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ULTRASTRUCTURAL CHANGES IN THE GILLS OF THE FRESHWATER CRAB, *Paratelephusa jacquemontii*, AFTER EXPOSURE TO SILVER NANOPARTICLES (AGNP)

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ABSTRACT: The present study involves the effects of Silver nanoparticles upon gills of freshwater crab, *Paratelephusa jacquemontii*. The study was carried out under laboratory conditions, and it was dose and time-dependent. The exposure of gills with AgNP for 15 and 30 days to sublethal concentrations of LC_0 and $1/10^{th}$ of LC_{50}. The acute toxicity study was carried out, i.e. 96 hours Lethal Concentration (LC_{50}) value was calculated for the study was 0.71 mg/l. The main aim of the study is to examine exposure of animals to AgNP on gill ultrastructure with the help of Scanning Electron Microscopy (SEM). The results showed that notable damages, such as vacuolation of the secondary lamella, fusion, hypertrophy, mucus secretion and necrosis. The severity of the effects increased with increase in dose and exposure period.

KEYWORDS: *Paratelephusa jacquemontii*, AgNP, LC_{50}, Gills, SEM.

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1.INTRODUCTION

The silver nanoparticles (AgNPs) are widely used nanomaterial, and worldwide production of silver nanoparticles (AgNPs) is estimated about 500 tons per year. Nowadays, the use of AgNPs is growing rapidly [1]. In the recent years, great development was occurred in nanotechnology also increased the use of silver nanoparticles [2]. The study carried out by [3] with silver nanoparticles were the most commonly referenced nanoparticles, which are used in various products Jonathan, 2013 [4] underlined 1,317 products containing nanoparticles, and of these products, 313 contain silver nanoparticles (AgNP)**.** The extensive application of silver nanoparticle (AgNP) in various fields is

receiving attention amongst researchers. The physical, chemical and mechanical properties of AgNP are distinctive and useful in the field of electronics, biotechnology, textile engineering, environmental and pharmaceuticals [5]. AgNPs are utilised commercially in such instances as textiles, disinfectants, chopping boards, washing machines and even for organ transplantation, which can release a significant amount of AgNP into the environment. Soil contamination with AgNP has been shown their effect on micro and macro-organisms from the terrestrial habitat [6]**.** The use of nanomaterials is also likely to result in their release into aquatic environments and may pose risks to the aquatic ecosystem. The aquatic ecotoxicology of engineered nanomaterials is a relatively new and evolving field [7]. Very little information is available on the environmental occurrence of AgNP, but their very significant production is likely that, AgNP is reaching to the aquatic environment where they may affect living organisms, as either particulate or as dissolved silver ions [8]. Nowack *et al.,* (2011), observed that the potential concentration of AgNPs has bigger in surface water up to 0.1 mg L^{-1} and in sludge up to 2.9 mg kg^{-1}. Despite its beneficial applications, numerous harmful effects of AgNPs have also been reported in plants and animals[9]. Imani *et al*., (2015) studied the short-term exposure of rainbow trout to AgNPs increased the number of RBCs, WBCs, and mean corpuscular haemoglobin concentration, but decreased the values of hematocrit, mean corpuscular volume, and mean corpuscular haemoglobin [10,11]**.** Significant alterations found in gene expression due to different AgNP treatments and several gene pathways affected, most notably those related to oxidative phosphorylation and protein synthesis, overlapped strongly among the treatments indicating similar mechanisms of toxicity for the forms of silver studied [8]. Several studies investigated the effect of AgNPs on haematological indices of fish. Venkatasamy *et al.,* (2013) showed that the amount of haemoglobin and the total count of blood cells considerably increased following exposure of *Labeo rohita* to AgNP [12, 13]**.** As biological processes affected by exposure to AgNP. A variety of organs, include induction of oxidative damage, alterations to the regulation of enzymes responsible for free radical scavenging, altered regulation of gene expression pathways involved in apoptosis, and disrupted the regulation of the cellular machinery involved in storing, detoxification and metabolism of metals [8]. The objective of the current study is to investigate alterations in the surface morphology of gills of freshwater Crab, *Paratelephusa jacquemontii* as a model organism with the chronic exposure of AgNP for different time interval using Scanning Electron Microscopy (SEM). The lethal concentration (LC_{50}) studied during the lab experiment which was essential to carry out the study.

