



## Effects of silver nanoparticles on the anti-oxidant status and histological alterations in different organs in Indian mud crab *Scylla Serrata*

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### ABSTRACT

In medicine and many other fields, the use of silver nanoparticles is inevitable. The negative impacts of silver accumulated silver nanoparticles on the environment and organisms need to be taken into consideration. The studies on the effect of silver nanoparticles on bio-indicators such as marine invertebrates pave the way to regulate the usage of silver nanoparticles. In the present study, the effect of silver nanoparticles on the hepatopancreas of Indian mud crab, *Scylla serrata* was studied. A 20 ppm of silver nanoparticles was exposed to *S. serrata* for ten days period. The morphological changes, the biochemical markers from hepatopancreas, histological analysis for hepatopancreas, gills and reproductive organs were examined. On the 2<sup>nd</sup> day exposure of silver nanoparticles showed the increased value of anti-oxidants and no morphological changes. But the 10<sup>th</sup>-day exposure shows the significantly reduced level of biochemical markers in the hepatopancreas and morphological changes were observed. The apoptotic cells and necrotic cells were recorded in hepatopancreas. Moreover, the cell architecture of gill tissues and reproductive tissues were observed. The silver nanoparticles in the concentration of 20 ppm caused significant changes in the hepatopancreas of *S. serrata* for ten days of exposure. The mechanism of silver nanoparticles needs to be studied to understand and protect models from the nanoparticles.

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### INTRODUCTION

Silver nanoparticles (AgNPs) gains more attention in medicine and other bio-related fields than any other nano-metals due to the excellent physical and chem-

ical properties such as stability, electrical conductivity, catalytic and antibacterial activity (Nakkala *et al.*, 2014). These nanostructures are used as anti-microbial agents in the pharma industry, cosmetics, food and beverage industry, textile coatings (Fortunati *et al.*, 2013; He *et al.*, 2012; Kejlová *et al.*, 2015). Since the broad utility of industrial products, AgNPs discharged into the oceanic environment either directly or through the river system (Baker *et al.*, 2014). Previous studies have demonstrated that the persistence of dissolved form of Ag<sup>+</sup> in the water system becomes highly toxic to freshwater, marine invertebrates and fish (Bianchini *et al.*, 2002). The formation of nanoparticles aggregation is mostly dependent on the surface area and collision forces, pH, ionic strength and surfactant (Peralta-Videa *et al.*, 2008; Rocha *et al.*, 2015; Vale *et al.*, 2016). The toxicity investigations

on embryo zebrafish revealed that the smaller size (20nm) of nanoparticles plays a role in inducing the toxicity than larger particles (100nm) (Kim and Tanguay, 2014). The environmental toxicity of silver nanoparticles and the effect on organisms have already been well studied in the in-vivo and in-vitro condition. AgNPs showed LC<sub>50</sub> values beneath 10 mg/L roughly to the aquatic organism such as shellfish and fish (Bondarenko *et al.*, 2013). Behavioural changes were observed in zebrafish after exposure of AgNPs (Powers *et al.*, 2011). Nanoparticles exposure induces the nonspecific oxidative stress in the host (Nel, 2006). The amplification loop between inflammation and oxidative stress causes an adverse effect due to nanoparticles exposure. Subsequently, DNA damage and cell death occurred (Franco *et al.*, 2009). The toxicity effect of silver nanoparticles on oxidative stress markers, apoptosis and genotoxicity, was reported by (Kim and Ryu, 2013). Electron spin resonance shows that the active surface of silver nanoparticles could trigger the production of free radicals (Zhang *et al.*, 2014). In a report, the silver ions released from silver nanoparticles in an aqueous medium can induce the generation of hydroxyl radicals (Zhou and Tang, 2018) and induction of apoptotic pathways (Hwang *et al.*, 2008). The marine system is the ultimate target of every pollution, including nanoparticles. The pollutants of seawater have a potent effect on aquatic animal survivability (His *et al.*, 1999). The present study demonstrates the toxic effects of silver nanoparticles on *Scylla Serrata*, the common edible Indian marine crab. Recently the toxic effect of cadmium nanoparticles was evaluated on *Scylla Serrata* (Sujitha *et al.*, 2017). In the present research evaluate the stress induction of silver nanoparticles in *Scylla Serrata* and histomorphological variations in hepatopancreas caused by AgNPs.

