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# Effect of Silver Nanoparticles on Haematological and Protein Metabolic Indices of Carp Fish, *Catla catla,* treated with Lihocin and *A. veronii*

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## Abstract

Fish is an important component of human diet and one of the cheapest sources of quality animal protein available to millions across the world. Fish serves as a major health food owing to its higher protein, beneficial fat and various micro nutrients. The freshwater fish, *Catla catla*, exposed to sub lethal concentration of Lihocin, *Aeromonas veronii*, and with Silver Naoparticles for a period of 45 days. In the present study the Lihocin striking behavioural, haematological, serological and antibacterial activity changes in the experimental fishes. After introduction of chemical, fishes showed interestingly try to jump out of aquarium to avoid the chemical stress followed by increased swimming, restlessness, surfacing and hyper activity. Lihocin and bacteria caused to significant ($P< 0.05$) decrease in haematological parameters such as Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Haemoglobin (Hb) and Packed Cell Volume (PCV) over the control fishes. The Lihocin and bacteria also caused a significant decrease in total proteins, whereas significantly increased free amino acids levels in gill, liver and muscle tissues of the fish *Catla catla*, indicating that increase of free amino acid levels were the resulting the breakdown of proteins for energy and impaired incorporation of amino acids in protein synthesis and increase in proteolytic activity. Reduced in tissue protein content suggests stress in metabolic process and impairment of protein synthesis machinery in fish and the catabolic process was initiated by increased proteolysis that led to rapid decline in protein content to meet the energy demand in extremely stressful medium. Increased activities of AAT and AlAT were observed in both lethal and sublethal concentrations of Lihocin and bacteria, *A. veronii* for over 45 days. Elevation of AAT and ALAT in different tissues of fish suggests either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or free fatty acids during Lihocin toxicity.

Key-Words: *Catla catla*, Lihocin, *Aeromonas veronii*, Silver Naoparticles, Macrocytic anemia, Leucopenia, Hypoprotenemia, Gluconeogenesis

## Introduction

The importance of the fisheries sector in India is demonstrated by the fact that it employs more than five million people contributes to food and nutritional security and employment, supports livelihoods, and raises the socioeconomic status of poor fishing communities. During the past two decades, the inland fisheries in India, which include both capture and culture fisheries, have registered tremendous growth and change. Until the mid-1980s, capture fisheries were major source of inland fish production[1].

The organochlorine insecticide 2-Chloro N,N,N, trimethyl ethano ammonium, commercially available as lihocin (OC), is used as a treatment against ectoparasite and as an insecticide for crops. Lihocin is poorly hydrolyze and as such, it biodegrades slowly in the environment.

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So, this compound persists for longer time in the food chain and cause severe effects at different levels of food chains. A review of the toxicological literature reveals that the exposure to toxic chemicals can produce unexpected effects in non target animals [2, 3]. It is well known that the most of the OC compounds and their derivatives adversely affect the nervous system.

Organochlorine insecticidal pollution and Bacterial fish diseases are the major threats to the sustainable development of aquaculture causing loss of millions of dollars annually[4]. Insecticides at high concentration are known to reduce the survival, growth and reproduction of fish, and produce many visible effects on fish[5]. Lihocin, an Organochlorine insecticide is highly toxic to fish which absorbs it directly from water or by ingesting contaminated food and bioaccumulate in their fatty acids due to its Lipophilic nature[6]. Lihocin entered in to the environment cause serious threatening to aquatic

organisms and also cause severe metabolic abnormalities in non target species like fish and freshwater mussels[7].

Nanotechnology is an emerging scientific field and considered to have potential to generate new and innovative materials. Nanotechnology provides the ability to engineer the properties of materials by controlling their size and this has driven research towards a multitude of potential uses for nanomaterials. Nanoparticles exhibit distinct optical, thermal, chemical and physical properties from that of the bulk material due to their higher surface area to volume ratio. Silver nanoparticles have been found many applications in therapeutics, antimicrobial drugs, microelectronics and biosensing devices because of their unique properties[8-10].

