ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2017; 3(6): 1266-1272
www.allresearchjournal.com
Received: 18-03-2017
Accepted: 24-05-2017

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

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

Effect in the kidney of the fresh water cat fish *Clarias batrachus* exposed to silver nanoparticles

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

Abstract
Despite increasing application of silver nanoparticles (NPs) in industry and consumer products there is still little known about their potential toxicity, particularly to organism in aquatic environment. To investigate the fate and effect of silver NPs in fish *Clarias batrachus*. The histological alterations in the kidney of a freshwater catfish *Clarias batrachus* exposed to sublethal concentration silver nanoparticle. The kidney exhibited shrinkage of glomeruli but the tubular structure was not affected after 10 days of 0.10mg/l silver nanoparticle. The glomeruli were further shurnkened along with the widening of tubular luman after 20 days of exposure. In the fishes exposed to 0.16 mg/l silver nanoparticle (10nm), the kidney showed distorted glomerular organization leaving a mass of cell and swollen tubular after 10 days of exposure. The epithelial cells of the tubules have lost the cell membranes and the area of lumen was now decreased after 20 days of exposure of silver nanoparticle. Therefore it is inferred that the harmful impact of silver nanoparticle on the kidney is dose and time dependent.

Keywords: Silver nanoparticles, *Clarias batrachus*

Introduction
Recently, a vast variety of nano-materials have been developed and nanotechnology has emerged as rewarding key research area in the modern scientific set-up. It is the science of nanoparticles that show new and different properties compared to what they exhibit on a macro-scale, enabling unique applications [1]. Due to the wide application of nano-materials in industry, agriculture, business, medicine and public health; nanotechnology has gained a great deal of public interest [2].

Silver found in the body of mammals (including humans) has no known biological purpose and is suspected of being a contaminant [3]. Silver, as ionic Ag+, is one of the most toxic metals known to aquatic organisms in laboratory testing, although large industrial losses to the aquatic environment are probably infrequent because of it's economic value as a recoverable resource [4]. Silver, however, is of concern in various aquatic ecosystems because of the severity of silver contamination in the water column, sediments, and biota.

Long-term industrial or medical exposure to silver and it's compounds may increase blood concentrations of silver to levels which can have toxic effects, such as induction of sarcomas, anemia, and enlargement of the heart [5]. It has been reported the toxicity of silver nanoparticles in zebrafish models. Their results suggest that silver nanoparticles induce a dose-dependent toxicity in embryos, which hinders normal development [6]. In fish and amphibian toxicity tests with 22 metals and metalloids, silver was the most toxic tested element as judged by acute LC50 values [7].

Regarding fast development of the nanotechnology and it's diverse applications, is very important having enough data on the probably it's side effects on the aquatic body organs. Therefore, these studies investigate the effects of nano-silver administration on histology of gill and kidney biochemical parameters in common carp.

Silver has been known to have antibacterial properties since Roman times, however, the increased use of nano-silver in a range of (as yet largely) experimental drinking-water treatment systems, its use in conjunction with ceramic filters and its perceived potential to be a water disinfectant that does not result in disinfection by-products (DBP) in the treated water have raised the profile of this chemical. Silver and AgNP have been shown to have general (i.e. not specifically water disinfection related) anti-bacterial properties against a range of both Gram-negative (e.g. Acinetobacter, Escherichia, Pseudomonas, Salmonella and Vibrio) and Gram-positive bacteria (e.g. Bacillus, Clostridium, Enterococcus, Listeria, Staphylococcus and Streptococcus) (2009) [8].

Some researchers have also demonstrated that fungi, such as Aspergillus Niger, Candida albicans and Saccharomyces cerevisia, are sensitive to silver In addition, a number of studies have suggested a biocidal action of AgNP against hepatitis B virus [9], HIV-1 syncital virus and murine norovirus.

Water-related applications

In terms of water-disinfection-related applications, silver is most commonly used in domestic water filters (either to reduce the level of biofilm growth within the filter or as an additional level of treatment). It is also quite commonly used in conjunction with copper ionization as a preventative measure against colonization of Legionella spp. in hospital hot water systems. AgNP are currently being tested in a number of experimental point-of-use (POU) treatment systems and ionic silver has been investigated for its potential for use as a secondary disinfectant (to reduce levels of chlorine) in drinking-water supplies. Silver ions (in combination with both copper and chlorine) have also been investigated for use in swimming pool disinfection.

