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HISTOPATHOLOGY IN HEPATOPANCREAS OF CRAB, *PARATELPHUSA JACQUEMONTII* EXPOSED TO SILVER NANOPARTICLES

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ABSTRACT: Study was conducted to determine the effects of Silver nanoparticles (AgNP) on the Crabs, *Paratelphusa jacquemontii*. The crabs were exposed to sub lethal concentration of LC_0 and LC_{50} (0.001 and 0.71 mg/l) of Silver nanoparticles (AgNP) for 30 and 15 days. Chronic exposure of crabs to the AgNP resulted prominent changes in the hepatopancreas structure observed by light microscopic study. The lumen showed more dilation with disintegration of connective tissue. Accumulation of haemocytes was seen in the connective tissue. Epithelial cells showed shrinkage of protoplasmic material. Connective tissue showed more accumulation of haematocytes and formation of necrotic spaces as well as occurrence of cell debris in between the tissue were also observed in the hepatopancreas due to chronic exposure to the AgNP. Moreover, our finding indicates that the toxic effects increased with the concentration, dose and time period of exposure.

KEYWORDS: AgNP, Crab, Hepatopancreas, Histology, Toxicity.

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1. INTRODUCTION

Nanotechnology deals with the manufacture of recent materials, devices, and distinctive technological structures with extensive variety of capacity packages on the atomic and molecular level. The widespread applications of nanoparticles in our daily life have unavoidably increase exposure to human and ecosystem [1]. In the environmental component silver released by the leaching, mining, and anthropogenic sources. Silver is traditionally corporate in the coins, jewellery, electronics and photographic manufacturing [2]. Release of AgNP to the environment has been through the production, transport, erosion, washing or direct disposal of AgNP containing

products [3]. A study mentions that 1,317 products enclosed nanoparticles, and out of these products, 313 contains AgNP. Silver nanoparticles used in the verity of the products [4]. The expanded use of AgNPs in consumer products, e.g. textiles, cosmetics, and personal hygiene, household appliances e.g., washing machines, vacuum cleaners and medical apparatus have led to their amplified discharge into the environment, in that way posing an environmental risk and human wellbeing concern [5]. The growing use of nanoparticles in industrial and domestic applications has been lead to the discharge of such materials into the environment. Therefore, studies are important to be conducted to understand and predict the environmental fate, transport, transformation, and toxicity of AgNPs [6]. AgNPs have been revealed to be toxic to numerous organisms. Ecotoxicological investigations of AgNPs have showed effects such as bio-uptake and reproductive stoppage in earthworms [7]. In the aquatic environment, AgNPs negatively affect prokaryotes, invertebrates, and fish. Toxicity mechanisms in aquatic animals have not yet been well-defined for AgNP but can include destabilisation of outer-membrane integrity, disruption of membrane potential, cytotoxicity, genotoxicity, interruption of energy transduction, and formation of reactive oxygen species [8]. Kim *et al.*, (2009) found changes of the expression level of genes fall into clinical indicators and histopathological changes [9]. The crustacean hepatopancreas has diverse functions including absorption, nutrients storage and vitellogenesis for the period of growth, and ovarian development [10]. Therefore, the study was carried out to know histological alterations in hepatopancreas of the crab, *Paratelephusa jacquemontii* after exposure to AgNP doses which can be applicable while evaluating effects of AgNP in human and other organisms.

2. MATERIALS AND METHODS

2.1 Material synthesis and characteristics

The nanoparticles synthesis method adopted for current study as described by Emanuala Filipo *et al.,* 2010 [11]. Green synthesis method was adopted for preparation of silver nanoparticles. Take 200 ml double distilled water and add it into 10 gm of sucrose. The mixture kept in water bath at 70-80^0 C for 10 minutes with continuously stirring condition. Prepare 10^{-1} molar $AgNO_3$ solution and add drop wise in the reducer solution. The colloidal solution heated up to next 30 minutes till solution appeares yellow in colour. The obtained yellow colour means the formation of silver nanoparticles (AgNP). The AgNP solutions characteristics confirmed with following techniques: UV-Vis Spectrum scanned from 800 to 200 nm using a spectrophotometer (Schimadzu UV 1800). The concentration of AgNP solution was measured with Inductive Coupled Plasma Atomic Emission Spectroscopy (ICP - AES) (SPECTRO Analytical Instruments GmbH, Germany). The size and distribution of AgNPs were confirmed with dynamic light scattering DLS (Malvern).

