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ALTERATIONS IN RENAL MARKERS OF TILAPIA FISH EXPOSED TO SILICON DIOXIDE NANOPARTICLE

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AUTHORS' CONTRIBUTIONS
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ABSTRACT

The present study investigated the impact of sublethal concentrations (60, 100 and 140ppm) of SiO_2 nanoparticle on freshwater fish *Oreochromis mossambicus*, an environmentally relevant and commonly available fish. Acute studies on the plasma renal markers revealed a dose dependent elevation of urea and creatinine level in the treated fishes compared to that of control group, which indicated a possible damage to the renal tissues of the fishes. Further histopathological studies revealed alterations in bowman's capsule, glomerulus, sinusoidal space, vacuolated proximal and distal tubules, haemorrhage, membrane damages in blood cells, deposition of melanomacrophages and degeneration of renal tubules, which helped us to ascertain the toxicological effects of SiO_2 nanoparticle.

Keywords: SiO_2 nanoparticle; histology; fish kidney; *Oreochromis mossambicus.*

1. INTRODUCTION

Nanoparticles are well known for environmental remediation of pollutants, however currently they have become one of the emerging contaminants in aquatic bodies [1]. Nano-ecotoxicology deals with the identification and prediction of the effects of nano-sized materials in the ecosystem [2]. Fishes being an important aquatic organism are considered as a good biological model for toxicological studies and various changes in their organs were reported as the early toxic effects due to the exposure of contaminants [3].

Kidney, being a vital fish organ well known for their excretory and osmoregulatory functions [4,5], which receives a huge amount of post-branchial blood and therefore the renal damages in fishes could certainly used as indicator of environmental toxicants [6,7]. Renal tissues involved in hematopoiesis and blood filtration [5,8]. Teleost kidney has the ability to filter blood borne particles including nanoscaled materials [9]. Renal function test are considered as an important toxicological biomarker to assess the level of creatinine and uric acid in fish kidney [10] and histopathological studies helps in the

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identification of structural alterations in renal tissues [11,12].

Silicon-based nanomaterials were predominantly used in a variety of consumer products owing to their unique characteristics features, which may results in the possible environmental discharge of these particles [13,14]. Silicon dioxide (SiO_2) nanoparticle toxicological studies to freshwater fishes were sparse and the available literature described about bioaccumulation [15], alterations in DNA, hematology, antioxidant mechanism [16,17] and also demonstrated histopathological conditions [18,19]. However very limited studies have conducted fish renal toxicity following different nanoparticle exposure and the available literature on SiO_2 NP induced renal damages are scanty, which prompted us to undertake this novel study on freshwater tilapia fish (*Oreochromis mossambicus*).

2. MATERIALS AND METHODS

2.1 Characterization and Sonication of NPs

Silicon dioxide nanoparticles received from Analytical Chemistry Division, Bhabha Atomic Research Centre (Trombay, Mumbai). Field emission scanning electron microscope (FESEM) analysis was conducted to understand the morphological nature of nanoparticle.

A stock solution of SiO_2 NP was prepared in distilled water and sonicated for 30 mins in a water bath type sonicator (100W, 50KHz) to disperse the particles. Different concentrations of NP solutions were obtained by diluting the stock solution with distilled water and further sonicated for 30 mins immediately prior to the test [20].

2.2 Experimental Fish and Acclimatization

The freshwater fish *Oreochromis mossambicus* is a staple food fish in Southeast Asia [21], which is rated as one of the most invasive species in the world [22] with high tolerance to wide range of ecological conditions and temperatures. Fishes were collected from local pond in Tiruchirappalli district, Tamil Nadu (South India) and acclimatized in the Environmental Research laboratory, Jamal Mohamed College, Tiruchirappalli.

2.3 Experimental Design

For the toxicity assay, healthy fishes were transferred to separate test vessels (30L, n=16) and allowed to adapt for one more day prior to the starting of the experiment. Different concentrations of sonicated nanoparticles (60, 100 and 140ppm) were used for the sublethal toxicity test along with a control. Fishes were not fed for 24 hrs prior to the commencement of the experiment and during the experimental period so as to minimize the risk of nanoparticle absorption in the food or fecal materials [23] and to minimize the dissolved organic carbon in the vessel [24]. Water parameters and temperature were maintained accordingly.

