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Smita Shukla (Nee Trivedi)
School of Applied Science, Shri
Venkateshwar University,
Gajraula, Uttar Pradesh, India

Rajesh Singh
School of Applied Science, Shri
Venkateshwar University,
Gajraula, Uttar Pradesh, India

SK Singh
Professor and Head,
Department of Civil
Engineering and
Environmental Technology,
Delhi Technical University,
Delhi, India

Corresponding Author:
Smita Shukla (Nee Trivedi)
School of Applied Science, Shri
Venkateshwar University,
Gajraula, Uttar Pradesh, India

Effect of aqueous silver nanoparticles on the gills of fresh water cat fish *Clarias batrachus*

Smita Shukla (Nee Trivedi), Rajesh Singh and SK Singh

Abstract

During the past development of Nano technology and the probably of its side effects on aquatic body organs, this study investigate the effect of nano silver administration on histology of gills in catfish *Clarias batrachus* following chronic exposure to sub lethal concentrations of silver nanoparticles. When fish were exposed silver nanoparticles size (10nm), (N10) 1/15th of 96 h Lc 50 value for 10 and 20 days. In the gills, the epithelial cells as well as the pilaster cells showed signs of degeneration. The epithelial cells at places lost their cell walls. The secondary lamellae became swollen and the epithelial cells became hypertrophied after an exposure to of 0.10 mg l⁻¹ of Silver nanoparticles for 10 days after 20 days of exposure the epithelial cells and basement membrane became further separated from pilaster cells. The secondary lamellae of the alternate side were more affected. The current findings indicate effect on *Clarus batrachus* chronic effect on *Clarias batrachus*; therefore preventing the entry of silver nano-materials into the aquatic environment would seem to be essential.

Keywords: Silver, *Clarias batrachus*, nano toxicology

Introduction

Effect on Cat Fish *Clarias batrachus* exposed to Aqueous silver nano particles in nano scale silver is the most important nanomaterial, as it is currently included in 23.52% of the nano products listed in consumer product inventories. As a result there is a greater risk of AgNPs being released into the environment worldwide estimates indicate that 63^t of nano-silver enters water bodies of annually [7]. Thus understanding the effects of nano silver on aquatic organism in particularly important while several studies have already focused on the toxic effect of various silver nanoparticles in human [4, 8] as well as different aquatic organism like fish [5, 6]. According to this study examined the histological changes in gills of cat fish *Clarias batrachus* following chronic exposure to sublethal concentrations (LC50) of silver nano particles.

The histological changes in gills due to silver nano particles have been studies by several workers [3, 6].

Material and Methods

The fresh water catfish *Clarias batrachus* which is locally known as mangur was selected (10+- cm) for the present study. They were kept in the aquarium and treated with 0.1% KMNO₄ to avoid any infection. The silver nanoparticle (Nanorex Janakpuri Delhi) will be procured from local market and used for the proposed study. The fish were fed with chopped goat liver (100g) and boiled egg (Half Egg/aquarium on alternate days except during the acute toxicity bioassay experiment. Fish of approximately equal size were selected for experimental and transferred to glass aquaria (40x30x35 cm). The experimental aquaria were set up with a parallel control. Food (as above) was given to both the experimental and control fish and water was renewed immediately after feeding. Six healthy specimens each were taken for the control as well as experimental groups.

Gill tissues were fixed for TEM adopting an in-house method. Briefly, tissues were fixed for 2 h in 2% paraformaldehyde/2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4), washed three times in 0.1M phosphate buffer for 5 min, and fixed in 1% osmium tetroxide in phosphate buffer for 1 h. The tissues were then washed in deionized water for 5 × 5 min, before being cut into 1-mm³ pieces, and subsequently suspended in 2% uranyl acetate for 1h. The tissues were then dehydrated in an ethanol series: 30, 50, 70, 90, and 100% (×2), in each for 10 min and embedded in TAAB resin.

The tissues were blocked in shallow planchets and placed in a 60 °C oven for 20 h. Tissues were sectioned on an ultramicrotome (Ultracut; Reichert) and examined for structural alterations and subcellular localization of silver using a Joel TEM 1400 transmission electron microscope.