Fish is an important component of human diet and one of the cheapest sources of quality animal protein available to millions across the world. Fish serves as a major health food owing to its higher protein, beneficial fat and various micro nutrients. Moreover, during the past several decades fisheries and aquaculture are contributing to global food security, poverty alleviation, rural livelihoods, employment and income generation [11].

The research has shown that fish is most frequently and most extensively contaminated with bacteria from the genus Aeromonas. Woo and Bruno[12]. Aeromonas sps species secretes many extracellular proteins, including amylase, chitinase, elastase, aerolysin, nuclease, gelatinase, lectinase, lipase and protease. These proteins are known as virulence factors that cause disease in fish and humans. Aerolysin is a representative virulence factor of Aeromonas and was reported to function as hemolysins and cytolytic enterotoxins[13]. The mesophilic *Aeromonas hydrophila* and *Aeromonas veronii* are recognized causative agents of fish diseases[14].

*A. veronii* are rod shaped motile, gram negative, facultative anaerobe. The bacteria are usually not found in groups or pairs but as individual cells[15]. *Aeromonas veronii* have a broad host range, and often have been implicated in the cause of numerous infections, such as humans with diarrhoea and fish with hemorrhagic septicaemia[16]. The diseased fish had similar clinical signs including open dermal ulcers on the body (ulcerative syndrome), haemorrhages over the skin, tail and fin rot, bacteria gill rot and dropsy[17] in the liver, kidney, swim bladder, spleen infracts fatty liver, ascetic fluid and lack of feeding, and visible pathological changes on the liver characterized by irregular hemorrhagic blots.

Due to the increase in the outbreak of bacterial diseases in the aquaculture industry and the development of bacterial resistance, new antibacterial agents are required. Silver nanoparticles have proved to be one of the most effective metallic nanoparticles and good antibacterial activity against some bacterial pathogens and fish pathogens[18]. The antibacterial effect of nanoparticles is independent of acquisition of resistance by the bacteria against antibiotics. Silver nanoparticles have properties, such as good conductivity, chemical stability, catalytic, antibacterial activity, antifungal, anti-viral, anti-inflammatory[19]. Nanoparticles (NPs) at the interface with aquatic organisms will interact with an extraordinary number of disease biological surfaces. These include skin, gills or gut tissues that have in common epithelial cellular structures in fish and invertebrates, as well as cell walls, which enclose the plasma membrane in microorganisms.

Transaminases form an important group of enzymes mediating carbohydrates, protein and lipid metabolism. Transamination represents the mechanism causing eventual deposition of nitrogenous waste products like ammonia and urea resulting in the production of carbon compounds, which contribute towards gluconeogenesis and fatty acid formation. AAT and ALAT are two important enzymes mainly involved in the inter-conversion of important compounds such as pyruvate, oxaloacetate, α-ketoglutarate and amino acids thus bringing the protein and carbohydrate metabolism on one hand and alanine, aspartic acid and glutamic acid[20]. The activity of aspartate and alanine amino transferases (AAT and AlAT), which serve as strategic links between protein and carbohydrate metabolisms, which is known to alter under several physiological and pathological conditions.

Silver nanoparticles are used in commercial products for their antibacterial and antifungal properties. Some of these products are likely to result in silver nanoparticles reaching the aquatic environment. Investigation cytotoxicity and genotoxicity of 30 nm diameter silver nanospheres are cytotoxic and genotoxic to fish cells[21]. The major mechanism through which silver nanoparticles manifested antibacterial properties is by anchoring to and penetrating the bacterial cell wall, and modulating cellular signalling by dephosphorylating putative key peptide substrates on tyrosine residues. The interaction of these nanoparticles with biologically active ligand in the animal system through chelation[22].

Silver nanoparticles can enter into cell then release the silver ion to combine with thiol, carboxyl, and hydroxyl group in cell for deactivate functions:

- Combine with respiratory enzyme to cause suffocate.
- Bind with protease enzyme and cause indigestion.
- Bind with DNAs and inhibit cell replication.
- After the bacterial cell functions disturbed by silver nano particles, these effects will lead to cell damage and death of bacteria cell.

The present study is aimed to evaluate the effect of silver nanoparticles on Antibacterial activity, Haematological indices, Free Aminoacids and Protein metabolism in various tissues of Lihocin and *A. vernoii* infected carp fish, *Catla catla* over an exposure period of 45 days. In the present investigation the levels of total proteins, protease activity and free amino acids were estimated in the Gill, liver, kidney and muscle of fish.