Water disinfection efficacy

Numerous studies have been conducted on the disinfection efficacy of silver and AgNP applications against a range of microorganisms found in water. Although the majority of these have focused on bacterial disinfection, some have also looked at the impact on bacteriophages and viruses. In addition to the material below, which focuses on water disinfection, there is also a short section (Appendix 1) on the general disinfectant mode of action of Ag and AgNP.

Ionic silver applications

Efficacy of ionic silver for disinfection of potable water In the studies outlined below silver ion (Ag+) efficacy (generated from silver salts [AgNO3, AgCl] or produced electrolytically) was tested against a range of bacteria and the inactivation was principally assessed by the log reduction in bacterial numbers. Initial bacterial concentrations ranged from 3.5 cells/ml up to 1.5 x 107 cells/ml. Hwang *et al*. (2007) looked at the efficacy of silver ions (up to 100µg/l), derived from silver nitrate, against Legionella pneumophila, Pseudomonas aeruginosa and Escherichia coli (all at 1.5 x 107 cells/ml) in synthetic drinking-water (pH 7, temperature 25 °C). After a three hour contact time with the highest concentration of silver the following log reductions were reported: • 2.4 log reduction - L. pneumophila; • 4 log reduction – P. aeruginosa; • 7 log reduction – E. coli. Similar work was conducted by [10], where the efficacy of silver ions, derived from AgCl, against 3 x 106 cfu/ml of P. aeruginosa, Stenotrophomonas maltophilia and Acinetobacter baumannii was investigated. A 5 log reduction in P. aeruginosa was seen with 80µg/l Ag (the highest concentration used) after 12 hours. S. maltophilia was more sensitive to Ag, with a 5 log reduction seen after 6 hours when exposed to 80µg/l. For A. baumannii, however, a 5 log reduction was only seen after 72 hours exposure to 80µg/l Ag. Silvestry (2007) [11], investigated the inactivation of Pseudomonas aeruginosa and Aeromonas hydrophila by silver in tap water, with a view to assessing the possibility for using silver as a secondary disinfectant to replace or reduce the level of chlorine.

Dechlorinated municipal water (obtained from a groundwater source) was seeded with 106 cfu/ml bacteria and silver nitrate added to a concentration of 100 µg/l. Experiments were performed at pH 7 and pH 9 at 24 °C for both bacterial species and at 4 °C for P. aeruginosa. In addition, 3 mg/l humic acid was added to the dechlorinated tap water (to simulate a surface water source). Inactivation of the bacteria was time Spring 2014 3 Silver: water disinfection and toxicity and temperature dependent and after 8 to 9 hours of exposure to 100 µg/l silver at 24 °C, there was more than a 6 log reduction in both bacteria (at 4 °C a 4.5 log reduction in P. aeruginosa was seen only after 24 hours). Silver was found to be almost as effective in reducing bacteria in the presence of humic acid (5.5 log reduction in P. aeruginosa at pH 7, 24 °C after 8 hours in the presence of 3mg/l humic acid). This group also looked at the potential for exposure to silver (100 µg/l) to reduce biofilm formation in drinking-water distribution systems. In this role, silver was found to be ineffective, and there was no difference seen between the silver treatment and the control [12], used flow cytometry to examine the minimum inhibitory concentration (MIC) of AgNO3 on E. coli, with a view to the methodology being used to examine water and wastewater disinfection. They reported a 24 hour MIC of between 60 and 80µg/l for silver. A 4 log reduction (approximately) was seen at 100µg/l after 24 hours of exposure [13], evaluated the efficacy of silver ions against E. coli. Bacteria (concentration - 1.75 x 103 cfu/ml) were exposed to various concentrations of silver ions (1, 2, 5, 10 and 20 µg/l), produced from silver electrodes, for up to 60 minutes. Complete bacterial inactivation was seen at neutral pH and ambient temperature after a 20 minute period for the 20 µg/l concentration. 100% bactericidal activity was also seen for the other silver concentrations (with the exception of 1 µg/l), although a longer contact time was required (10 µg/l – 40 minutes; 5 µg/l – 50 minutes; 2 µg/l – 60 minutes). Disinfection was most efficient at pH values between 8 and 9 and at temperatures greater than 20 °C [14]. Looked at the efficacy of silver (AgNO3) in removing P. aeruginosa and E. coli in rooftop harvested rainwater supplies. Prior to disinfection, samples were found to contain between 350-440 cfu/100ml P. aeruginosa and 740-920 cfu/100ml E. coli. The disinfection rate and residual effect of silver was determined using final silver concentrations between 10-100 µg/l over a period of up to 168 hours. Samples were taken for microbial analysis every two hours for 14 hours after the application of silver and then daily for 1 week, to examine regrowth. At higher concentrations (80-100 µg/l) complete inactivation of both microorganisms was seen in 10 hours, with no regrowth of E. coli seen after 168 hours. Inactivation was slower at lower concentrations (95-99% inactivation for silver concentrations between 10-40 µg/l after 14 hours) and regrowth was also observed (e.g. 7.5% survival of P. aeruginosa exposed to 10µg/l silver for 168 hours compared to approximately 4.5% survival at 14 hours), thus, at the lower concentrations, silver only delayed bacterial reproduction and did not cause permanent damage [15], also looked at the effectiveness of silver disinfection as part of rainwater harvesting treatment. Ten rainwater harvesting systems in Mexico, equipped with silver electrodes were evaluated for a number of water quality parameters. The silver electrodes were located in line with the filtering system (after a mesh filter, designed to remove large particles, and before an activated carbon filter). On average, the ionisers reduced the level of total coliforms by approximately 1 log and E. coli by approximately 0.4 log and resulted in a silver concentration of approximately 0.01mg/l in the final water. The systems, as a whole, delivered water containing zero E. coli and less than 10/100ml total coliforms. In a comparative study of disinfectants, the potency of silver ions, derived from AgNO3, was examined in a batch disinfection test of ground water using 106 cfu/ml E. coli [16]. It was found that for a 6