## METHODS

### Animal Collection

The male and female species of *Scylla Serrata* were collected from Pulicate Lake, Pulicate, Tamil Nadu, India. The collected animals were kept in separate tanks filled with seawater with the provision of aeration.

The seawater was changed periodically, and crabs were fed with commercial fish feed. The crab was acclimatized for further studies for ten days before starting the experiment.

During the acclimatization period, the animals were fed twice a day. Water temperature was maintained within a range (27.5 ± 0.5°C) as recommended for optimal growth of mud crabs.

### Acute toxicity test

The acute toxicity of AgNPs was determined by using the standard methodology which was described by Food and Agriculture Organization (FAO) (Ward and Parrish, 1982) and the American Public Health Association (APHA, 1992). Crabs were made to adopt the animal house condition for 14 days. Semi-static toxicological bioassays were carried out for 120 h. A series of ten concentrations, 4, 8, 12, 16, 20, 25, 50, 75, 150 and 300ppm of Silver nanoparticles (AgNPs) suspension of 40nm in size (Sigma and Co) was injected intraperitoneally per kg of crab weight. Five replicates of at least ten animals were exposed to the above-stated concentrations. Mortality was recorded every 24 h period after which dead crabs were removed. Probit analysis was used to estimate the concentration and 95% confidence limits of AgNPs that kills 50% of the exposed crab (LD<sub>50</sub>)

### Silver Nanoparticles treatment

Healthy adult male and female crabs with a homogeneous size (carapace width 14-16cm, weight 200-300g) were selected for experimental studies. Silver nanoparticles at the concentration of 20 ppm/kg of crab weight were used to expose to crabs while control animals also maintained separately. The acute exposure lasted for ten days. During the experiment, crabs were fed, and dead animals were removed in time. The hepatopancreas was collected every two days exposure of silver nanoparticles, and it was immediately excised. The hepatopancreas tissues homogenized in Tris-HCl buffer (0.1M, pH 7.4) to prepare 10% (w/v) homogenate. Then the homogenate was centrifuged at 12000 g for 30 min at 4° C, and the supernatant was used for further analysis. A part of the tissues was stored in 4% formaldehyde for histological evaluation.

### Evaluation of biochemical markers

The hepatopancreas tissue homogenates subjected to various biochemical assays such as alkaline phosphatase (ALP) (Bianchi *et al.*, 1994), Succinate Dehydrogenase (Schirawski and Unden, 1998), Lactate Dehydrogenase (Gloster and Harris, 1962), Superoxide Dismutase (SOD) — (Beyer and Fridovich, 1987).

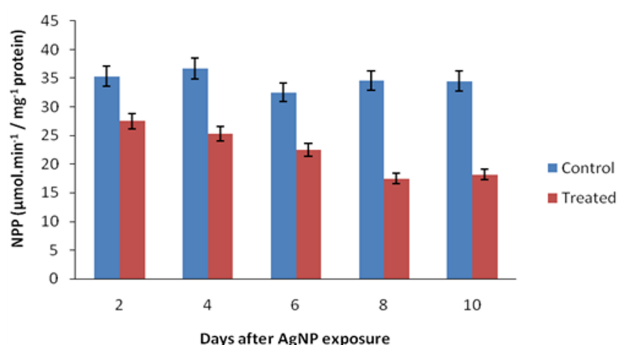
### Histology analysis

Hepatopancreatic tissues were dehydrated and replace the tissue in ethanol by gradient transfer. The tissues were embedded in paraffin blocks and made 5 mm thick sections using a microtome. The sections were deparaffinized in two changes of xylene for 10 minutes, rehydrated and stained with hematoxylin and eosin.

