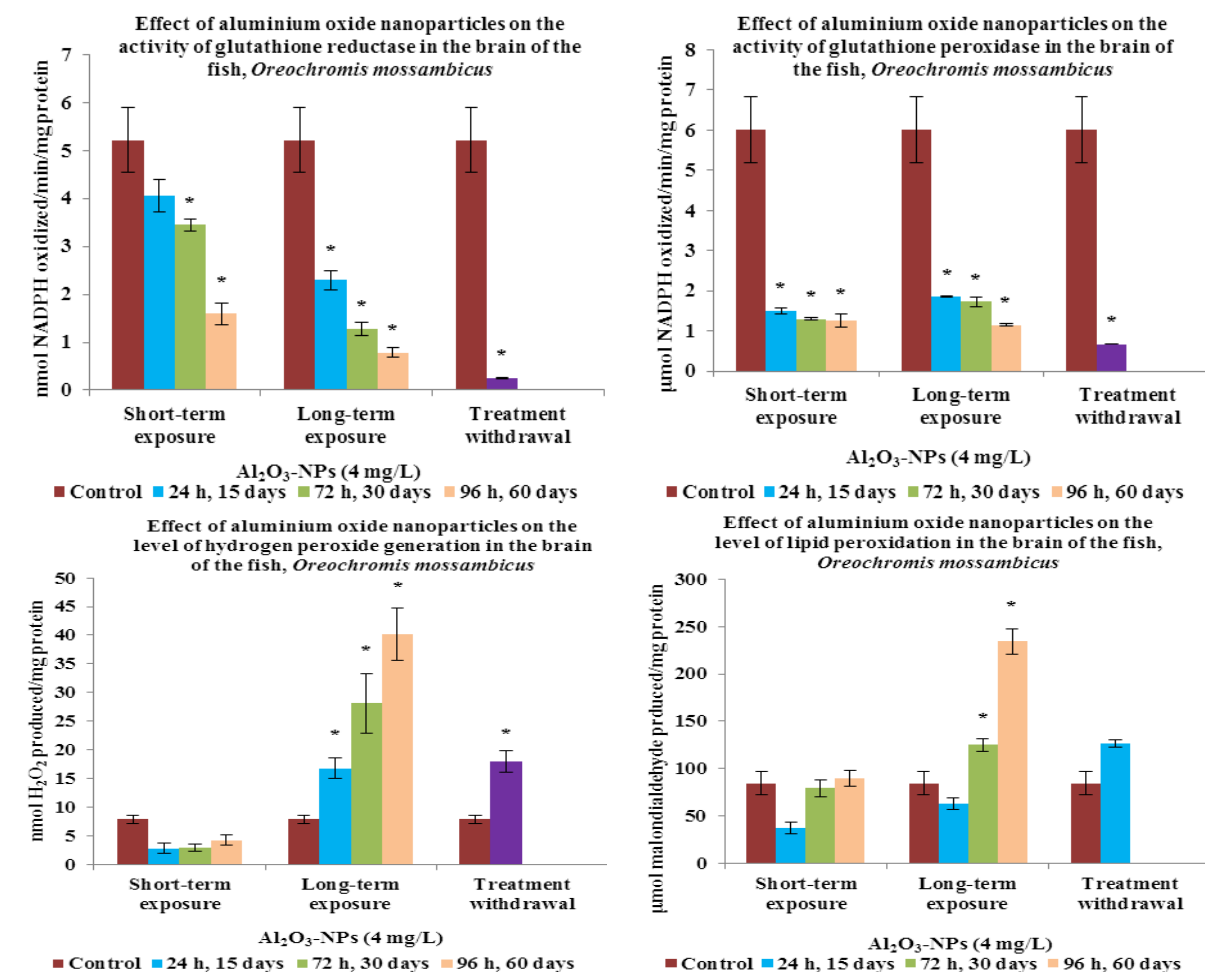
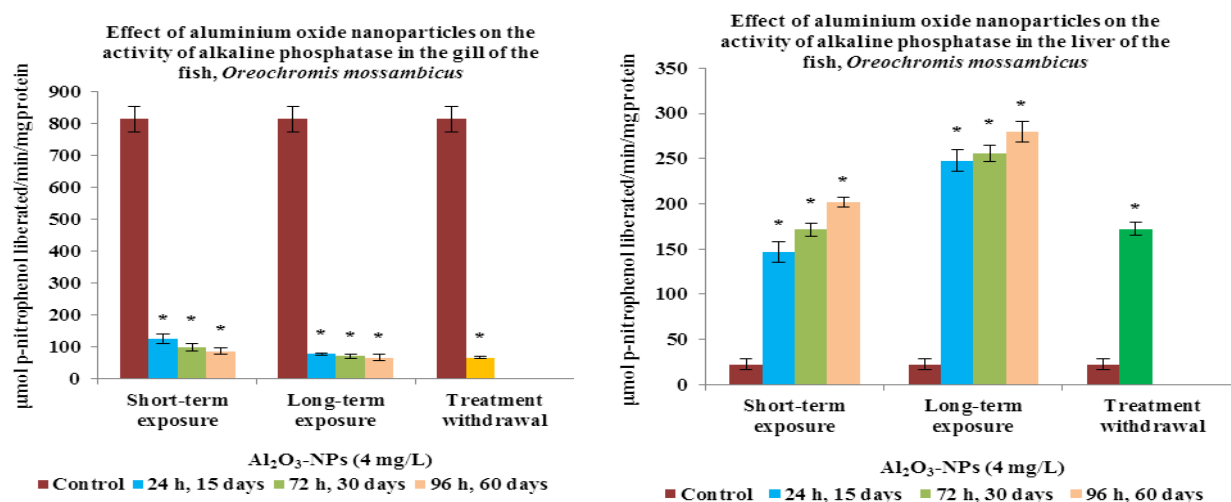