2. MATERIALS AND METHODS

2.1 Material characteristics

The study was carried out with the colloidal AgNP solution which was synthesised by 10^{-2} molar $AgNO_3$ solution, and reducing agent used sucrose as described by Emanuala *et al.,*(2010), for a detailed process of AgNP synthesis, are advised to refer the Emanuala Filipo *et al.,*(2010) [14]. The

characterisation of synthesised material was done with the UV –Vis Spectrophotometer, ICP-AES, and DLS.

2.2 Experimental Design

Crabs were purchased from local market of the mean total length of 6 - 8 (± 0.8) cm and mean weight 80 - 120 gm. Before beginning, the experimental crabs were acclimatized to laboratory condition for 48 hrs and then exposed to various concentrations of AgNPs to find out LC_{50} value as per OECD guidelines. Five experimental groups were made with each group containing ten animals. Experimental groups were made as, one control group, group with LC_0 (0.01mg/l) and group with $1/10^{th}$ of LC_{50} (0.07mg/l) and exposed for 15 and 30 days respectively. The experimental groups were studied in triplicates. In each trey added 3 litres of tap water, which comes from the university lakes and addition of AgNP solution, to maintain concentration every time. The static experimental protocol was adopted and water was changed once every day. After 15 and 30 days of the exposure period, five healthy crabs were randomly sampled to assess effects on the surface of gills. The gills were removed and kept for 24 hrs in Bouin's fixative for fixation. Then the tissues were dehydrated by passing through ascending grades of alcohol and finally drying for 24 hrs in the oven. The dried samples were mounted on stubs, gold coated and examined under JEOL JSM-6360 SEM Japan.

2.3 Data Analysis

Mortality observations were made for every day upto 96 hrs. The LC_{50} value was calculated with Trimmed Spearman-Karber computer programme (V 1.5) developed by Ecological Monitoring Research Division of USEPA and Biostat v5 software.

3. RESULTS AND DISCUSSION

3.1 Characterisation of AgNP

The results obtained for various characteristics of the synthesised material are as follows. Fig 1A reveals the UV–Vis absorption spectrum displayed as a sharp peak with maximum absorbance at 400 nm. The DLS measurements exhibited that the hydrodynamic diameter of the AgNPs ranged from 25 to 150 nm, with an average value of 65.76 nm (Fig. 1B). Fig. 1C exhibits ICP-AES analysis peak which confirmed the concentration of AgNP with 0.031 ppm. With the dilution factor multiplication, the original sample was 248 ppm concentration of AgNP. This finding indicates that the colloidal suspension of synthesised material is AgNP. These data clearly confirmed a homogeneous dispersion of the AgNPs in aqueous solutions.