## Material and Methods

### Experimental Animal

Live specimens of *Catla catla* of 12 ± 1.5 g and body length of 8.5 ± 1.5 cm were collected from AP Govt. Fish Breeding and Hatchery Centre, Kalyanidam, near Tirupati, Chittoor district and immediately transferred to transparent polypropylene tank of 500L capacity filled with filtered, well aerated and dechlorinated bore well water. They were stored in large glass aquaria and water in the tanks was changed daily. The water quality is maintained constantly throughout the experimental period. The average temperature of water was 24-26°C. They were fed with rice bran, oil cake and soya bean in the ratio of 2:2:1 daily. The physico-chemical parameters of water are given in Table 1.

### Experimental Chemical

Technical grade Lihocin [2-Chloro-N, N, N-trimethylethanaminium chloride] is a chlorocholine chloride which is used as a planned growth regulator (PGR) is obtained from Kisan Agro Agencies, Anantapur. They are highly insoluble in water, but are attracted to fats. Lihocin is very stable in both fresh and salt-water environments[7]. $LC_{50}$ of Lihocin for 96h is calculated by the static bioassay method[23]. Fish were starved 24h prior to the experimental period to nullify the metabolic changes. Five replicates of each containing twenty fish were subjected to Lihocin at various concentrations for 96 hours. The lethal concentration of Lihocin ($LC_{50}$/96hrs) and identified as 7.63mg/L and 1.56mg/L was selected as $1/5^{th}$ sub lethal concentration for the further analysis.

### Experimental strain

Bacterial fish pathogens *Aeromonas Veronii* were obtained from Central Institute for Brackish water Aquaculture (CIBA), Chennai, Tamil Nadu, India. 100 µL of the overnight culture of bacteria were transferred to 10 mL TSB (tryptone soya broth) and incubated at 35°C with shaking. Absorbances of the cultures were measured at 600 nm after 5 hours and the viable cell count at this absorbance was determined by plating onto tryptic soy agar (TSA). According to the correlation between absorbance and viable cell count, approximately 105 - 106 cfu/mL of bacteria was inoculated into the wells of the microplates based on reports[24].

**Table 1: Physico-chemical parameters of water used for the present study**

| S. No. | Parameter | Value |
|---|---|---|
| 1 | Turbidity | 7.85 ± 0.04 Silica units |
| 2 | Electrical Conductivity at 28°C | 821micro ohms/cm |
| 3 | pH at 28°C | 7.42±0.02 |
| 4 | Alkalinity | |
| | 1. Phenolphthalein | Nil |
| | 2. Methyl orange | 468 |
| 5 | Total hardness as (CaCO3) | 254± 1.25mg/l |
| 6 | Calcium hardness (as N) | 78 ± 0.25 mg/l |
| 7 | Sulphate (as SO4) | Trace |
| 8 | Chloride (as Cl) | 34 ± 0.16 mg/l |
| 9 | Fluoride (as F) | 1.8 ± 0.012mg/l |
| 10 | Iron (as Fe) | Nil |
| 11 | Dissolved Oxygen | 9.58 ± 0.56 ppm |
| 12 | Temperature | 27 ± 2°C |

### Preparation of Silver Nanoparticles

The silver nanoparticles (NPs) were synthesized in a one-step reduction process in an aqueous solution. In a typical preparation, a 400-µL aliquot of a 0.1-M AgNO3 aqueous solution was added into 100 mL of an aqueous solution containing 0.10 wt. % of the soluble starch and vigorously stirred for 1 h. The pH of the resulting solution was adjusted to 8.0 by adding 0.1 M NaOH solution. Under this experimental condition, the initial reaction mixture was colourless, and the growth of the AgNPs was monitored at different intervals using UV-Vis absorption spectroscopy. After about 1 h, the solution turned light yellow, which indicated the initial formation of the AgNPs. The mixture was

maintained at 50°C for 24 h, and the colour of the reaction solution became yellow.

**Experimental Regime:**

The acclimatized fishes were randomly divided in to 3 groups and each group contains 45 animals.