Fig. 3: Effects of $\text{Al}_2\text{O}_3\text{NPs}$ in the antioxidant defense system of brain tissue in *Oreochromis mossambicus*.

Sublethal effects of $\text{Al}_2\text{O}_3\text{NPs}$ in the tissue marker enzymes in *Oreochromis mossambicus*

The activity of alkaline phosphatase showed significant ($P < 0.05$) decrease in all treatment groups of gill tissue after $\text{Al}_2\text{O}_3\text{NPs}$ exposure (Fig. 4). Liver tissue showed significant ($P < 0.05$) increase in the activity of the alkaline phosphatase in both short-term and long-term

exposure groups (Fig. 4). The activity of brain marker enzyme, acetylcholinesterase decreased significantly ($p < 0.05$) after short-term and long-term exposure of $\text{Al}_2\text{O}_3\text{NPs}$ (Fig. 4). The activities of all marker enzymes in gill, liver and brain tissues were found similar to that of treatment groups after the treatment withdrawal period of 60 days (Fig. 4).



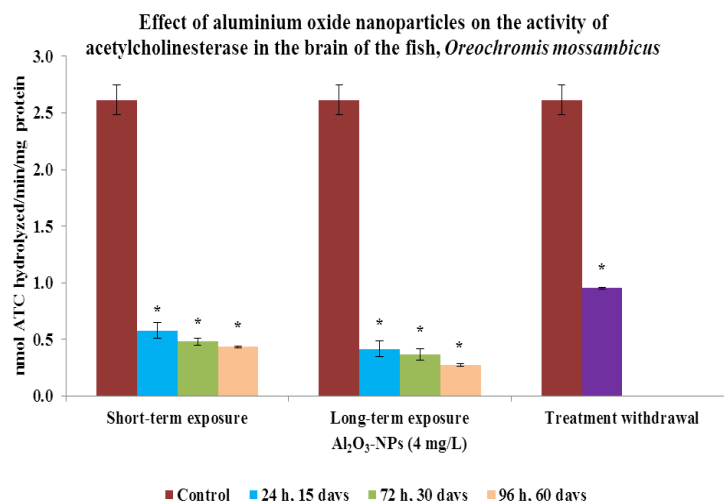


Fig. 4: Effect of Al_2O_3 NPs in the tissue marker enzymes in the fish, *Oreochromis mossambicus*.