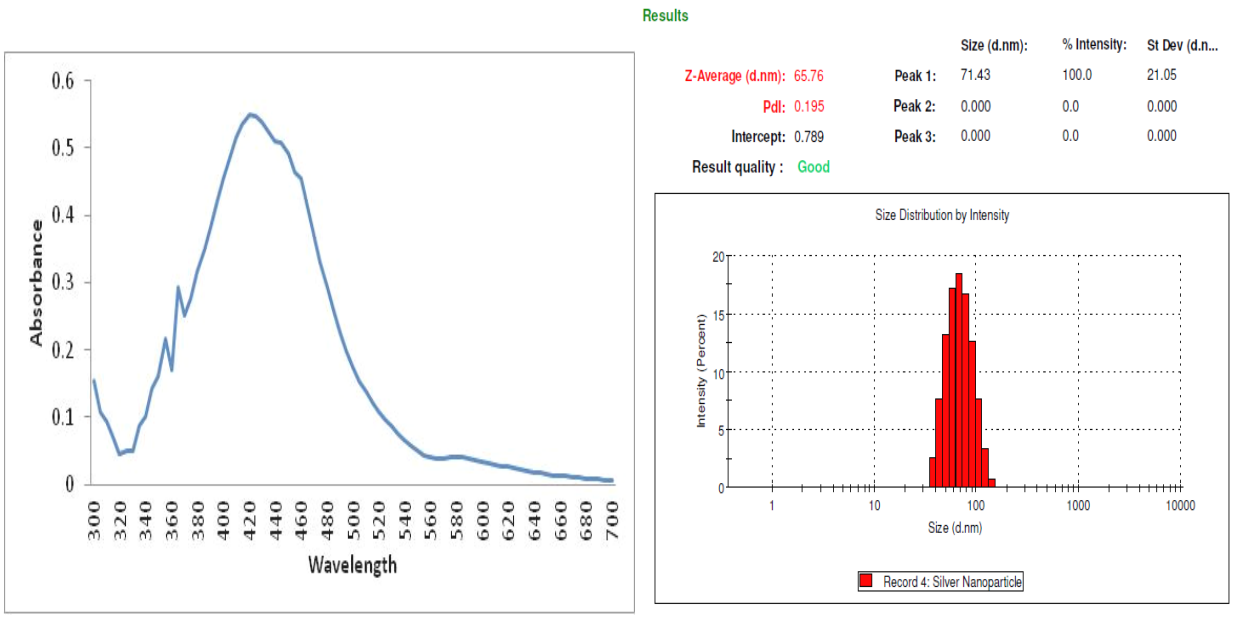

Fig 1: (A) UV–Vis spectrum of synthesized AgNP. (B) DLS measurements of synthesized AgNP

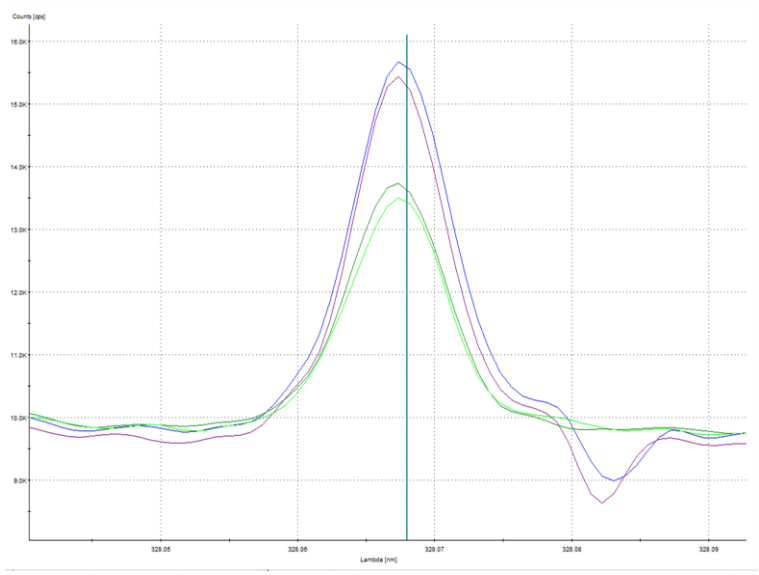

Fig 1: (C) ICP-AES analysis peak the confirmation and concentration of AgNP

3.2 Lethal Concentration 96 hours Study

The 96 hrs acute toxicity test was carried out, and results are shown in (Table 1). The lethal concentration value LC_{50} was calculated with Biostat v5 software, for AgNP solution with the test animal Crab, *Paratelphusa jaquemontii,* i.e., 0.77 PPM. This study is the evidence of toxicity of colloidal AgNP to the freshwater crab. During the study, behaviour of the control animals was normal, but the crabs introduced with AgNP concentrations showed abnormal behaviour. In crustaceans, the exposure routes are mainly via ingestion and adsorption to surface epithelia such as gills. The toxic effects of AgNP increases with the dose and time.

