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Hematology of freshwater fish, *Labeo rohita*, fingerlings exposed to silica oxide nanoparticles

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Abstract
The fate and result of nanomaterials within the surroundings has raised concern pointing towards their environmental risk to aquatic organisms. Silica nanoparticles (SiO_2-NPs) have wide applications in diverse fields such as medicine, cosmetics, engineering etc and are unavoidably released into the environment. However, the ecotoxicological effects of SiO_2-NPs on the freshwater fish remain out of action. The aim of this study was to determine the effect of Silica oxide nanoparticles on hematology and survival of freshwater fish *Labeo rohita* fingerlings. The fingerlings of average length 7.5 ± 1.5 cm, weighing 50±10 g were exposed to different Silica oxide doses (T1, T2 and T3) along with a Control group (without any Silica oxide dose) in triplicates and further effects on survival and hematology were recorded after an exposure period of 15 days. The result of the present study suggested that survival of the fish and alterations in hematology are both, dose dependent and time dependent in terms of exposure to the Silica oxide nanoparticles. A significant decrease in the blood parameters (Total erythrocyte corpuscles, Total leucocyte corpuscles, Hemoglobin and Mean corpuscular hemoglobin) of fish were recorded, both, at the end of the experiment and with increasing concentration of nanoparticles in different treatment groups. Meanwhile the of the fish fingerlings survival was not affected in any of the SiO_2 treated groups during the experimental period.

Keywords: *Labeo rohita,* Survival, SiO_2 NPs, Hematology

1. Introduction

Nanotechnology is one of the quickly developing multidisciplinary science encompassing the fields of physics, chemistry, biology and engineering, that involves the assembly and unharness of several nano-sized particles into the setting. Nanoparticles have different characteristics from those of corresponding bulk materials and are utilized in varied industries and consumers goods [1]. In recent years, particularly the production and application of some particular nanoparticles like Silver has increased, accounting for over 50% of the global nanomaterial products in 2015, with expectation in rise nearly 13% until 2024 [2]. Some nanoparticles are identified to exist naturally and are thus, in direct and continuous contact with the living systems. Whereas many nanoparticles are synthesised by artificial means and therefore the increase within the rate of synthesis and therefore the intentional or unintentional unharness of nanoparticles into the system may adversely have an effect on living organisms as well as bacterium, algae, insects, birds and mammals; like synthetic amorphous silica, an attractive manufacturing nanomaterial have penetrated into people's lives and productions [3]. The properties, behaviour and environmental fate of designed nanoparticles are totally different, which lead to an unforeseen impact on setting and living system [4]. Engineered nanoparticles embrace nanotubes, nanospheres, nanowires, quantum dots and are characterised for its novel physical, optical, thermal and biological properties. Among the creation of manipulated nanoparticles, silica nanoparticles (SiO_2-NPs; nano silica) are wide employed in engineering, industries and biomedicine, notably as tools for targeted drug and cistron delivery.

However, the toxicity of silicon dioxide nanoparticles might be due to altered chemistry properties such as size, cell type, dose and even specific coatings, substance incorporation, or surface modifications, that cause health hazards in non-target organisms [5]. Silicon dioxide nanoparticles are the advisedly made nanoparticles having applications within the medicine fields. Crystalline silica as compared to natural amorphous silica is generally considered as more harmful, since the toxicological potential of silica has so far been linked to its crystallinity [6]. It is widely used for in vitro and in vivo drug and cistron delivery, siRNA delivery, biosensing, sunblock lotions, food, nanomedicine, cancer medical aid and chemical

Industries [7, 8]. The main route of exposure is from different sources like producing laboratories, sewerage treatment plants, landfills and runoff water. Thanks to the tiny size, silicon dioxide nanoparticles have potentialities of acquisition through different penetration routes and it has been rumoured that nanosized particles are additional poisonous than the bulked size [9]. Silicon dioxide nanoparticles are extremely stable and protracted, where the particle size influences the toxicity, tissue distribution, metabolism and excretion. One of the studies has ascertained that submicron-sized silicon dioxide particles (100 or 200 nm diameter) considerably increased the incidence and severity of liver inflammation, whereas the consequences of nano-sized particles (50 nm diameter) were non-significant [10]. Therefore, the toxicity and environmental persistence of nanoparticles in living organisms depend on numerous factors like stability, bio-persistence and bioaccumulation properties [11, 12, 13]. Once free into the environment, the particles can move with the environment like air, water and land. It has been earlier rumoured that silica nanoparticles additionally cross the barrier in mice [14]. In vitro studies, exploitation human cell line models additionally pointed that SiO_2-NPs induced mitochondrial pathology, aerobic stress and necrobiosis [15]. In endothelial EAHY926 cell line, SiO_2 exposure resulted in minimized cell viability and toxicity [16]. In the rat, intra nasal administration of silica nanoparticles resulted in hepatotoxicity characterised by depletion of inhibitor system and loss of traditional liver architecture [17]. Similarly, one in every of the nanomaterials C60 has been shown to induce aerobic stress in hepatocytes of the seafood, *Pseudetroplus maculatus* [18]. Inexperienced synthesis of silver nanoparticles from pepper vine at sublethal concentrations has led to the build-up of silver and elicited aerobic stress and histopathological alterations in gill, liver and excretory organ of the fish, *Labeo rohita* [19]. Aquatic organisms, particularly fishes, are extremely prone to the uptake of nanoparticles from the exposed aquatic setting.