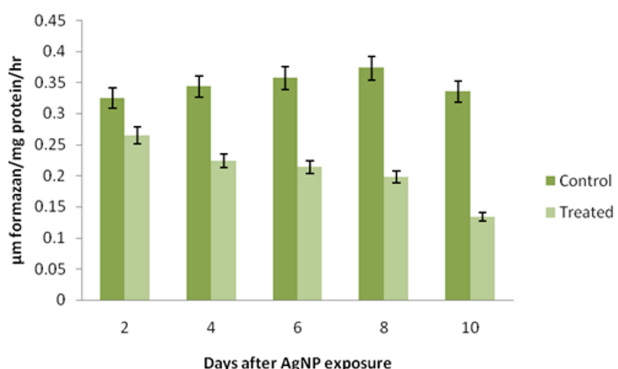
**RESULTS**

**Effects of AgNPs on alkaline phosphatase activity of hepatopancreas**

Silver nanoparticles (20ppm) exposure to *S. Serrata* showed that the alterations in the activity of biochemical markers in hepatopancreas when compared to that of in control crabs. Alkaline phosphatase (Figure 1), SDH (Figure 2) and LDH (Figure 3) activity were started to decrease on day two exposure and reached the lowest activity on ten days exposure of silver nanoparticles. SOD (Figure 4) activity started increasing on day two and reached a peak on day 10 of exposure.



**Figure 1: Effect of AgNPs (20ppm) on Alkaline phosphatase activity in the hepatopancreas of *S.serrata***

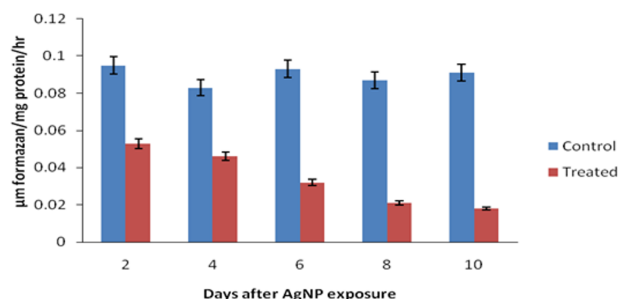


**Figure 2: Effect of AgNPs (20ppm) on Succinate dehydrogenase (SOD) activity in the hepatopancreas of *S.Serrata* exposed to AgNPs (20ppm)**

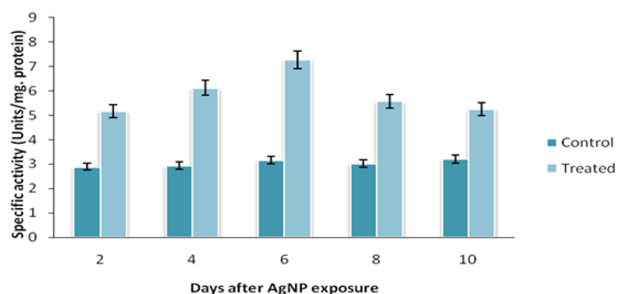
**Histological changes of hepatopancreas**

The standard hepatopancreas shape looks like a circle or ellipse, and the inner surface was irregular and continuous cells were lined up in the order in the control tissue (Figure 5 A). In crabs exposed to silver nanoparticles on day 2, the epithelia were slightly damaged with lots of broken or swollen cells (Figure 5 B). In increasing days of AgNPs exposure showed cellular swelling and necrosis emerged as

the day progresses compared to its respective control (Figure 5 C & D). On day six, classical apoptosis was identified, and the cells were become smaller (Figure 5 E-J). A) Day 2 C) Day 4 E) Day 6 G) Day 8 I) Day 10 of control B) Day 2 D) Day 4 F) Day 6 H) Day 8 J) Day 10 after AgNPs treatment T- tubular cell; R - R cell; Hi - Haemolytic infiltration; B - B cell; Lu - lumen; H - Haemolytic clumps; Ct - Connective tissue; He - Haemafodium; Ci - Cytoplasmic inclusions.