log reduction (i.e. complete inactivation), the minimum concentration of silver required was 10mg/l with a contact time 3 hours. It can be seen from these studies that log reductions varied widely with some bacteria being more sensitive to silver (i.e. more easily killed or inactivated) than others. Generally, relatively long contact times were required to effectively reduce bacterial concentrations (e.g. 3 hours or longer), the exception being the study of [17] where silver ions were generated electrolytically (Rather than from silver salts), and complete inactivation (3 log) was seen after 20 Spring 2014 4 Silver: water disinfection and toxicity minutes at a relatively low silver concentration (20µg/l). In contrast to the lab spiked samples, where generally good log reductions were reported, relatively poor results (lower log reductions) were seen in harvested rainwater samples (low initial bacterial concentration) used by [14] and they suggested that this may result from greater resistance to disinfection in microbes grown in low nutrient systems.

Human exposure

It is clear that silver (largely irrespective of the route of exposure or form) can distribute widely within the body and cross both the blood-brain and placental barriers in experimental animals. Silver, has also been found to be widely distributed throughout the body in exposed humans. Human data relating to silver ingestion is largely limited to a number of case reports where people have ingested varying amounts of colloidal silver, generally over a protracted period [18]. The most common presenting feature is argyria [19]; [20] where tissues become impregnated with silver sulphide, which forms a complex in elastic fibres; large amounts of this complex under the skin give it a bluish, grey-blue or (in extreme cases) a black colour. Generalised argyria results from increased serum silver levels and silver granules can be detected in all body tissues, with the highest concentrations found in the skin, liver, spleen and adrenal glands [21]. Silver has also been found to cross the placental barrier in humans [22]. Looked at liver samples, collected at autopsy, and found significant levels of silver (Median 15.5ng/gww) in livers of children under 6 years old. It was speculated that silver (probably from maternal mercury amalgam fillings) is accumulated from the mother during pregnancy and lactation.

Kidney

A number of different kidney cell types have been subjected to silver, these include embryo kidney cells, which are a heterogeneous mix of almost all the types of cells present in the body (Although most are endothelial, epithelial or fibroblasts), proximal tubule cells and renal epithelial cells [23]. AgNP on primary cells. Cells exposed to 11µg/ml (a concentration below the LC50 value) showed altered morphology and a 9% increase in the early apoptotic population compared to control cells the renal epithelial cells were sensitive to 44nm AgNP, with a significant reduction in viability.