However, the cells of fish are extremely equipped with inhibitor defence system to eradicate the toxicant-induced aerobic stress. The inhibitor implements consists of each catalyst and non-enzymatic defensive mechanisms. The non-enzymatic inhibitor system as an example consists of antioxidant, vitamin E, carotenoids, amino acids and therefore the catalyst antioxidant system includes enzyme, catalase, glutathione enzyme and oxidase and others [20]. Impairment of inhibitor system ends up in the generation of reactive oxygen species and aerobic stress, that are liable for the peroxidation of membrane lipids and thereby inflicting tissue damages [21]. The present study was applied to analyse the acute toxicity of silica nanoparticles on the freshwater fish *Labeo rohita*. The results obtained from the fish model will be thought of as an indirect end to extrapolate the impact of such nanoparticles on higher organisms, as well as humans.

2. Materials and Methods

2.1 Experimental site

The experiment was conducted in the Wet Lab of College of Fisheries, Gobind Ballabh Pant University of Agriculture and Technology, Pantnagar, with coordinates, 290ºN latitude, 79.30ºE longitudes and present in the Tarai belt of Himalayan foothills at an altitude of 243.3m above mean sea level (MSL). Pantnagar's humid sub-tropical climate is characterised by dry, hot summers and bitterly cold winters, with mist usually occurring in late December 2020 and early February, 2021.

2.2 Procurement of fish and acclimatization

Labeo rohita fingerlings weighing 50±10 g and length 7.5 ± 1.5 cm were collected from the hatchery of College of Fisheries, G.B.P.U.A.T, Pantnagar, Uttarakhand. Fishes were acclimatised in glass tanks filled with 50L water, supplied with good aeration and dechlorinated water prior to the experiment for 2 weeks.

2.3 Experiment design

SiO_2-NP were obtained from Department of Chemistry, G.B.P.U.A.T, Uttarakhand, India. The nano-dispersions were prepared and maintained as stock. The fishes were exposed at different concentrations of Silica oxide and a control (without Silica oxide). 20 fishes each in 3 treatments and a control group were distributed randomly in triplicates.

2.4 Survival

Fishes were randomly selected from the stock and exposed to different concentrations of SiO_2 NPs in different glass aquaria. For the present study 2, 4 and 6 mgl^{-1} SiO_2 concentrations were selected based on the survival to mortality ratio and the experiment was conducted for a period 15 days. Zero mortality was found in the *Labeo rohita* fingerlings at the end of the experiment.

2.5 Collection of blood

Blood from control and SiO_2 NPs treated groups was drawn and the collected blood sample was transferred into small plastic vials, which were previously coated with EDTA. The whole blood was used for the estimation of Hb, MCH, RBC and WBC counts. Hb estimation was done using Sahli's hemometer while RBC and WBC counting was done using Neubauer's Chamber.

3. Results & Discussion

At the end of 15 days the RBC, Hb and MCH was found to be lower while an increase in WBC count was observed in groups exposed to SiO_2 NPs when compared to control groups and groups with lower concentration of Silica oxide nanoparticles as shown in Fig. 1, Fig. 2, Fig. 3 and Fig. 4 respectively.

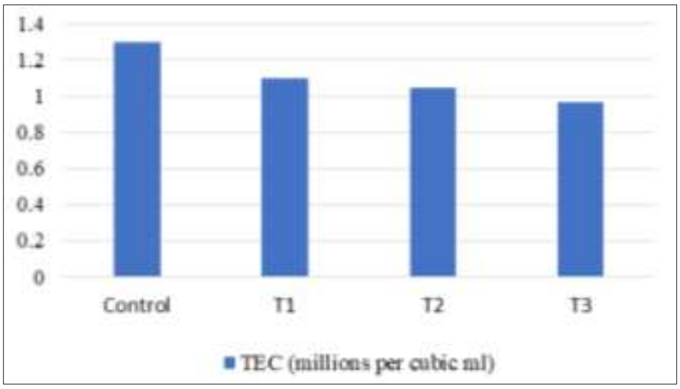

Fig 1: TEC (millions per cubic ml)