**-Group-1:** served as control,

**-Group-2:** treated with Lihocin

**-Group-2:** treated with pathogenic bacteria *Aeromonas veronii*

**-Group-3:** treated with silver nanoparticles against Lihocin and pathogenic bacteria

After expiry of exposure period of 45days with bacteria and silver nanoparticles, the necropsy is done to the animals and collected blood for immune-haematology and tissues like Gill, Liver, Kidney and muscles are collected for free Amino acid and protein metabolism.

**Acute toxicity of AgNPs**

A 48-hour acute toxicity ($LC_{50}$) test of silver nanoparticles for 96hrs on *Catla catla* (weighing, 30.0 ± 1.5g) was conducted in a static water renewal experiment according to Organisation for Economic Cooperation and Development (OECD) guideline for testing of chemicals[25] (OECD, 1992). Fish were starved 24h prior to the experimental period to nullify the metabolic changes. Five replicates of each containing twenty fish were subjected to silver nanoparticles at various concentrations for 96 hours. The sub lethal concentration of AgNP's ($LC_{50}$/96hrs) and identified as 84µg $L.^{-1}$ was selected for further analysis based on other reports.

**Haematological and Serological Analysis**

The blood was collected from the fishes by puncturing the heart by using 1ml insulin syringe. For serological analysis the collected blood were centrifuged at 2500 rpm for 14min.

**a) Total Erythrocyte Counts (TEC)** ($10^6 mm^{-3}$) were estimated using diluted with Dacie's fluid in the ratio 1:4 as per Blaxhall and Daisley[26] with haemocytometer under a microscope.

**b) Total Leucocyte (TLC)** (in $mm^3 x10^3$) cells counted using by Neubaur Haemocytometer[27]. Blood was diluted to 1:20 with Turks diluting fluid and placed in haemocytometer at 640x.

**c) The Haemoglobin content** (mg/100) of blood was analysed following the cyanmethaemoglobin method using Drabkins fluid and the absorbance was read in spectrophotometer at 546nm.

**d) The Hematocrit** (PCV) is used to deliberate the volume percentage (%) of red blood cells in blood, and determined by the Microhematocrit tube method.

**e) The Total Proteins** were estimated by Folin phenol reagent method of Lowry et al[28].

**f) Protease activity** was estimated using Ninhydrin method of Davis and Smith[29].

**g) The Free Amino Acids** were estimated by the Ninhydrin method of Moore and Stein[30].

**h) Aminotransferase activities:** AAT and AlAT activities were estimated using the method of Reitman and Frankel[31].

**Statistical Analysis:**

Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for difference between means significance at $p < 0.05$[32].

## Results and Discussion

**Behavioural changes**

In the 20th century, many thousands of organic trace pollutants, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans (PCDFs) and dibenzop-dioxins (PCDDs) have been produced and, in part, released into the environment. Since the early sixties mankind has become aware of the potential long-term adverse effects of these chemicals in general and their potential risks for aquatic and terrestrial ecosystems in particular. The ultimate sink for many of these contaminants is the aquatic environment, either due to direct discharges or to hydrologic and atmospheric processes[33]. .

The behavioural abnormalities and state of fishes in both the control and experimental fish was recorded at every hour of first 24 hours. The fishes showed marked changes in their behaviour when exposed to various concentrations of the Lihocin. Fishes exposed to Lihocin had whitish, slimy mucous like substances covering most parts of the body especially over the pectoral and dorsal fins. After introduction of chemical, fishes showed interestingly try to jump out of aquarium to avoid the chemical stress followed by increased swimming, restlessness, surfacing and hyper activity. In lower concentration of Lihocin the fishes showed a slow swimming than the control group. Behavioural manifestations of acute toxicity like erratic swimming, restlessness and surfacing movements were observed in *Catla catla exposed* to higher concentration of Lihocin over the 24 hours. After 24 hours fishes exhibited lathery and erratic swimming movements suggesting loss of equilibrium at higher concentration of Lihocin. At the time of death evanescent hyper activity was also observed. The reaction and survival of aquatic animal depends not only in the biological state of animals and physiochemical characteristics of water but also on the kind, toxicity, type of exposure to the toxicants. The fish treated with *Aeromonas veronii*, also exhibited increased erratic movements, restlessness and gasping

the mouth. However after the treatment with AgNPs, the behavioral manifestations of fish showed a gradual recovery and animals tried to come normal level indicating that the AgNPs caused to increase the immunity, so the fish overcome the pesticide stress and come to normal level.