2.2 Experimental Design

For the current study aquatic animal crab, *Paratelphusa jacquemontii* was used. The selected size (mean length 6 - 8 (± 0.8) cm and mean weight 80 - 120 gm) crabs were purchased from local

market. Prior to the start of experiment, crabs were acclimatized to laboratory conditions for 48 hrs and then exposed with AgNP to find out LC_{50} as per OECD guidelines. Five experimental groups were made with each group containing ten crabs. Experimental groups were made as, 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. Individual trey contains 3 litres of lake water 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 selected to assess effects on hepatopancreas and were fixed in Boiun's fixative. After fixation tissues were dehydrated using a series of graded ethanol solutions. They were cleared in xylene and embedded in paraffin wax. Slices of 5 µm were taken from paraffin blocks with the help of rotary microtome. These slices were spread on glass slides and then stained with haematoxylin-eosin and examined microscopically.

2.3 Data Analysis

The mortality study was done carried out 96 hrs and LC_{50} was calculated by value done 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

The UV-Vis spectroscopic results showed maximum absorbance of synthesized material at 420 nm in Fig 1. These findings confirmed that the synthesised material is AgNP. The ICP-AES confirmed the concentration of AgNP with 0.031 ppm which showed in Fig 2. Dilution was adopted for the analysis of AgNP concentration. After analysis the actual calculation found 248 ppm of AgNP. The DLS measurements exhibited that the hydrodynamic diameter of the AgNPs ranged from 60 to 120 nm, with an average value of 65.76 nm (Fig 3). This data clearly confirmed a homogeneous dispersion of the AgNPs in aqueous solutions.