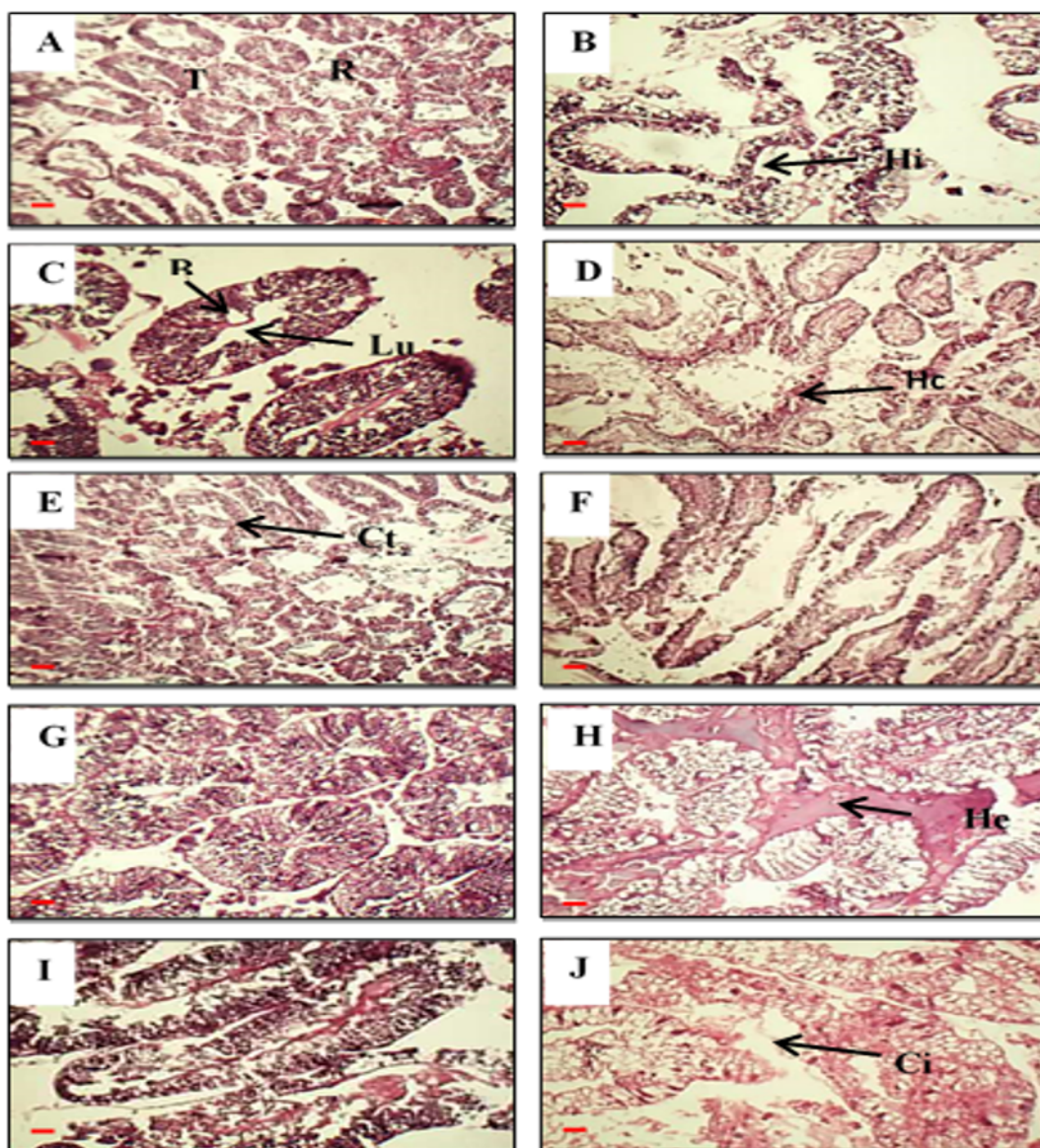
**Figure 3: Effect of AgNPs (20ppm) on Lactate dehydrogenase (LDH) activity in the hepatopancreas of *S.Serrata***