**Haematological Indices**

Blood is highly susceptible to internal and external environmental fluctuations as it is acted as vehicle for the transport of pollutant[34]. The fish serves as bio indicator of water quality and the impact of pesticide can well be understood by analyzing either blood or serum. The toxic effect of pesticides to the blood of fishes has been studied by many researchers[35]. .

In the present study the results revealed a striking behavioural, haematological, serological and antibacterial activity changes in the experimental fishes. Changes in haematological indices of control and fishes treated with lihocin and *A.veronii* are depicted in **Figures: 1-4**. The haematological parameters such as Total Erythrocyte count (TEC), Total Leucocyte count (TLC), Haemoglobin (Hb) and Packed Cell Volume (PCV) revealed a significant decrease ($P< 0.05$) over the control fishes. TLC is known to defend the body against toxic and foreign substances and antibody production.

Panigrahi and Mishra[36] observed reduction in the Hb% and RBC count in the fish, Anabas, when treated with mercury. Decrease in haemoglobin, RBC count was observed in the fish Tinca tinca exposed to mercuric chloride and lead[37]. Lowering of Hb percentage might cause anaemia. This may be due to the decreased rate of production of red blood cells or increased loss of these cells. It is evident from the results that the sub lethal concentration of Lihocin caused that there is a gradual and significant decrease in all haematological indices in all exposure periods and also in treated with *Aeromonas veronii* whereas increased in the fish treated with AgNps (**Figures: 1-4**).

ANOVA results reveal that the changes in TEC were highly significant ($P<0.01$) at all intervals of Lihocin exposure in all the treated groups. Similar decline has also earlier been reported in the fishes by Das and Mukherjee[38], Verma[39], Raina[40] following exposure to different xenobiotics. Decline in TEC present author feels seemingly appears to be due to combined effect of haemolysis of RBCs and malfunctioning of haemopoietic organs. Decline in TEC also appears to be the outcome of i) an increase in the rate of erythrocyte destruction due to their lysis and ii) reduced surface area of RBC due to their abnormal shapes. The results may also indicates the distorted shape of erythrocytes, due to decrease of PCV, may cause an imbalance in the respiratory physiology of the fish by reducing the surface area of haemoglobin and its access to oxygen. It can, therefore, be very safely inferred that Lihocin has induced conspicuous alterations, both qualitatively and quantitavely, in TEC of *Catla catla.*

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Similar to TEC, Hb and Hct (**Tables: 1-3**) also exhibited similar decline throughout the exposure period. Present author proposed that decline in Hb seemingly appear to cause rapid oxidation of haemoglobin to methaemoglobin and or release of oxygen free radical. Free radical of oxygen by causing hemolysis may lead to reduced oxygen carrying capacity of blood. Prolonged reduction in Hb content may be deleterious to oxygen transport.

The present results are in good agreement with earlier reports that a significant decrease in RBC's hemoglobin and packed cell volume of fresh water fish exposed to heavy metals[41]. The variations in the blood indices may be attributed to a defense reaction against toxicity through the stimulation of erythropoiesis. The related decrease in hematological indices proved the toxic effect of heavy metals that affect both metabolic and hemopoietic activities of *Catla catla.* Our results are supported by previous research work that various toxicants and toxins enter into the aquatic system exerted a specific toxic effect on fish blood and tissues[42, 43].

The Packed cell volume (PVC) appears to be positively correlated with erythrocyte count of the fish treated with Lihocin and bacteria. Fall in the number of red blood cells followed by PCV confirms anemia in *Catla catla*. The decrease in PCV in fish exposed to the pesticide was maximum on day 45 might be due to decreased erythrocyte numbers, which in turn might be due to pesticide and bacteria exposures. The decrease in PCV indicates anaemia or oligohanaemia. The anemia developed by *Catla catla* in this study could be

regarded as macrocytic anemia. The probable mechanisms for developing anemia in *Catla catla* could be due to the loss of erythrocytes as compensatory erythropoiesis could not be observed, which was reflected in the absence of immature erythrocytes in the peripheral blood[36, 41].