Table 1: Median Lethal concentration value of colloidal AgNP to *Paratelphusa jaquemontii.*

Sr. No.	Duration of hrs.	LC_{50} ppm
1	96	0.77
2	72	1.88
3	48	2.70
4	24	3.62

3.3 SEM study of Gills

The scanning electron microscopic study of gills exposed to AgNP is presented in Fig. 3 to 12. The images of scanning electron microscope confirm the surface alteration observed in exposed gills with severe loss of micro ridges. Microphotographs of gills of control crab showed the normal network of the haemal channel (HC) and normal architecture of micro-ridges (M) (Fig 3). While high power microphotographs showed normal network (NN) on the surface of the haemal channel with mucus free surface of the gills (Fig 4). On exposure at LC_0 concentration of AgNP for 15 days shows bulging of the haemal channel (HC) (Fig 5) and high power microphotographs showed denatured network (DN) with mucus layer secreted on the surface of the gills, also loss of regular pattern of micro ridges under stress condition (Fig 6). In 30 days exposure of AgNP at LC_0 concentration, microphotographs showed the bulging (B), and a disturbed architecture of the Micro ridges (Fig 7) and high power microphotographs showed Degeneration Network (DGN) with mucus layer secreted on the surface of gills Micro ridge (M) (Fig 8). Which leads to produce hypoxic condition in epithelial cell of the gill surface. As concentration of AgNP dose increases with increase in exposure time for sub lethal concentration of LC_{50} for 15 days showed the degeneration (DGN), bulging (B) haemal channel (HC) with the distortion observed due to stress with extensive swelling (Fig 9), and high power microphotographs showing Swelling and Denatured Network (DN) with heavy exudation on the surface of the gills Micro ridge (M) (Fig 10). The slight increase in exposure period i.e. 30 days for sub lethal dose LC_{50} of AgNP exposure, the microphotographs showed disturbed network (DN) with Necrosis (N) of the haemal channel (Fig 11) and high power microphotographs showed fusion, Degeneration Network (DGN) and swollen micro ridges with heavily mucous laden on the surface of the stubby Micro ridge (M) (Fig 12). The higher concentration and long exposure showed the complete loss to the regular pattern of micro ridges under stress condition of AgNP exposure. During the present investigation, it was observed that the ultrastructural alterations increase with the increase in concentration of the AgNP and exposure period. On 30 days exposure of the Crab, *Paratelphusa jaquemontii,* to different concentrations of AgNP, severe ultrastructural alterations were observed. The gill filament revealed heavy exudation of mucous and disorganised lamellae.

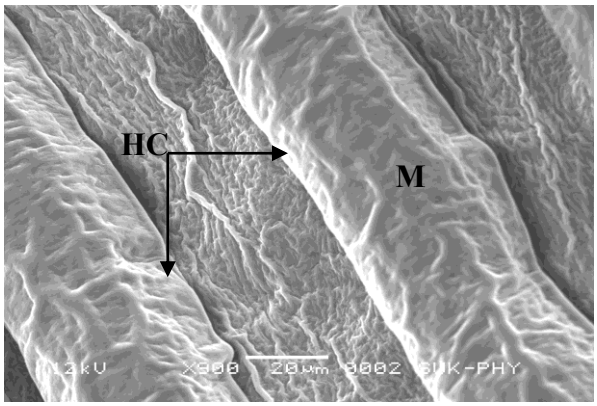

Fig. 3: Scanning electron microphotograph of control gill of freshwater crab, Paratelphusa jacquemontii showing network of haemal channels (HC). Note normal architecture of micro ridges (M). X 900

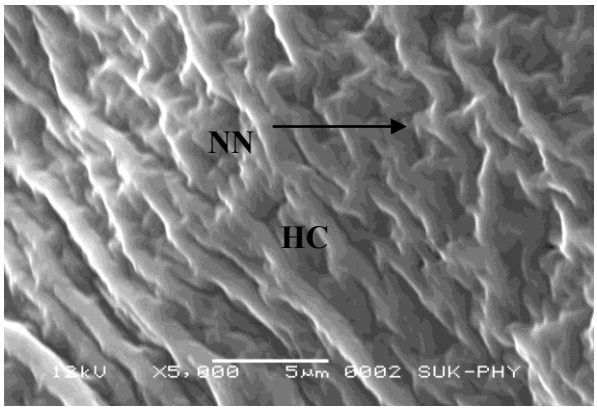

Fig.4: High power SEM microphotograph of control gill of freshwater crab, *Paratelphusa jacquemontii* showing surface of haemal channels (HC). Note normal network (NN) and distinct appearance of haemal channels.X 5000