DISCUSSION

The present study provides information on the toxic effects of one of the metal oxide nanoparticles, Al_2O_3 NPs in the freshwater fish, *Oreochromis mossambicus*. The mechanism of toxicity of nanoparticles were evaluated by assessing the antioxidant defense system in gill, liver and brain tissues after short and long exposure periods. It is well-documented that exposure to nanoparticles generated reactive oxygen species (ROS) in cells or tissues. ROS generation is governed by several factors such as internalization of nanoparticles, size and surface area, solubility, aggregation, agglomeration, bioconcentration and biomagnification.^[27] Despite the larger sized nanoparticles, the small size nanoparticles undergo structural modifications and alterations in the electronic properties in the surface of the particle, which ultimately result in the formation of more reactive groups on the particle surfaces.^[28] As a result of the surface modifications with more reactive groups, the oxidants and free radicals present on the particle surface modulate in the generation of ROS. The concentration, duration and size of the nanoparticles also play major role in the generation of ROS and intensity of toxicity in organisms. Exposure to low concentration of nanoparticles for acute period has been shown a potent antioxidant defense mechanism to overcome the oxidative stress so that capable of recovering the redox balance.^[29] However, exposure to high concentration of nanoparticles even for short duration overwhelms antioxidant defense system that results in the induction of oxidative stress.^[29] The particle size is another factor for the toxicity of nanoparticles, where small sized nanoparticles undergo internalization in cells or tissues easily and resulted in more adverse effects when compared to the large-sized nanoparticles. This is evident by the conflicting findings on the median lethal concentration of Al_2O_3 NPs in the fish, *Oreochromis mossambicus*.^[13,14] Nanotoxicity of particles not only results in oxidative stress, but also leads to other disruptions such as cytotoxicity, unregulated cell signaling, DNA damage, apoptosis,

tumour formation, metastasis etc.^[30] Thus the present study was undertaken to investigate the toxic effects of Al_2O_3 NPs in the fish, *Oreochromis mossambicus*. The modified antioxidant defense system in gill, liver and brain attribute to the imbalance of redox potential in tissues, followed by the induction of oxidative stress. Further, the persistence of nanotoxicity is proved by the treatment withdrawal over a period of 60 days, which facilitate to highlight the crucial role of Al_2O_3 NPs induced ROS modulation in the fish.

The body weight and organ weights are important parameters in toxicology as it gives the direct impact of toxicant in the body as well as in different organs. Body weight of the fish remained unaltered whereas the weight of the gill and brain tissues showed alterations after Al_2O_3 NPs treatment and also in the treatment withdrawal group. However, the hepatosomatic index showed reduction only after 60 days of treatment and this could be due to the treatment related toxicity in the fish. The gill tissue is the primary target of toxicants as it is continuously exposed to the surrounding environment and gill performs the first line of defence against toxicants.^[31] Exposure of Al_2O_3 NPs for short-term and long-term durations decreased the activities of antioxidant enzymes as superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase. Superoxide dismutase and catalase are considered as the first set of defensive enzymes against the free radicals.^[32] The decrease in the activities of superoxide dismutase and catalase could be due to the generation of excessive reactive oxygen species as a result of Al_2O_3 NPs exposure. Reactive oxygen species generation plays an important role in the induction of oxidative stress in tissues.^[33] The depletion in the activity of superoxide dismutase in turn could result in the accumulation of reactive metabolites like superoxide, hydrogen peroxide etc. which in turn diminishes the activities of other antioxidant enzymes as catalase, glutathione reductase and glutathione peroxidase. Failure in the neutralization of excessive ROS by the reduction in the activities of

antioxidant enzymes are reflected by the rise in the levels of hydrogen peroxide generation and lipid peroxidation in gill tissues. There are evidences that nanoparticles alter the prooxidant-antioxidant system through reactive oxygen species generation and oxidative stress.^[34] Lipid peroxidation is believed as the first step of membrane damage^[35] and is used as important biomarker of tissue damage in toxicology. Increase in the level of lipid peroxidation in gill clearly demonstrates that Al₂O₃NPs exposure resulted in the destruction of polyunsaturated fatty acids thereby could alter the amount of polar lipids in the cell leading to change in the permeability of gill epithelial membrane. The activity of alkaline phosphatase in gill tissue decreased in all treatment groups reflecting the cellular oxidative damage induced by Al₂O₃NPs exposure. Similar observation has been reported in the gill of fish *Pseudotroplus maculatus* treated with fullerene (C₆₀).^[36] Thus the study reflects the nanotoxicity in gill tissues when exposed at sublethal concentration of Al₂O₃NPs in the fish, *Oreochromis mossambicus*.