Experimental model *Clarias bactricas*

Fig 1: Experimental model *Clarias bactricas*

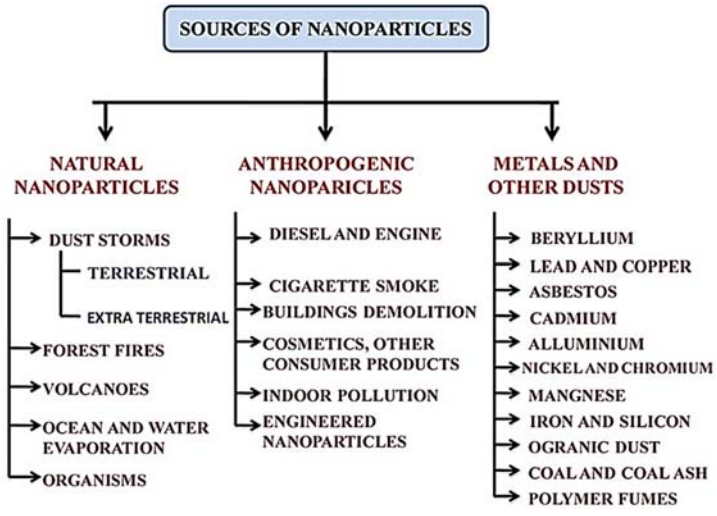


Fig 2: Source of nano particles

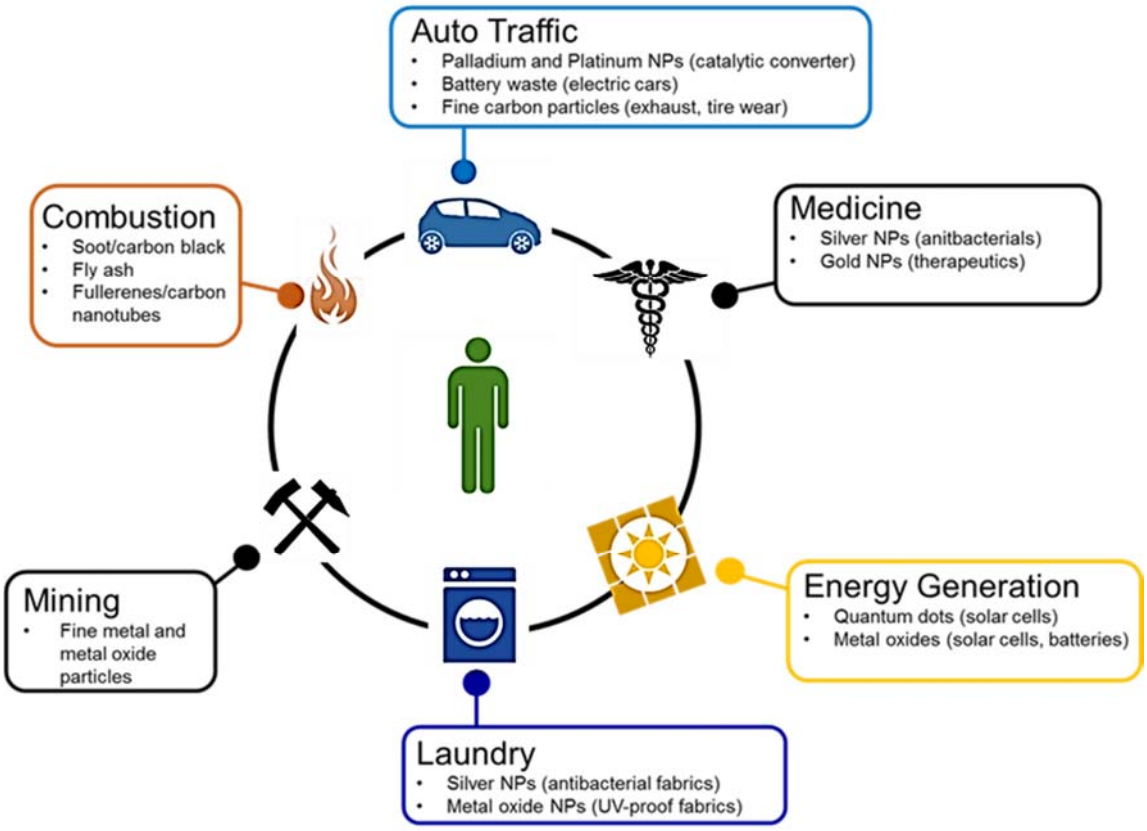


Fig 3: Pathways by which human-made nanoparticles (NPs) are released into the environment

The other most likely source of nanomaterial contaminants is nanoparticle-enabled consumer products. More than several thousand consumer products, including many items of clothing, personal care products, next-generation batteries, and sporting goods now contain nano-materials [6]. In the next ten years, nanoparticles will likely be incorporated into many more sophisticated products, including pharmaceuticals and next-generation solar cells or batteries. While the inclusion of nano-materials in these products can enhance their performance, the breakdown of these products at the end of their useful life also provides several key points of entry for synthetic nanoparticles into the environment. When nanoparticles are incorporated into products intended for domestic use (like anti-microbial fabrics or UV-blocking clothing), nanoparticles can end up in landfills or washed down the drain when clothes have been laundered. Nanoparticles that end up in drain water or in landfills can then enter the environment by many different routes.