**Figure 4: Effect of AgNPs (20ppm) on Superoxide dismutase activity in the hepatopancreas of female *S.Serrata***

**Histological changes of gills of male *S. Serrata***

Results of AgNPs on the gills of *S. Serrata* were presented in Figure 6. Regular crabs displayed usual architecture in all haemal lacunae of the gill lamellae and central shaft (Figure 6 A). On day 2 of AgNPs exposure, sections of gill lamellae showed increased haemal space with little tissue damage (Figure 6 B). Epithelium, pillar cells and haemal vessels were relatively discernible at this stage of the infection. More intense histopathology was observed in an increased day of exposure (Figure 6 C-F). The increased concentration of AgNPs in crab causes the disorganization of pillar cells, prominent deterioration of pillar cells and haemal canal could be observed in the haemocytes (Figure 6 F). A) Control B) Day 2 C) Day 4 D) Day 6 E) Day 8 F) Day 10 after AgNPs treatment. Ha - haemocytes; Hc - Haemal canal; Hs - Haemal space; Dpc - Degenerated Pillar cells; Pc- Pillar cells.

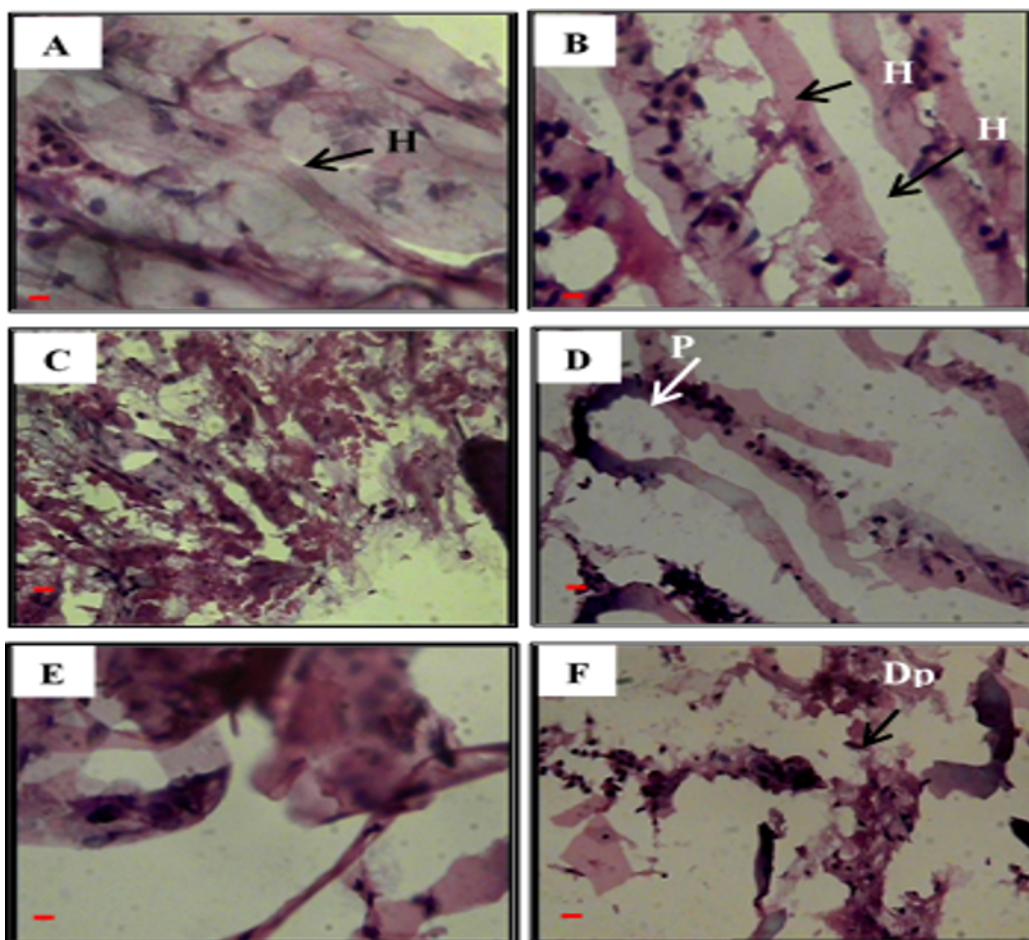


**Figure 5: Effects of AgNPs on the structure of hepatopancreas *S.Serrata* by light microscope. (Scale bar-10µm)**

**DISCUSSION**

The present research demonstrates the effect of silver nanoparticles (AgNPs) on Indian mud crab *Scylla Serrata* and the alterations in the activity of biochemical markers. In this study, the morphological changes could be observed in *Scylla Serrata* during the silver nanoparticle exposure. Earlier of 20<sup>th</sup> century, a new shell disease called rust spot, which was found in *Scylla Serrata* when exposed to AgNPs, which was reported in Australia (Andersen *et al.*, 2000). We aimed the alterations in the hepatopancreas of *S.serrata* against the exposure of AgNPs. The hepatopancreas involves nutritional absorption and detoxification in crustacean animals (Vogt, 1994). Hence, we hypothesized that the hepatopancreas might show the immediate response against silver

nanoparticles exposure in animals. After the exposure of AgNPs, the activity of anti-oxidant enzymes in hepatopancreas were evaluated. The total activity of anti-oxidants was reduced after ten days of AgNPs exposure. At the beginning of exposure, the activity of SOD was increased immediately, but after ten days, the activity was decreased significantly. The sudden expression of anti-oxidant might be due to the activation of the defensive system as a result of free radical generation after AgNPs exposure to remove the scavenging the free radicals. A similar phenomenon occurred in the liver SOD activity of three species of fish exposed to Cd (Palace and Klaverkamp, 1993). The reduction in the activity of SOD realized that interruption in the conversion of highly reactive free radicals to hydrogen peroxide. Kumar *et al.* (2009) reported that the mitochon-