The decreased haemoglobin concentration observed in this study is an indication of impaired oxygen delivery to the tissues. The observed decrease in the PCV, Hb and erythrocyte count of *Catla catla* exposed to Lihocin, could be indicative of heamodilution due to erythrocyte sequestration.

It is observed that when the fish also exposed to AgNPs all the hematological parameters are reversed and showing the recovery trend indicating that AgNPs are induced and boost up the immunity of the fish. So the fish are recovered to sustain in the pesticide contaminated aquatic medium. A reduction in leukocyte counts (i.e. leucopenia) was observed in *Catla catla* after exposure of Lihocin. The observed leucopenia was due to increased activity of the pituitary internal stress axis. Therefore, reduction in the number of circulating leukocytes is a response of the fish to increased levels of circulating ACTH and corticoid stress hormone, where as the increase in leukocyte count, when the fish treated with AgNPs, was correlated with an increase in antibody production that helps in survival and recovery of the fish exposed to a sublethal concentration of pesticide. The present findings also show hypersensitivity of leucocytes for Lihocin and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by Lihocin.

**Protein Metabolism and Enzyme Indices**

Proteins are complex nitrogen containing macromolecules. They are the basic building block of animals. The survival ability of animals exposed to stress majorly depends on their protein synthetic potentials. The proteins are the major source of energy during chronic conditions besides carbohydrates. Proteins are primary importance of the living world and only because of their peculiarity but also because of the fact that they appear to confer their biological specificity among various types of cells[44] . These are responsible for many metabolic changes in every one. In present study, an attempt has been made to examine the sub lethal toxic effect of organochlorine pesticide Lihocin on protein metabolism in terms of tissue proteins in fish. There is significant decrease in proteins in gill, liver and muscle of the fish *Catla catla* after 24 hours exposure relative to control.

Proteins are indeed of primary and paramount importance in the living world not only because of their peculiars but also because of the fact that they appear to confer their biological specificity among various type of cells[44]. Environmental stress invokes compensatory metabolic activity in the organs of an animal through modification and modulation of the quantity and quality of problems. Gill is an important organ because of its direct contact with water, which allows the pesticides to enter through it and get accumulated in the fish body. The percentage decrease of protein is greater in gill. It is maximum in 72 hours. Ganeshwade[45] reported that the alteration in protein value may also be related to some structural changes in the liver, the arrangement of hepatic words leading to the alteration of liver metabolism. The decrease in liver protein is also attributed to the inhibition of protein synthesis. The decrease in protein content suggests an increase in proteolytic activity and possible utilization of its products for metabolic purpose. The fall in protein level during exposure may be due to increased catabolism and decreased anabolism of proteins[45]. A significant reduction was observed in the levels of proteins and glycogen [46]. Jha and Verma[47] reported that the effect of the pesticidal mixture of Endosulfan, Malathion and grafun at the ratio of 1:1:1, on total protein content in the stomach, intestine and ovary of the fish *Clarias batrachus* acute, sub chronic and chronic exposures and found that depleted the total protein profiles in treated fish.

Reduced in muscle tissue protein content suggests stress in metabolic process and impairment of protein synthesis machinery in fish and the catabolic process was initiated by increased proteolysis that led to rapid decline in protein content to meet the energy demand in extremely stressful medium[48]. Hypoprotenemia was also observed in the selected tissues of fish exposed to various pesticides by many workers, supporting the findings of the present results [49, 50]. The decrease in protein content of Lihocin treated fish in the present study also indicates the physiological adaptability of the fish to compensate for pesticide stress. To overcome the stress the animals required high energy. This energy demand might have led to the stimulation of protein catabolism.

The Lihocin even at sub lethal concentration affected the levels of free amino acids of gill, liver and muscle in that in both the tissue the level increased significantly. Toxicant induced similar alterations in the quantity of free amino acids have also been reported in *Leucipus cephalus*[51] and in *Salmo giardneri*[52]. In fact during this period the protein level decreased in all tissues intensively suggesting that the

Lihocin induced proteolysis to meet the increased energy demand and the similarly postulated by Saravanan et al.[53] and Kumar et al.[54].