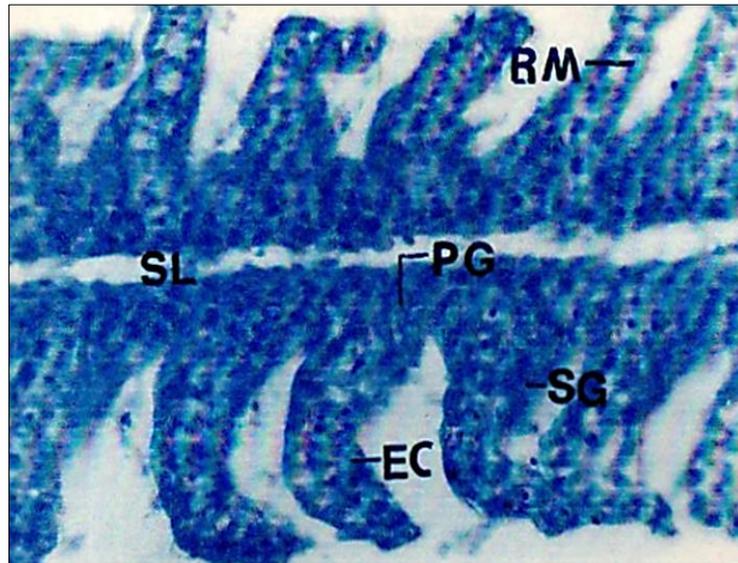
Result

Several histological changes to occur to fish exposed to silver nanoparticles. These responses involves damage in major organ such as a gills. The change in histopathological aspects are described below and the microphotographs showing the changes are presented the changes observed

this investigation were time dependent after exposure to Silver nanoparticles.

A. Gills of control fish

Histological, each hemibranch consists of a row of long thin filaments called primary gill lamellae. From primary gill lamellae a number of leaf like structure arise on both the sides to form secondary lamellae, which are the major seat of gaseous exchange. The primary lamellae are enveloped by a layer of mucous cells. Below the primary gill lamellae it consist a single layer of epithelial cells. (Fig. 1)



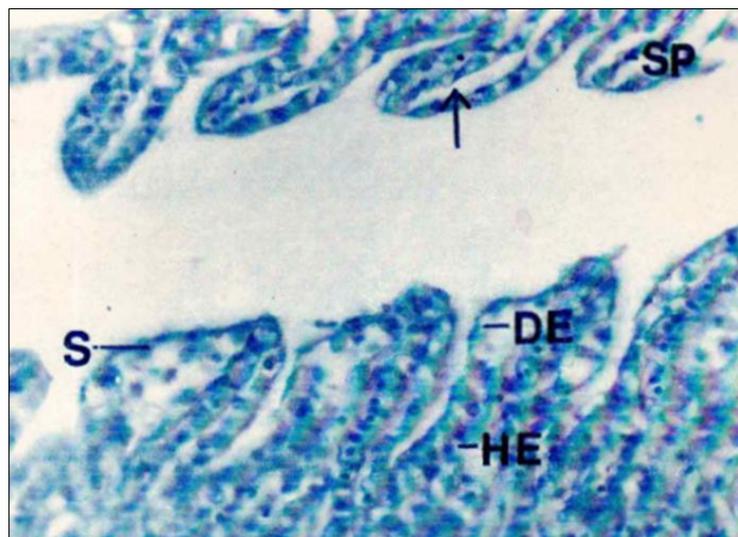
BM = Basement membrane, EC = Epithelial cell, PC = Pilaster Cell, PG = Primary gill lamella, SG = Secondary gill lamella, SL = Shrinkage of primary gill lamella

Fig 1: Photomicrograph of longitudinal section of gill of freshwater catfish, *Clarias batrachus* showing normal histological structure (x 400)

B. Gills after an exposure to 0.10 mg l⁻¹ of silver nanoparticles (1/15th of 96 h LC₅₀ value) for 10 days

When fish were exposed to 0.10 mg l⁻¹ of Silver Nanoparticles for 10 days the epithelial cell as well as the pilaster cells showed signs of degeneration in the epithelial

cells at places and have lost their cell walls. The separation of gills epithelium along the basement membrane from the pilaster cells took place leaving behind a space in between. The secondary lamellae had become swollen (Fig. 2).