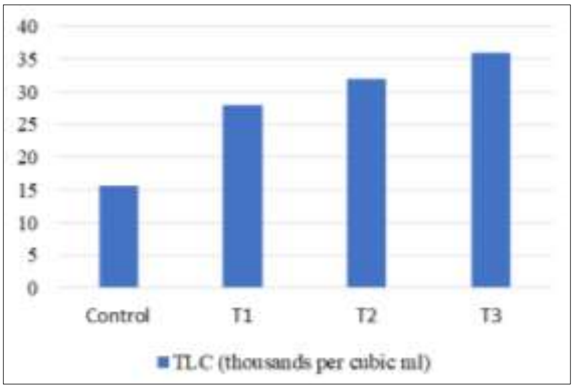

Fig 2: TLC (thousands per cubic ml)

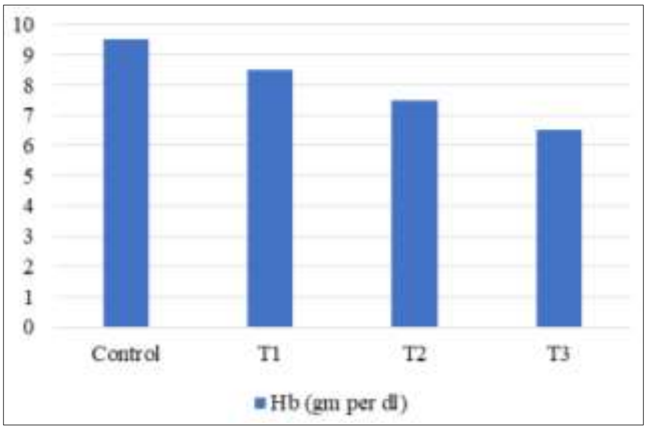

Fig 3: Hb (gm per dl)

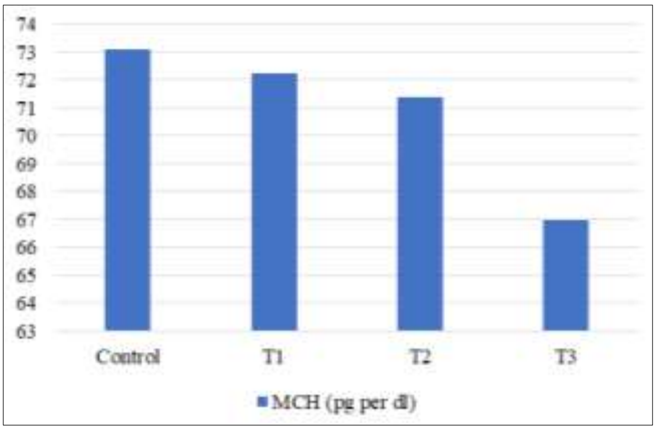

Fig 4: MCH (pg per dl)

A similar decline in RBC along with Hb was also recorded in *Labeo rohita* fingerlings exposed to Silicon dioxide nanoparticles (1, 5 and 25 mgl^{-1}) at the end of 72 hrs [22]. A 21-day exposure of common carp to Silver nanoparticles showed significantly lower number of red blood cells (RBCs) in fish exposed to 25% and 50% of Ag-NPs LC_{50} (96h) than the treatment of 12.5% and the control [23]. From the haematological investigation of *Labeo rohita* exposed to Silver nanoparticles concluded that the levels of all the haematological parameters (RBC, WBC and Hb) were reduced at 25 mgkg^{-1} concentration when compared to control fish samples [24]. In a study, Nile tilapia (*Oreochromis niloticus*) fingerlings were exposed to Silver nanoparticles, and at the end of 1 week exposure, the amount of red blood cells (RBC) and the percent hematocrit (%Hct) were decreased significantly particularly in those exposed to 100 mg AgNPkg^{-1} [25]. The alterations in the haematological parameters were supposed to be the result of stressful circumstances which disrupts the metabolism and normal functioning of the fish physiology [26]. A decline in WBC count was also recorded in zebra fish exposed to TiO_2 [27]. Significant decrease in blood cell-related parameters was observed in rainbow trout (*Oncorhynchus mykiss*) exposed to Cu-NPs [28]. The observed haematological disturbances in this study may have been caused by the accumulation of SiO_2 NPs in the gills, which could lead to respiratory problems. Nanoparticles accumulating in the gills can cause organ damage and respiratory disturbances [29, 30, 31, 32]. Hematological disruptions can also be caused by oxygen deficiency caused by gill damage.

4. Conclusion

The present study demonstrated that the hematology of the exposed fingerlings of fresh water fish, *Labeo rohita*, was deteriorated when exposed to different and increasing concentration of SiO_2 nanoparticles. Therefore, the results showed that status of fish health was remarkedly impaired with higher concentrations. However, the concentrations selected for different treatments does not have any lethal and sub lethal effects on the exposed fingerlings during the experimental period. The findings may contribute to the status of Silica nanoparticle toxicity on aquatic organisms.

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