The protein content decreased in the liver, brain and kidney tissues during lihocin treatment (Tables 1, 2 and 3). Krueger et al.[55] reported that the fish can get the energy through the catabolism of proteins. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction in liver, brain and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis. The toxicants may have effect on hormonal balance, which could directly or indirectly affect the tissue protein levels[56, 57].

The present study also indicate that the free amino acid (FAA) pool was increased in the tissues of the fish during exposure to Lihocin and bacteria *A.veronii*, while the elevated FAA levels were utilized for energy production by supplying them as keto acids into TCA cycle through aminotransferases to contribute energy needs during toxic stress. Increases in free amino acid levels were the result of breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis[58]. It is also attributed to lesser use of amino acids and their involvement in the maintenance of an acid-base balance[59] and stress conditions induce elevation in the transanimation pathway. The increase in FAA levels of tissues indicates stepped up proteases activities and fixation of ammonia into keto acids[60]. It is evident that the enhanced FAA might be due to diminution of reserved glycogen, so the fish can try to yield metabolic energy by gluconeogenesis process. Similar findings were observed by Vijuen and Steyn[61] in various animals during different toxic conditions. The increased FAA also attributed that increased free amino acid levels in various tissues of the fish, *Catla catla*, could be due to degradation of proteins by proteolysis or due to decreased protein synthetic potentials in the pesticide induced pathological condition also supports the present trend in protein metabolism. However the protease activity levels are gradually decreased and animal tried to come normal under the treatment of AgNPs, indicating that an increase in protein content may also help to invigorate the organs for developing resistance to the imposed toxic stress and synthesis of enzymes necessary for detoxification. In the present study the shifts in protein metabolism might to compensate with situation shown by the animal for its survival.

Total serum proteins in liver, kidney, gill and muscle tissues of the fish treated with Lihocin and bacteria, *A. veronii* are decreased over control which is graphically represented in **Figure-5.** The highest percentage of reduction was observed on day 45. The level of total protein was depleted probably because of renal excretion (albuminuria) and impaired protein synthesis or was due to liver disorder after the pesticide exposure. Proteins are indispensable constituents of the body and their metabolism is almost confined to the liver. Fall in serum protein level may be due to impaired function of kidney or due to reduced protein synthesis owing to liver cirrhosis

The activities of AAT and AlAT during the toxic exposure of Lihocin pesticide and bacteria were enhanced (**Tables-8 and 9**). The elevated activities of AAT and AlAT were observed by Narasimha et al.[62] in *Anabas testudineus* during treatment of organophosphates pesticides and in *T. mossambica* under lindane toxicity and by Sajal et al.[63].

Increased activities of AAT and AlAT was observed in both lethal and sublethal concentrations of Lihocin and bacteria, *A.veronii* for over 45 days (**Tables-8, 9**). Elevation of AAT and ALAT in different tissues of fish suggests either increased operation of transamination or increased synthesis of amino acids from other sources like glucose or fattyacids during Lihocin toxicity . In the present study, ALAT activity is found to be relatively higher than AAT in all tissues of control and test fish, suggesting pyruvate contribution is slightly more than oxaloacetate formation. The increase in activities of aminotransferases as observed in the present study were in agreement with earlier reports, demonstrating a consistent increase in the activities of these enzymes under conditions of enhanced gluconeogenesis [64].

The activity levels of transaminases are gradually increased with increasing the exposure period of Lihocin pesticide and the both transaminases were showed maximum increase in liver tissues on day 45. The increase in AAT and AlAT activity levels may be attributed to either cellular damage, increased plasma membrane permeability or altered metabolism of enzymes[65]. The increased activity of AAT and AlAT may be compensatory mechanism for the impaired mitochondrial oxidation. Moreover increased transaminase activities have indicated that there is a conversion of amino acids into ketoacids than that of utilized energy synthesis. It is apparent that the increased AAT and AlAT activities are an indication of an adaptive physiological response to combat energy demand[66]. As the liver is the major metabolic centre, the conversion of amino acids into ketoacids was more

in liver tissues than other tissues and conversion gradually increased, with increase of Lihocin exposure period is justified.