**Figure 6: Effects of AgNPs on the structure of gills in *S. Serrata* by light microscope. (Scalebar-10 $\mu$ m)**

dria are the primary target of AgNPs because of the decreased level of such enzymes. Mutant SOD accumulation paves the way to mitochondrial vacuolation and swelling (Jaarsma *et al.*, 2001).

GPx removes the hydroxyl free radicals in the conversion reaction of hydrogen peroxide to water. Thus GPx could reduce the tissue injury. Similarly, other free radicals such as singlet oxygen, superoxide and per hydroxyl can inhibit the CAT (Kono and Fridovich, 1982).

The histological technique is the promising field of aquatic toxicology as it provides the direct view of changes occurred in tissue level. Histology analysis of AgNPs exposed *S.serrata* hepatopancreas demonstrated that blind-ending tubules. The histology results were comparable with previous reports on *Crangon crangon* (Andersen and Baatrup, 1988). Other cell irregular forms such as cellular disruption, the disappearance of lateral cell membranes and folding of the basal lamina also reported. This morphological dysregulation occurs due to the cell decomposing by necrotic initiation (Asztalos *et al.*, 1988)

AgNPs exposed gills of *S. Serrata* damaged deeply such as vacuole formation in the gill stem, and a rupture occurred in gill lamellae, destruction and congestion of haemocytes and these observations of the present study were comparable with previous reports (Jadhav *et al.*, 2007). The heavy metal copper causes the swelling and fusion of the lamellae, structural changes in gill tips and necrotic lamellae in *Macrobrachium rosenbergii* (Li *et al.*, 2006). Such changes observed in the gills of field crab, *Paratelphusa hydrodromus* on exposure to nickel (Kurian and Radhakrishnan, 2002).

## CONCLUSIONS

There are number of reports have been made on the clinical applications of silver nanoparticles (AgNPs). The usage of AgNPs in different field pave the way to accumulate the nanoparticles in the environment and causes the pollution. The present paper have demonstrated the negative effects of AgNPs in *S. serrata*. The alteration in the level of antioxidant and tissue structure due to AgNP exposure revealed that the toxicity of AgNPs at cellular level. The study help

to carry out the research on biochemical path way of AgNPs in cells. This report might be the preliminary research to study the genetic alterations *S. seratta* in future.

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We authors now declare that we have no funding support for this study.

### Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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