Once nanoparticles have been disposed of in household garbage or down the drain, their journey through the environment is just beginning. Nanoparticles that escape with water down the drain will eventually enter a wastewater treatment facility. Here, nanoparticle-contaminated water will undergo several purification processes, including mechanical filtering and settling treatments (designed to remove large particles [larger than a grain of sand]), followed by digestion with microbes, and ultimately chemical disinfection. Unfortunately, none of these treatment stages are specifically designed to eliminate nanoparticles from the waste stream. As a result, following waste water treatment, nanoparticles may remain in the purified water that is released back into the environment, or nanoparticles may remain trapped in the microbe-bearing sludge left over from the purification process. Often, the bio-sludge left over from wastewater purification is repurposed as fertilizer for farm land, which can potentially allow man-made nanoparticles to enter soils or small rivers. Alternatively, when nanoparticle-enabled products end up in a landfill, the original product can break down, allowing man-made nanoparticles to leach into soil in and around the landfill area, providing a route for synthetic nano-materials to enter new environments via soils or even ground water sources. [Fig. 3]

Sources of silver nanoparticle in the environment

Engineered NPs may be found in the form of metals, other dust or various compounds where they are used (Figure 2). Synthesis of the NPs in laboratory or industry is one of the important sources of its release in the environment [24]. Physical and chemical methods of NP synthesis are not eco-friendly and may contaminate the surrounding environment whereas biological synthesis of NPs is rather eco-friendly. By using strong reducing and stabilizing agents, the chemical methods have an undesirable effect on biotic components; [25]. However, the NPs synthesized from plant extract do not include any reductants or stabilizing agents [26]. An outline of the various point and non-point sources of AgNPs has been given in Fig. 2.

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. Fist 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.

For the histopathological study the tissue kidney were taken from the fishes exposed to sublethal concentration of Silver Nanoparticles as well as from control group of fish. The fish were divided into three groups of 6 individuals each. The sub lethal concentration of Silver Nanoparticles Selected was 1/15 and 1/10 of 96h LC50 value respectively. (Table 1.)

Table 1: Concentration and duration of exposure of fresh catfish *Clarias batrachus* to silver nanoparticles

S. no.	Concentrations of silver nanoparticles	No. of Fish	Control Duration (Days)		Experimental duration (Days)	
1.	0.10 mg1-1 (1/15th of 96 h LC50 value)	6	10	20	10	20
2.	0.16 mg1-1 (1/10th of 96 h LC50 value)	6	10	20	10	20

Processing of tissues

The most important steps of tissue processing by paraffin wax are dehydration, clearing, infiltration and embedding. After fixation, the tissue was processed for the paraffin embedding which gives the tissue necessary hardness for microtomy. The water is removed gives from the tissue by dehydration, and cleaning was done before paraffin infiltration (Since paraffin is not miscible in water.

Requirements

1. Stainless Steel or plastic perforated containers (Cassettes or Capsules).
2. Ethanol- 30%, 50%, 70% and 90% and absolute alcohol.
3. Xylene
4. Paraffin Wax
5. Embedding Oven (Temperature 50 - 60 °C)
6. Pencil and Paper
7. Embedding boxes (Blocks)
8. Tissue (Kidney)

Procedure

1. Tissues were thoroughly dehydrated before they were ready for embedding. In this procedure, water was completely removed from fixed tissues. Further the procedure was carried out as follows;
2. Tissue blocks were placed in capsules.
3. The identification number was written with a pencil on a piece of papers.
4. These tissues passed through a series of increasing concentrations of alcohol with the changes in the each concentration.
5. Tissues were first treated with 80% alcohol twice and were kept for two hours.
6. In the third step, tissues were transferred to vails containing 90% of alcohol for one hours.
7. In the fourth, fifth and sixth steps, the tissues were dehydrated by placing them into absolute alcohol (100%) thrice for a period of two hours

Clearing

Since xylene is miscible in both paraffin wax and alcohol, it was used as clearing agent. It helps to replace alcohol and makes room for the paraffin during infiltration and impregnation. The tissues were treated with xylene twice for two hours.