The liver is the important organ that performs many functions necessary for survival of the animal, which includes breakdown of nutrients to builds up body tissues, as storage reserve, to eliminate harmful micro-organisms, regulation of several metabolic processes and for the disposal of toxins by detoxification processes. In present study Al₂O₃NPs induced alterations in the antioxidant defense system of liver tissue was evaluated. The activities of all antioxidant enzymes except glutathione peroxidase showed no significant changes after short-term exposure. This could be due to defensive mechanism of liver tissue to eliminate the free radicals formed as a result of Al₂O₃NPs exposure. However, when the treatment period is prolonged for more than 15 days showed reduction in the activities of all antioxidant enzymes. This reflects the failure of hepatic antioxidant enzymes to eradicate the free radicals formed, which is evident by the increase in the level of hydrogen peroxide generation. Lipid peroxidation was also found increased in time-dependent manner after Al₂O₃NPs exposure. Lipid peroxidation is an autoxidation process initiated by the generation of free radicals such as hydrogen peroxide on phospholipids or polyunsaturated fatty acids, resulting in the formation of various aldehydes, which are highly reactive with other cellular components and the extracellular matrix. Among reactive aldehydes, malondialdehyde is a toxic aldehydic end product of lipid peroxidation which is evaluated in the present study. The increase in the production of malondialdehyde reflects alterations in tissue functioning and Al₂O₃NPs associated oxidative stress in liver tissues. The present observations coincides with other studies where exposure to fullerene (C₆₀) and silicon dioxide nanoparticles induced alteration in the antioxidant status of liver tissues in the fish, *Pseudotroplus maculatus* and *Oreochromis mossambicus*, respectively.^[37, 38] Alkaline phosphatase is one of the liver marker enzymes widely used to diagnose the normal function of liver tissues.

Phosphatases catalyze the hydrolysis of organic esters of phosphoric acids, and the highest activity is associated with many pathologic conditions such as liver diseases or tissue damage.^[39] In the present study, the activity of alkaline phosphatase enzyme was found increased in liver tissues of treatment groups which may be due to Al₂O₃NPs induced tissue damage.

It is well known that fish are more intelligent than they appear and holds many areas involved in memory, cognitive power that match or exceed than those of higher vertebrates. The protection of brain from the exposure to environmental toxicants is achieved by the restriction in the entry of molecules by an effective system called blood-brain barrier. Owing to the small size of the nanoparticles, it can easily cross the blood-brain barrier and accumulate in the brain tissue. In the present study, the activities of antioxidant enzymes superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase decreased in the brain tissues after Al₂O₃NPs exposure in both short-term and long-term durations. The levels of hydrogen peroxide generation and lipid peroxidation increased as a result of failure of antioxidant defense enzyme system. The present findings indicate the induction of oxidative stress in brain tissue after Al₂O₃NPs exposure. The results coincides with another study that exposure to silver nanoparticles induced toxicity in brain tissue by altering the activities of antioxidant enzymes in *Oreochromis niloticus* and *Tilapia zillii*.^[40] Oxidative stress is responsible for several neurodegenerative diseases in brain including Alzheimer's disease.^[41] Acetylcholinesterase (AChE) is a key enzyme in the nervous system which are specialized carboxylic ester hydrolases that break down esters of choline. Decreased activity of acetylcholinesterase indicates the neurotoxicity of exposed nanoparticles in the fish, *Oreochromis mossambicus* and the results coincides with another study when nonylphenol exposed to the brain of *Pseudotroplus maculatus*.^[42] Irreversible alterations in the antioxidant defense system as a result of Al₂O₃NPs exposure was further proved by the treatment withdrawal for a period of 60 days, which showed the decrease in the activities of antioxidant enzymes in gill, liver and brain with concomitant increase in the levels of hydrogen peroxide generation and lipid peroxidation. The results confirm the persistent toxic effects of Al₂O₃NPs thereby leading to irreversible tissue damage to the exposed animal.

CONCLUSIONS

The present findings suggest that sublethal exposure of Al₂O₃NPs caused oxidative imbalance and thereby induce oxidative stress in gill, liver and brain tissues of the fish *Oreochromis mossambicus*. Moreover, the oxidative damage of Al₂O₃NPs is proved as irreversible by treatment withdrawal study. Thus Al₂O₃NPs exposure in the aquatic ecosystems may pose a threat for the life of the organism when exposed even at sublethal concentration. It is evident from the present observations

that induction of oxidative stress as the mechanism behind nanotoxicity of Al_2O_3 NPs and further clarification is needed for the prediction of exact mechanism behind the nanotoxicity.

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Conflict of interest

No conflict of interest.

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