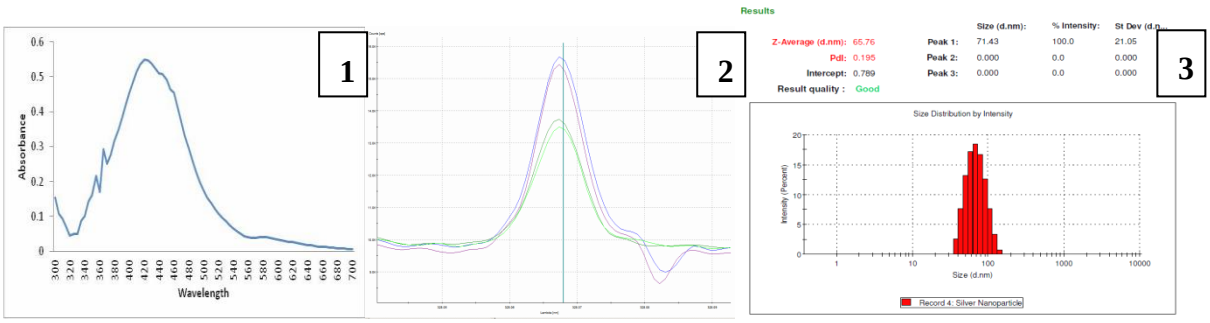


Fig 1: UV–Vis spectrum of synthesized AgNP. **Fig 2:** ICP-AES analysis peak the

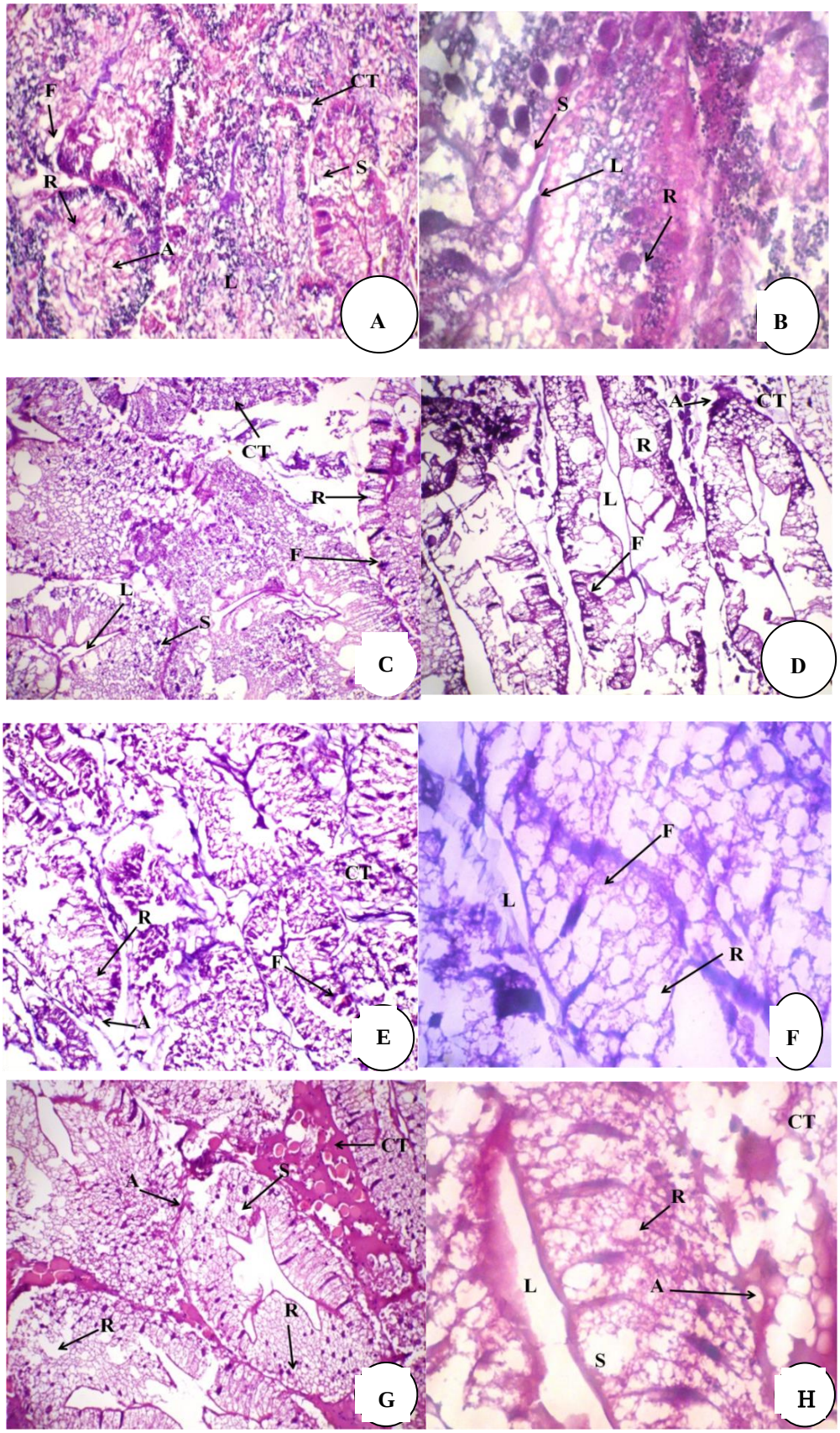
3.1 Lethal Concentration 96 hours Study

The 96 hrs acute toxicity test was carried out for AgNP toxicity study. Mortality was recorded for 24, 48, 72 and 96 hrs and lethal concentration value LC_{50} was calculated with Biostat v5 software, for AgNP solution with the test animal crab, *Paratelphusa jacquemontii* i.e., 3.62, 2.70, 1.88, 0.77 PPM respectively. This study is the evidence of toxicity of colloidal AgNP to the freshwater crab. Behaviour of the control animals was found normal throughout the study but the crabs introduced with AgNP's showed abnormal behaviour. In crustaceans, the exposure routes are mainly passing through ingestion and adsorption to surface epithelia such as gills. The toxic effects of AgNP are bigger with the dose and time.