Infiltration and impregnation

The xylene was, eliminated from the tissues by diffusion in the surrounding wax (impregnation) this procedure was carried out in the oven for 2 h. The temperature of paraffin was maintained between 60 to 62 °C, period of 2h. Care was taken to check inadequate impregnation and temperature during infiltration and impregnation of paraffin wax.

Embedding

Embedding is casting or blocking in this procedure, the in filtered and impregnated tissue are placed in warm liquid paraffin, which formed a firm block after cooling. Embedding enables the tissues to be cut with the microtome. This procedure is carried out as follows:

1. Leukhard embedding box (Consisting of two L shaped metallic brass) was arranged on glass plate to obtain a rectangular box.
2. The tissue were placed at the bottom of cavity. Orientation of the tissues was achieved according to the instructions given by the pathologist. The identification number was written by a pencil on a paper. The identification number was placed adjacent to the tissue.
3. Paraffin was (with high melting point 60 – 62 °C) was first melted a filtered through a course filter paper. The filtered paraffin was then poured in to the cavity of the box containing the specimen.
4. The mould was then placed in a container of cold water or kept in a refrigerator, until that was harden (It took about 10-20 minutes).

Microtomy

The section of the tissues (Kidnely) were cut at 6.0 um thickness) with the help of microtome knife. The section were mountain the glass sledes with the help of egg albumin and were stretched on a hot plate. The section was taken and hydrated through decreasing concentrations of alcohol baths and water. The concentrations of alcohol used were 100%, 90%, 70% and the sections were placed for 30-60 seconds in these alcohol solutions. These were washed in tap water and rinsed in distilled water. The section was drained before staining.

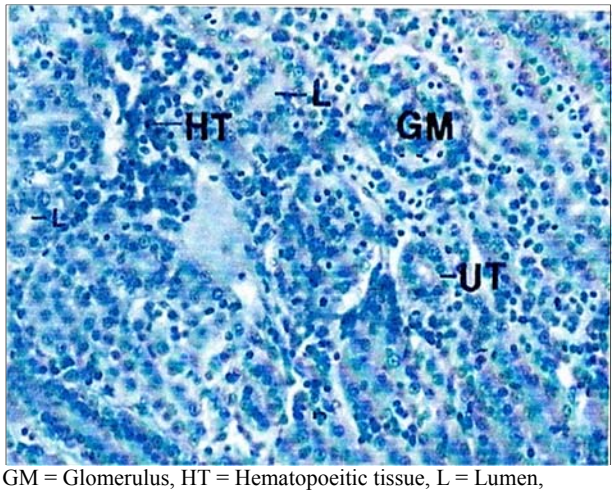
Staining

Haematoxylin and eosin were the prominent stain used for the demonstration of nuclear and cytoplasmic inclusions. Alum acts as a mordant and haematoxylin consists of alum that stains the nucleus light blue, which turns red in presence of acid. The cell differentiation was achieved by treating the tissues with acid solution. Using eosin solutions, which imparts pink colour to cytoplasm, the counter staining was performed.

The section of kidney was stained with haematoxylin solution for 3-5 minutes. They were washed with running

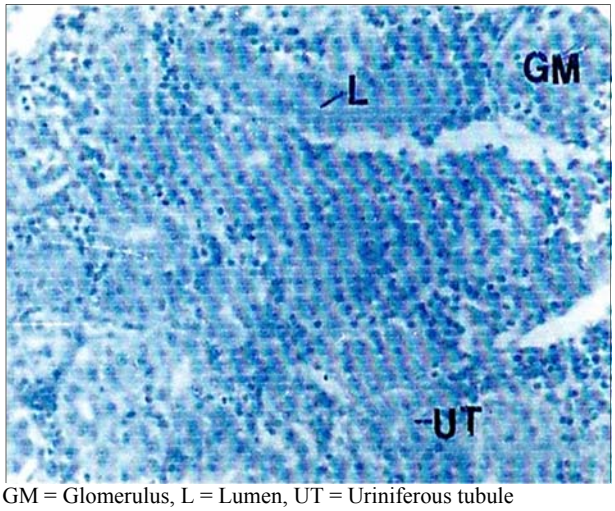
tab water and slide were treated very briefly with 0.5% v/v hydrochloric acid to remove excess stain. Then the slide were dipped several times in dilute ammonia water (then the section appears blue.) thereafter it was washed in tap water and then rinsed in 95% alcohol, the slides was agitated in eosin solution for 10-60 seconds, and the staining solution was drained.