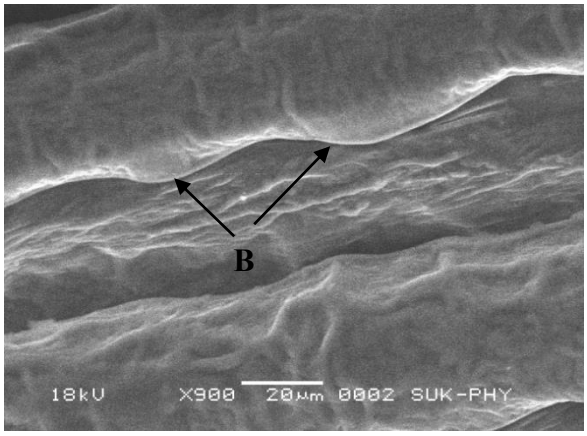

Fig. 5: SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP at LC_0 for 15 days showing network of haemal channels (HC). Note disturbed architecture of micro ridges (M) and bulging (B) haemal channels (HC). X 900

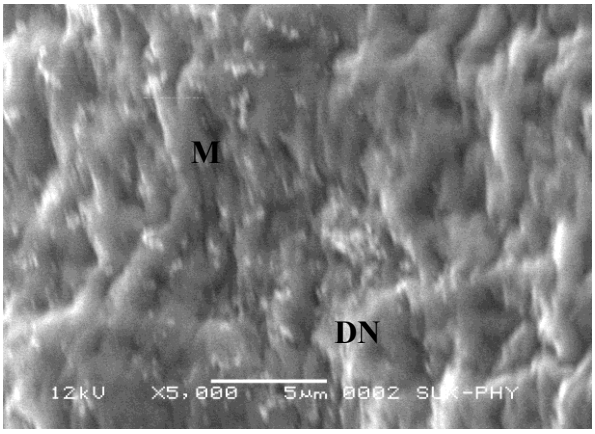

Fig. 6: High power SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP at LC_0 for 15 days showing surface of haemal channels (HC). Note highly degenerated network (DN) and surface of haemal channels. X 5000

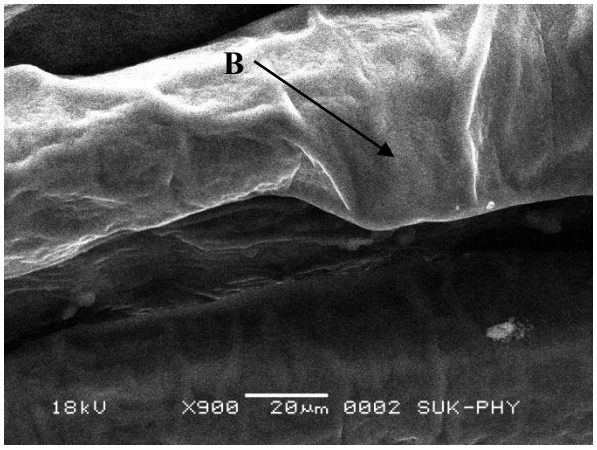

Fig. 7: SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP at LC_0 for 30 days showing network of haemal channels (HC). Note disturbed architecture of micro ridges (M) and bulging (B) haemal channels (HC). X 900