2.4 Blood Collection and Renal Marker Analysis

Blood samples collected from the control and treated group fishes through vein puncture after 96 hrs were immediately transferred into EDTA vials [25]. Blood plasma was collected from the EDTA container and used for renal biochemical marker (creatinine, urea) analysis at Clinical lab, SMS Hospital (Woraiyur, Tiruchirappalli) using a semi-automatic biochemical analyzer [26].

2.5 Assessment of Renal Histological Alterations

The dissected kidney tissues of control and treated fishes were immediately fixed in 10% formalin for 48 h. The preserved tissues were subjected to standard histological procedures [27]; dehydrated in an alcohol series and cleared in xylene, infiltrated with liquid paraffin at 62°C, and finally embedded in paraffin blocks. Sections of 5-8 µm thick slices were made using an ultra-microtome (Leica Microsystems) and stained with Haematoxylin and Eosin. Then the slides were mounted with DPX and observed under a light microscope (Leica DM750, Switzerland) for assessing the histological alterations.

2.6 Statistical Analysis

Renal function marker values were expressed as mean with standard error (SE) and significance differences between control and treated groups were analysed using the One way ANOVA tool in SPSS software (version 20.0) and values found significant at $P<0.001$.

3. RESULTS

3.1 Characterization of NP

FESEM analysis revealed SiO_2 NP morphology showing amorphous nature with an average particle size of 32nm as shown in Fig. 1.

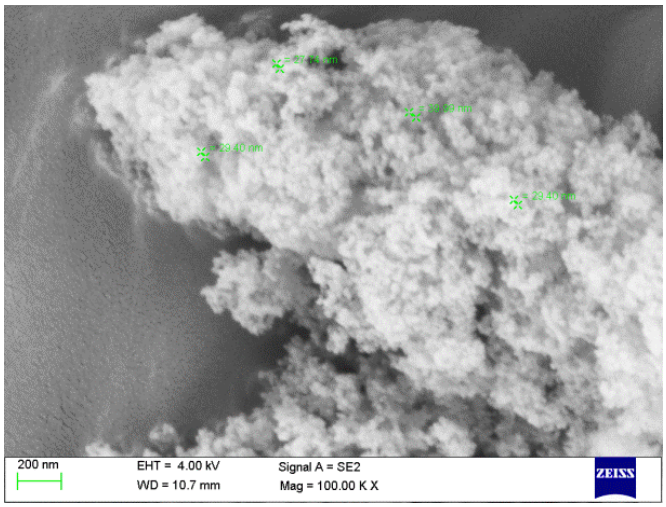

Fig. 1. FESEM image of SiO_2 NP

Table 1. Blood plasma renal markers in SiO_2 NP treated *Oreochromis mossambicus* (n=12)

Experimental concentration of SiO_2 NP	Blood plasma renal markers	
	Urea (mg/dL)	**Creatinine** (mg/dL)
Control	5.2620±0.011	0.3992±0.003
60ppm	6.0733±0.010	0.4367±0.004
100ppm	6.9008±0. 005	0.5825±0.005
140ppm	7.6430±0.006	0.6550±0.008

**Data were expressed as mean±standard error (SE)*

3.2 Blood Plasma Renal Markers

Table 1 depicted the blood plasma renal marker profile of *Oreochromis mossambius* after 96 hours sublethal experimental exposure to SiO_2 NP. Results revealed a dose dependent increase ($p<0.001$) of urea and creatinine concentrations among the treated fishes when compared to control group, with a maximum value recorded in fishes exposed to higher concentration of SiO_2 NP (140ppm).

3.3 Renal Histological Observations

Kidney of control fishes showed normal morphology of glomerulus (G), proximal and distal tubules (T) as showed in Fig. 2, whereas the SiO_2 nanoparticle treated fishes revealed renal histopathologcial conditions (Fig. 3).