The slides were dehydrated in 70% alcohol from 30-60 seconds each and the same slide was transferred into 95% alcohol container for 30-60 seconds. Then was placed in absolute alcohol for 30-60 seconds and repeated twice. For cleaning the slide were placed in xylene for 30-60 seconds each. After thorough cleaning the slide was subjected for mounting with DPX and Canada Balsam with cover slip. The slide was allowed to dry and the DPX was cleared with xylene. The slides were checked under microscope to observe histopathological details of kidney.



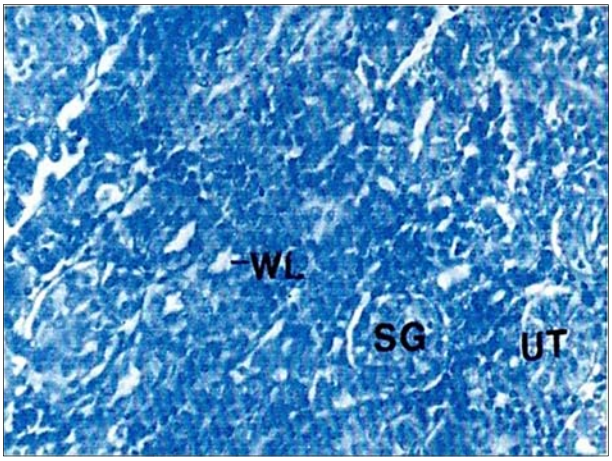
GM = Glomerulus, HT = Hematopoeitic tissue, L = Lumen, UT = Uriniferous tubule

Fig 4: Photomicrograph of transverse section of kidney of freshwater catfish, *Clarias batrachus* showing normal histological structure (x 400)



GM = Glomerulus, L = Lumen, UT = Uriniferous tubule

Fig 5: Photomicrograph of transverse section of kidney of freshwater catfish, *Clarias batrachus* showing condition of glomerulus, uriniferous tubules and lumen of tubules after an exposure to 0.10 mg 1-1 of Silver Nanoparticles. For 10 days x (400)



SG = Shrunkened glomeruli, UT = Uriniferous tubules, WL = Wide Lumen

Fig 6: Photomicrograph of transverse section of kidney of freshwater catfish, *Clarias batrachus* showing wide lumen and shrinkage of glomeruli after an exposure to 0.10 mg 1-1 of Silver Nanoparticles for 20 days (x400)

Result

In the kindey, after 10 days of 0.10 mg 1-1 Silver Nanoparticles. exposure, A little shrinkage in glomerulus but not much change in the tubular structure was observed. The glomeruli of the nephrone were further shrnkened and tubules showed a wide lumen after 20 days of exposure.

When the fish were exposed to 0.16 mg 1-1 Silver Nanoparticles for 10 days, the glemureli of uriniferous tubules had become swallened. The glomerular organization was distorted leaving a cellular mass within the glomerulus. The swelling of glomeruli had further increased their size. The epithelial cells of the tubules lost their cell walls and the area of lumen was decreased after 20 days of exposure.

References

1. Maynard AD, Baron PA, Foley M, Shvedova AA, Kisin ER, Castranova V. Exposure to carbon nanotube material: aerosol release during the handling of unrefined single-walled carbon nanotube material. J Toxicol Environ Health Part A 2004;67:87-108. DOI: 10.1080/15287390490253688.
   [PubMed] [CrossRef] [Google Scholar]
2. Ju-Nam Y, Lead JR. Manufactured nanoparticles: an overview of their chemistry, interactions and potential environmental implications. Sci Total Environ 2008;400(1-3):396-414.
   DOI: 10.1016/j.scitotenv.2008.06.042.
   [PubMed] [CrossRef] [Google Scholar]
3. Smith IC, Carson BL. Trace Metals in the Environment. Ann Arbor: An Arbor Science Publishers 1977, P469. [Google Scholar]
4. Nebeker AV, McAuliffe CK, Mshar R, Stevens DG. Toxicity of silver to steelhead and rainbow trout, fathead minnows and Daphnia magna. Environ Toxicol Chem 1983;2:95-104. DOI: 10.1002/etc.5620020111. [CrossRef] [Google Scholar]
5. Aoki T, Nihonyanagi K, Oba T. Involvement of thiols in the induction of inward current induced by silver in frog skeletal muscle membrane. Experientia 1993;49:792-794. DOI: 10.1007/BF01923550. [PubMed] [CrossRef] [Google Scholar]