3.2 Hepatopancreas Histopathology

The light microscopic structure of hepatopancreas of the control crab shows a typical crustacean midgut gland structure showing a large number of acini. The lumen of each acinus is surrounded by layer of epithelial cells, which are further identified into four types viz. embryonic cells (E cell), fibrillar cells (F cell), juvenile cells or mature absorptive cells (R cell) and secretory cells (S cell) (Fig. A). The secretory cells are more in number showing extensive secretory activity. The connective tissue is uniform showing blood vessels intermittently. The lumen is filled by translucent secretory material (Fig. B). The light microscopic structure of hepatopancreas after chronic exposure at LC_0 concentration for 15 days period with the AgNP showed hypertrophy of acini. Connective tissue, acini, dialeted lumen, secretory cells fibrillar cells and storage cells were observed (Fig. C). The secretory material in the lumen is reduced and connective tissue showed vaccuolation (Fig. D). The light microscopic structure of hepatopancreas after chronic exposure for 30 days period with the AgNP for the concentration of LC_0 showed with connective tissue, acini, dialeted lumen, secretory cells, fibrillar cells and storage cells. The secretory cells are in the secretory phase. Connective tissue shows disintegration with vacuolisation (Fig. E). Lumen is with reduced amount of secretory material (Fig. F). The light microscopic structure of hepatopancreas after chronic exposure to LC_{50} concentration for 15 days period with AgNP for the concentration of $LC_{50,}$ showed prominent histological changes. The lumen increased in its size. The acini show four different types of cells as embryonic cell, secretory cell, absorptive cell and fibrillar cell. The secretory cell is enlarged in its size with shrinkage of cytoplasm (Fig. G). It also shows vacuolization. The absorptive cell also showed changes like nuclear enlargement, disintegration of cytoplasm and vacuolization (Fig. H). The light microscopic structure of hepatopancreas after the chronic exposure for 30 day period with the AgNP for the concentration of LC_{50} the hepatopancreas observed with prominent histological changes. The connective tissue, acini with dilated lumen, secretory cells, fibrillar cells and storage cells were observed. Lumen shows more dilated and connective tissue disintegration (Fig. I). The epithelial cells showed shrinkage of protoplasmic material. Connective tissue showed more accumulation of haematocytes. Some of

depositions of AgNP are also seen in the cells (Fig. J).



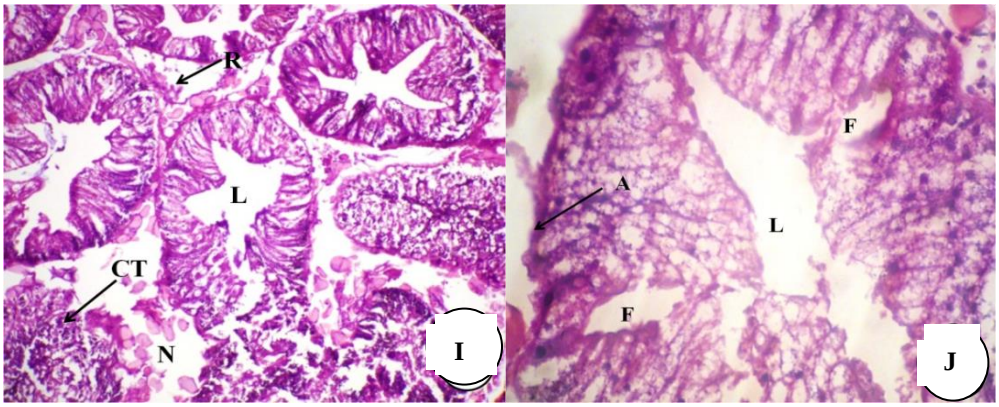


Fig. A, B: Light photomicrograph of control hepatopancreas of the crab, *Paratelphusa (Barytelphusa) jacquemontii* (Rathbun) showing acini (A), connective tissue (CT), Lumen (L), secretory cells (S), fibrillar cells (F) and storage cells (R). X25, X40

Fig. C, D: Light photomicrograph of the hepatopancreas of the crab, *Paratelphusa (Barytelphusa) jacquemontii* (Rathbun) exposed to AgNP for LC0 15 days showing acini (A) with scattered and widened connective tissue (CT). Note the dilated lumen (L). Also note secretory cells (S), fibrillar cells (F) and storage cells (R). X25, X40

Fig. E, F: Light photomicrograph of the hepatopancreas of the crab, *Paratelphusa (Barytelphusa) jacquemontii* (Rathbun) exposed to AgNP for LC0 30 days showing enlarged acini (A). Note scattered connective tissue (CT), lumen with scanty secretory material. Also note secretory cells (S), fibrillar cells (F) and storage cells (R). X25, X40

Fig. G, H: Light photomicrograph of the hepatopancreas of the crab, *Paratelphusa (Barytelphusa) jacquemontii* (Rathbun) exposed to AgNP for LC50 15 days showing acini (A) with scattered and widened connective tissue (CT). Note the dilated lumen (L). Also note secretory cells (S), fibrillar cells (F) and storage cells (R). X25, X40

Fig. I, J: Light photomicrograph of the hepatopancreas of the crab, *Paratelphusa (Barytelphusa) jacquemontii* (Rathbun) exposed to AgNP for LC50 30 days showing enlarged acini (A). Note scattered connective tissue (CT), lumen with scanty secretory material. Also note secretory cells (S), fibrillar cells (F) and storage cells (R). X25, X40.