Fishes exposed to lower concentration of nanoparticle (60ppm) displayed mild alterations in bowman's capsule (BC), glomerulus (G), proximal (P) and distal (T) tubules along with initiation of vacuolation (V). Degeneration of the renal tubules (D), membrane damages in the blood cells (MD) as well as the presence of melanomacrophages (M), haemorrhage (H), alterations in sinusoidal space (SS) and increased space in between glomerulus (G) was observed in 100ppm group. Severe alterations in the proximal tubules (P), increased haemorrhage (H), elevated membrane damage in blood cells (MD), enlarged bowman's capsule (BC), higher deposition of the melanomacrophages (M), vacuolation (V) and severe degeneration of renal tubules (D) were observed in fishes at the higher concentration group (140ppm).

4. DISCUSSION

This is first study of this kind, in which the renal biomarkers of *Oreochromis mossambicus* was assessed to identify the toxicological impact of SiO_2 nanoparticle. Our results demonstrated increased kidney functional markers (urea and creatinine) in the blood plasma samples of treated fishes and were in accordance with the findings of Hadi et al. [28], who reported similar blood plasma changes in *Tilapia zillii* upon aluminium exposure. Elevated levels of serum creatinine and uric acid were observed in *O. niloticus* following sublethal exposure of zinc nanoparticle [29-31] and silver nanoparticle [32]. Similar effects were also observed in *Clarias gariepinus* exposed to mercury chloride [33] and in *O. niloticus* due to the exposure of methyl-testosterone [34] and copper sulfate [35]. The above elevation in urea and

creatinine among the blood samples of treated fishes may be associated with the glomerular insufficiency, impaired carbohydrates metabolism, increased catabolism of protein and muscle tissues [28,36].

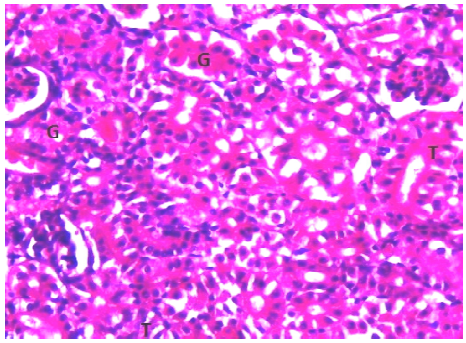

Fig. 2. Renal histology of *Oreochromis mossambicus* in control group showing normal morphology of glomerulus (G), proximal and distal tubules (T)