6. Asharani PV, Wu YL, Gong Z, Valiyaveettil S. Toxicity of silver nanoparticles in zebrafish models. Nanotechnol 2008;19(25):255102. DOI: 10.1088/0957-4484/19/25/255102.
   [PubMed] [CrossRef] [Google Scholar]
7. Birge WJ, Zuiderveen JA. Transport, fate and effects of silver in environment [Abstracts]. 3rd International conference 1995, P6-9.
8. Wijnhoven SWP, Peijnenburg WJ, Herberts CA, Hagens WI, Oomen AG, Heugens E *et al*. Nano-silver – a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology 2009;3(2):109-138.
9. Liau SY, Read DC, Pugh WJ, Furr WJ, Russell AD. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. Letters in Applied Microbiology cited by Park *et al*. 2009 1997;25:279-283.
10. Huang HI, Shih HY, Lee CM, Yang TC, Lay JJ, Lin YE. *In vitro* efficacy of copper and silver ions in eradicating Pseudomonas aeruginosa, Stenotrophomonas maltophila and Acinetobacter baumannii: Implications for on-site disinfection for hospital infection control. Water Research 2008;42:73-80.
11. Cunningham JH, Cunningham C, van Aken B, Lin, LS. Feasibility of disinfection kinetics and minimum inhibitory concentration determination on bacterial cultures using flow cytometry. Water Science and Technology 2008;55(4):937-944.
12. Pathak SP, Gopal K. Evaluation of bactericidal efficacy of silver ions on Escherichia coli for drinking-water disinfection. Environmental Science and Pollution Research 2014;19:2285-2290.
13. Nawaz M, Han MY, Kim T, Manzoor U, Amin MT. Silver disinfection of Pseudomonas aeruginosa and E. coli in rooftop harvested rainwater for potable purposes. Science of the Total Environment 2012;431:20-25.
14. Adler I, Hudson-Edwards KA, Campos LC. Evaluation of a silver-ion based purification system for rainwater harvesting at a small-scale community level. Journal of Water Supply and Technology – AQUA 2013;62:545-551.
15. Patil RA, Kausley SB, Balkunde PL, Malhotra CP. Comparative study of disinfectants for use in low-cost gravity driven household water purifiers. Journal of Water and Health 2013;11(3):443-456.
16. Pathak SP, Gopal K. Evaluation of bactericidal efficacy of silver ions on Escherichia coli for drinking-water disinfection. Environmental Science and Pollution Research 2012;19:2285-2290.
17. Chung IS, Lee MY, Shin DH, Jung HR. Three systemic argyria cases after ingestion of colloidal silver solution. International Journal of Dermatology 2010;49:1175-1177.
18. Brandt D, Park B, Hoang M, Jacobe HT. Argyria secondary to ingestion of homemade silver solution. Journal of the American Academy of Dermatology 2005;53:S105-S107.
19. Wadhera A, Fung M. Systemic argyria associated with ingestion of colloidal silver. Dermatology Online Journal 2005;11(1):12.
20. Brandt D, Park B, Hoang M, Jacobe HT. Argyria secondary to ingestion of homemade silver solution. Journal of the American Academy of Dermatology 2005;53:S105-S107.
21. Lyon TDB, Patriarca M, Howatson AG, Fleming PJ, Blair PS, Fell GS. Age dependence of potentially toxic elements (Sb, Cd, Pb, Ag) in human liver tissue from paediatric subjects. Journal of Environmental Monitoring 2002;4:1034-1039.
22. Gopinath P, Gogoi SK, Sanpui P, Paul A, Chattopadhyay A, Ghosh SS. Signalling gene cascade in silver nanoparticle induced apoptosis. Colloids and Surfaces B: Biointerfaces 2010;77:240-245.
23. Bhaduri GA, Little R, Khomane RB, Lokhande SU, Kulkarni BD, Mendis BG *et al*. Green synthesis of silver nanoparticles using sunlight. J Photochem. Photobiol. A Chem 2013;258:1-9. DOI: 10.1016/j.jphotochem.2013.02.015
24. Kuppusamy P, Ichwan SJ, Parine NR, Yusoff MM, Maniam GP, Govindan N. Intracellular biosynthesis of Au and Ag nanoparticles using ethanolic extract of *Brassica oleracea* L. and studies on their physicochemical and biological properties. J Environ. Sci 2015;29:151-157. DOI: 10.1016/j.jes.2014.06.050
25. Carlson C, Hussain SM, Schrand AMK, Braydich Stolle L, Hess KL, Jones RL *et al*. Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. J Phys. Chem. B 2008;112:13608-13619. DOI: 10.1021/jp712087m