DISCUSSION

Hepatopancreas is called as digestive gland or midgut gland which functions as similar to human liver and pancreas [12]. The metabolic processes in body of crustaceans regulated by the hepatopancreas and thus any change occurring in to the hepatopancreas and their processes can occurs the reflection of cellular destruction of the animals. In crustacean, the hepatopancreas is the main organ responsible of absorption and storage of ingested substancess. This organ is also concerned in the creation of digestive enzymes and the detoxification of xenobiotics [13]. The histological changes can give valuable information on the nature of harm to the cells and tissues and it is a good indicator of pollution hazard. LC_{50} of the AgNP were calculated for 96 hours and

exposed crabs to sub lethal concentration of LC_{50} concentration for 15 days and 30 days time period. Light microscopic study was taken into account to know about histopathological alterations occurred in hepatopancreas of freshwater crab, *Paratelphusa jacquemontii*, which was exposed to AgNP's for LC_0 and LC_{50} concentration for 15 days and 30 days. Histopathological study was carried out with control and exposed gills of crab. Histological alterations observed in the hepatopancreas of fresh water crab, *Paratelphusa jacquemontii* (Rathbun) exposed to pollutants the hepatopancreas revealed infiltration, formation of huge lumen and disappearance of haemocytes [14]. The nanoparticles exposure with hepatopancreas of crustaceans showed abnormal structural alterations in the hepatopancreas such as widespread vacuolation, necrotic lamellar lesion formation and haemocytic infiltration [15]. Likewise, hemocytic infiltration in the interstitial sinuses, an increased number of haemocytes, thickening and ruptures of the basal laminae, and necrosis of the tubules were observed in the hepatopancreas of Giant Freshwater Prawn, *Macrobrachium rosenbergii* [16]. In the present study AgNP has been observed accumulated in to the hepatopancreas at the higher concentration of exposure i.e LC_{50}. Hepatopancreas or mid gut gland in crustacean is organ of regulation of metabolism. Cellular damage induced as toxic effect is reason for disintegration of this organ leading to death of exposed organism. Similarly with hepatopancreas of the prawn, *Palaemonetes turcorum* was confirmed histopathological changes by Kutlu *et al.,* (2005) after exposure to lead acetate [17]. Hepatopancreas is storage site for vitellogenin precursors before its release in to ovary in vitellogenesis process. Organic and inorganic contaminants in polluted environment have produced morphological changes in the hepatopancreas of the crustaceans after exposure [18]. There were necrotic cells, disruption of the secretory cells. As studied by Manisseril *et al.,* (1996), further dameges were seen with the mitochondria, endoplasmic reticulum and nuclear membrane in midgut gland [19]. In currunt study the deceased hepatopancreas showed infiltration, formation of large lumen and disappearance of haemocytes which one also seen by Sanageetha *et. al.,* 2016 [20]. Thus, hepatopancreas is organ of paramount importance in crustaceans. Normal function of this organ is necessary for protective mechanism against pollutants in their surroundings. The present study supports to the histopathological observations of work carried out by other authors in respect to the effects of the accumulations of nanopartilces into the hepatopancreas.

4. CONCLUSION

This study shows that the sublethal dose of LC_{50} of AgNP's indicates severe toxic effects on the hepatopancreas of freshwater crab, *Paratelphusa jacquemontii*. The histological study of hepatopancreas shows scattered connective tissue, vacuolisation and lumen with scanty secretory material which has lead to changes in cellular level. Thus cellular damage induced as toxic effect is reason for disintegration of this organ leading to death of exposed organisms. More extensive studies are recommended for deeper understanding of the mechanisms and effect of nanomaterial

hazards when other nanoparticles and environmental pollutants are present in the environment.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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