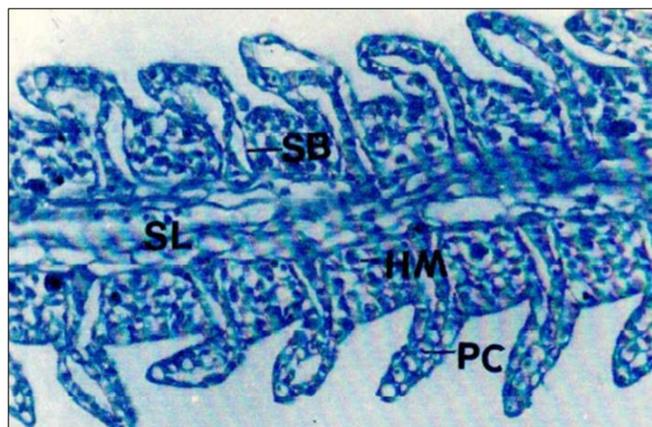


DE = Degeneration of epithelial cells, HE = Hypertrophy in the epithelial cells SL = Separation of epithelial cells from the pilaster cell, S = Swelling in secondary gill lamella

Fig 2: Photomicrograph of longitudinal section of gill of freshwater catfish *Clarias batrachus* showing shrinkage of pilaster cells, hypertrophy in epithelial cell separation of epithelial cells from the pilaster cells and degeneration of epithelial cells after an exposure to 0.10 mg l⁻¹ of silver nanoparticles for 10 days) x 400)

C. Gills after an exposure to 0.10 mg l⁻¹ of silver nanoparticles (1/15th of 96 h Lc50 value) for 20 days

When fish were exposed to 0.10 mg l⁻¹ of Silver Nanoparticles for 20 days, their pilaster cells in general had shrunken. The epithelial cells and basement membrane became further separated from pilaster cells. The epithelium at the base of the secondary lamellae became swollen due to appearance of vacuoles. The mucous cells become hypertrophied with vacuolization. Here again the secondary lamellae of the other side were more affected (Fig. 3)



HM = Hypertrophy of mucous cell, SB = Separation of basement membrane, SL = Skeleton of primary gill lamella, SP = Shrinkage of pilaster cell

Fig 3: Photomicrograph of longitudinal section of gill of freshwater catfish *Clarias batrachus* showing shrinkage of pilaster cells, separation of basement membrane and epithelial cells, hypertrophy of mucous cells after exposure to 0.10 mg l⁻¹ of silver nanoparticles for 10 days x 400)

Discussions

Gills being the primary sites of osmoregulation and respiration remain the main target organs for aquatic toxicants. The gills are vital organs of the fish responsible for respiration, accessory exertion and regulation of water salt balance. The toxicants, including silver nanoparticles which cause histopathological damage to the delicate structure of the gills.

In the present study, experiments were performed to find out pathological changes induced by Silver nanoparticles in gills of *Clarias batrachus*. Silver Nanoparticles brought about detachment of epithelial cells and basement membrane from the pilaster cells, haemorrhage between pilaster cells and hypertrophy of mucous cells. With the increased concentration of silver nanoparticles, the damage was accentuated as the gap between epithelial cells and pilaster cells, shrinkage of pilaster cells and haemorrhage were further increased. Branchial uptake of ionic silver and copper has been well documented in freshwater fish and appears to occur primarily through apical membrane sodium channels and the copper transporter protein^[1, 2]. The amount of copper associated with the gill after exposure to either copper nanoparticles or soluble copper was greater than control, but was the same in both cases. This suggests that gill is primarily taking up dissolved copper released from particles and that cellular uptake of intact copper nanoparticles or enhanced dissolution of copper nanoparticles is not a major factor. In contrast, exposure to silver nanoparticles produced significantly higher levels of silver associated with the gills than did exposure to only the soluble fraction. This suggests that the nanoparticles themselves are contributing to the gill burden of silver. There are several mechanisms by which nano particulates

may increase the gill silver levels. Nanoparticles may be trapped in the mucus layer of the gill as demonstrated for larger particles^[9, 10]. Nano particle trapped in this manner may not actually enter the cells but mucus entrained particles can also increase intracellular metal content by enhanced dissolution due to changes in water chemistry in the gill microenvironment including mucus complication^[11].

Conclusion

This study found that chronic exposure to silver nanoparticles caused various microscopic structural changes in tissue of *Clarias batrachus* therefore more attention should be paid to preventing the accidental or intentional release of silver nanoparticles into aquatic biomes.

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