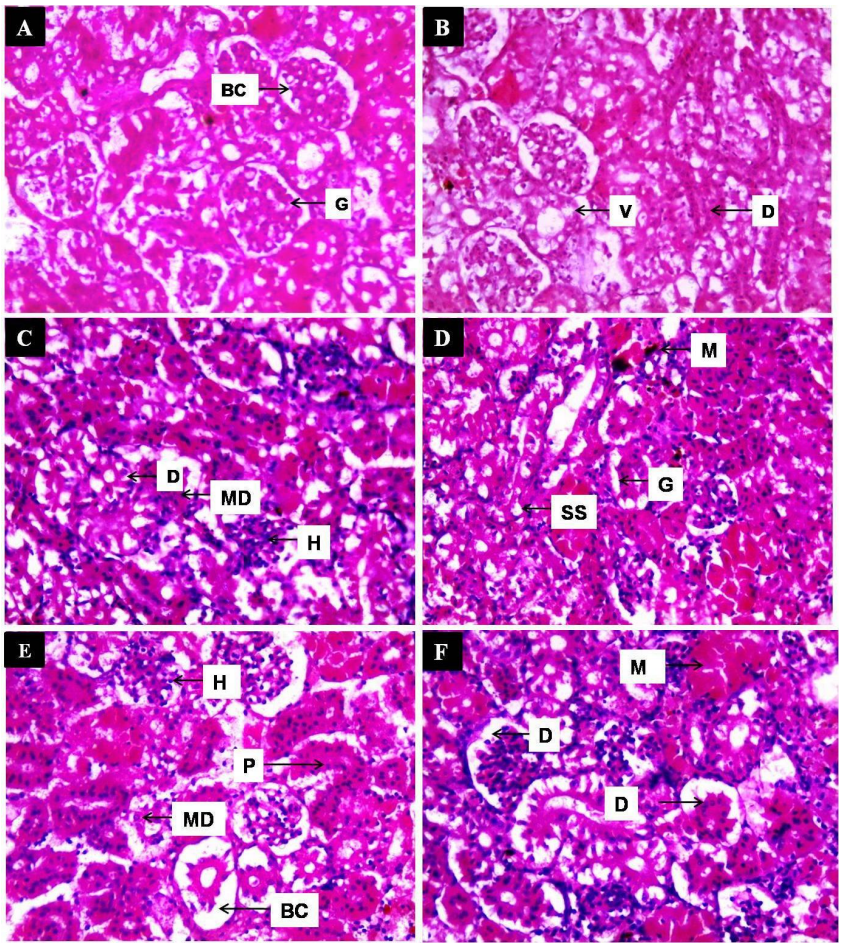

Fig. 3. Renal histology of *Oreochromis mossambicus* following SiO_2 nanoparticle exposure at 60ppm (A-B), 100ppm (C-D) and 140ppm (E-F) showing alterations in bowman's capsule (BC), glomerulus (G), sinusoidal space (SS), proximal (P) and distal (T) tubules along with vacuolation (V), haemorrhage (H), membrane damages in blood cells (MD), deposition of melanomacrophages (M) and degeneration of renal tubules (D)

The present study revealed renal histopathological conditions in the treated tilapia fishes, which are in agreement to the elevated levels of plasma renal markers in the experiment. A previous study has reported similar renal tissue damages in the same species after sublethal exposure with aluminium dioxide nanoparticle [37]. It is quite worth to note that the previous reports on experimental exposure to different nanoparticles on various freshwater fishes have resulted in diverse renal histological alterations. Degenerated renal tubules, enlarged bowman's space and sinusoidal spaces were observed in copper nanoparticles treated *Cyprinus carpio* [38]. Sub-chronic exposure of zinc nanparticle resulted in mononuclear cell infiltration, tubular deformations, glomerular deformation and hemorrhage, necrosis, melanomacrophage deposition and glomerular expansion in *O. niloticus* [39].

Cellular degeneration, shrinkage in malpighian corpuscles, dispersed infiltration of leukocytes and presences of melanomacrophages were observed in silver nanoparticle treated *C. gariepinus* [40]. Al-Bairuty et al. [41] has reported epithelial damages in renal tubules and increased bowman's space in *Oncorhynchus mykiss* following the exposure to copper nanoparticles and copper sulphate. Copper nanopartcle treated *Rutilus rutilus caspicus* showed shrinkage of glomerulus, severe degeneration of tubules, interstitial tissues and glomerulus along with higher aggregation of macrophages and increased interstitial cells [42]. Mild histopathological changes such as congestion, haemorrhage as well as tubular degeneration and intra-tubular pigment accumulation were observed in *C. carpio* exposed to zinc oxide nanoparticle [43]. ZnO nanoparticle exposure has also resulted in necrosis and deformations of renal tubule epithelium of *O. niloticus* [44]. Necrosis, damages in epithelial cells of the renal tubules and increased bowman's space were observed in the copper nanoparticle treated *Prochilodus lineatus* [45]. Similar renal histopathological changes were also observed in different fishes exposed to various environmental contaminants [7,12, 46-51].

5. CONCLUSION

The present study has revealed blood plasma alterations of renal markers and the structural damages in kidney tissues as evidenced from histological studies, which helped us to establish the renal tissue damages in *Oreochromis mossambicus* due to silicon dioxide nanoparticle exposure at sublethal concentrations. Our study has proven that the freshwater tilapia fish could be used as a bioassay organism to evaluate the toxicity of nanoparticle, and renal markers could be utilized as an efficient eco-toxicological tool. The results of our work can serve as a baseline data for further toxicological studies using different biomarkers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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