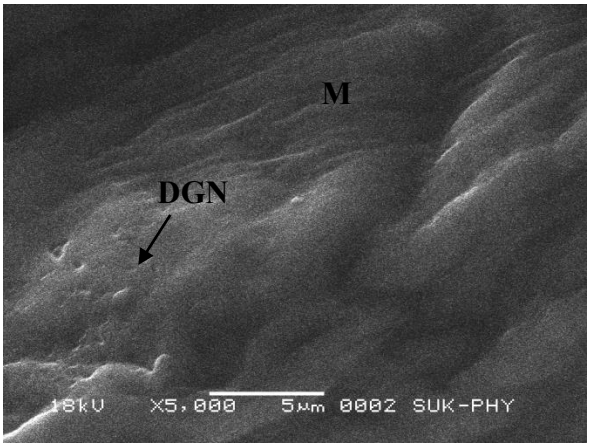

Fig. 8: High power SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP at LC_0 for 30 days showing surface of haemal channels (HC). Note highly degenerated network (DGN) and surface of haemal channels. X 5000

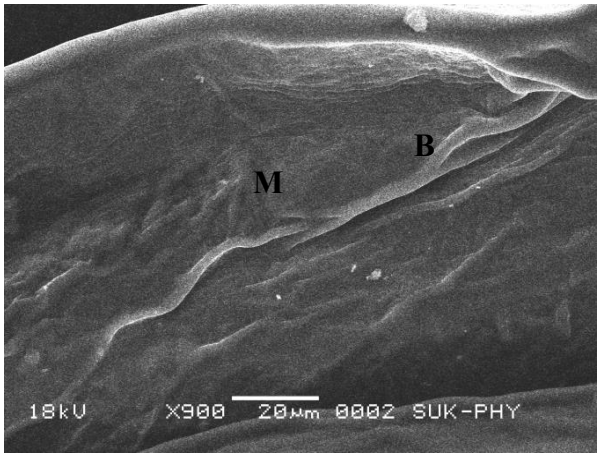

Fig. 9: SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP a LC_{50} for 15 days showing network of haemal channels (HC). Note disturbed architecture of micro ridges (M) and bulging (B) haemal channels (HC). X 900

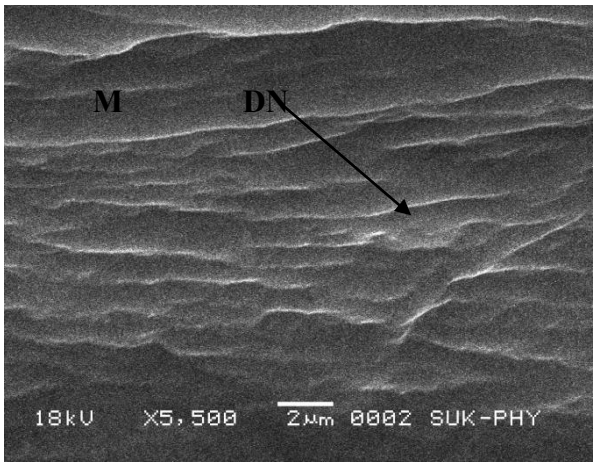

Fig. 10: High power SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP a LC_{50} for 15 days showing surface of haemal channels (HC). Note highly degenerated network (DN) and surface of haemal channels. X 5000

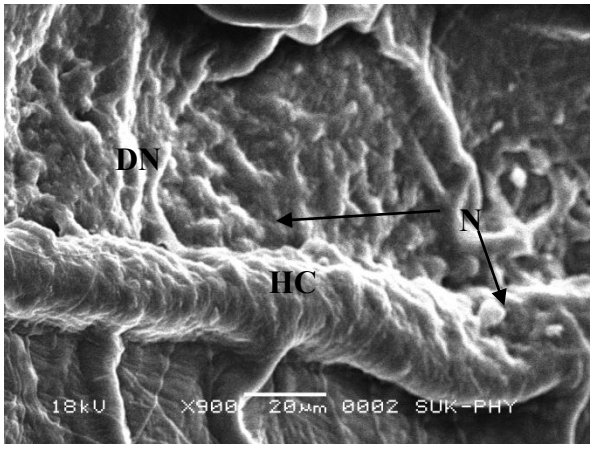

Fig. 11: SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP at LC_{50} for 30 days showing network of haemal channels (HC). Note disturbed architecture of micro ridges (M) and haemal channels (HC). X 900

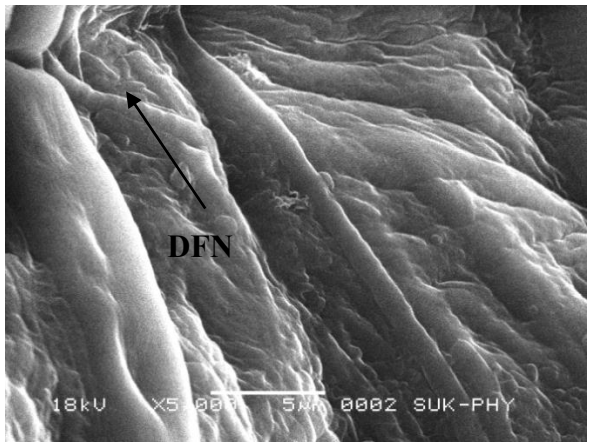

Fig. 12: High power SEM microphotograph of gill of freshwater crab, *Paratelphusa jacquemontii* exposed to AgNP at LC_{50} for 30 days showing surface of haemal channels (HC). Note highly defragmentation network (DFN) and surface of haemal channels. X 5000

DISCUSSION

Crustacean gills are important organs which do manifold functions and number of physiological processes such as a base for hemolymph osmoregulation, acid-base balance, and excretion of ammonia [15]. Gills are the first sites by which pollutants enter in the body of aquatic organism and therefore necessitate studying of this vital organ as a part of exposure analysis [16, 17]. Studies on metal toxicity and accumulation in gills are carried out by many researchers in fishes, but very few studies in metal toxicity of crustaceans [18]. Metals are one of the toxicants, which represents major environmental problems causing long-term effects on aquatic ecosystems [19]. As studies revealed that the major route of AgNP entry is via direct passage across surface epithelia and that the gills are

key target organ for Ag accumulation, which causes changes in the gills of aquatic animals [20]. Researchers have observed the effects of toxicants in the gills (disruption of pilaster cells and collapse of gill lamellae etc.). Gill surface alterations such as shrinkage, detachment of epithelium, degeneration of micro-ridges had reported due to various stress conditions [21, 22]. In the present study, the surface of the gill epithelium showed a disintegrated network of haemal channels and distorted architecture due to stress conditions of AgNP. It is due to a loss of structural integrity of epithelial cells causing breakage of primary gill filament from gill arch and shortening of secondary gill lamellae [23, 24, 25]. These changes were severe in the LC_{50} concentration of 15 and 30 days exposure period than the LC_0 concentration of 15 and 30 days exposure period to gills. AgNP toxicity mechanism imparts surface of the organ as ion convey and ion uptake concerned with gills in aquatic organisms. Further, this changes results in loss of respiratory area leading to a hypoxic condition of metabolism in exposed organisms. Oxygen deficiency creates stress, which results in necrosis, gill fouling and mortality. Similar changes in the bronchial architecture of *Ctenopharyngodon idella* were observed ultrastructurally [26]. Bulging of the gill filaments in an edible estuarine clam, *Meretrix casta* followed by exposure to heavy metal which was observed by Saravanan *et al.,* (2013) [27]. Griffitt *et al.,* (2007) confirmed that CuNP damaged the gill lamellae, characterised by proliferation of epithelial cells as well as oedema of primary and secondary gill filaments [28]. As studied by Deepasree and Nair, 2003, observed the gills of *Channa punctatus* exposed to pesticide exhibited lamellar fusion at the tip of gill filaments. Also, a few aneurysms observed in gill lamellae. The gills showed curved secondary gill filaments resulting in distortion of the lamellae and the epithelial layer detached completely from the central portion of gill lamellae [29]. Similar results were also been confirmed with the study.

5. CONCLUSION

The sublethal dose of LC_{50} of AgNP produces severe toxic effects on the respiratory organ of the freshwater crab, *Paratelphusa jacquemontii*. The finding of present study indicates that ultrastructural changes observed provides as “Biomarkers” for assessing AgNP toxicity in the aquatic organisms. The study also warns use of AgNPs for medical purpose be used with caution.

6.ACKNOWLEDGEMENT

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