The elevated transaminases may indicate that fish can utilize the free amino acids from amino acid pool for energy production. Abdul Naveed et al.[7] (2004) reported that if any malfunction occurs in energy yielding compounds, the cell switches over to the gluconeogenic process with the help of transaminases. The change in transaminases activities suggest a possible change in protein metabolism in the tissues of *Catla catla* with the exposure of Lihocin. The overall decrease in protein content in proteases and enhanced levels of amino acid transaminases might suggest the utilization of proteins under toxic stress conditions of the fish, *Catla catla*.

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**Table-1: Variations in Total Erythrocyte Count (TEC) levels of *Catla catla* exposed to sub lethal concentra- tion of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

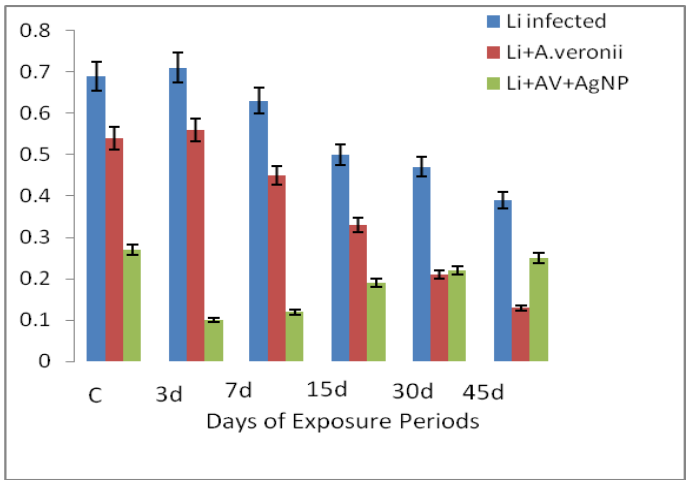



**Table-2: Variations in Haemoglobin Content (Hb) of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

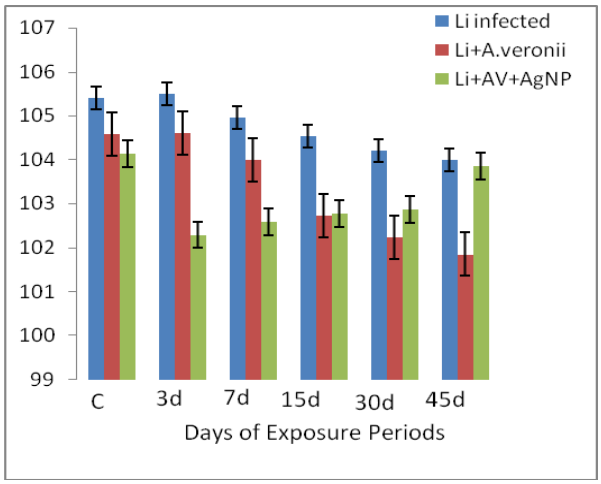



**Table-3: Variations in Hematocrit (Hct) of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

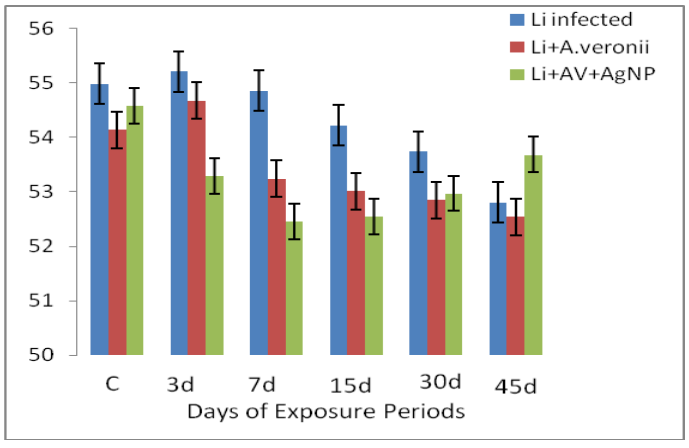



**Table-4: Variations in Total Leucocyte Count (TLC) of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

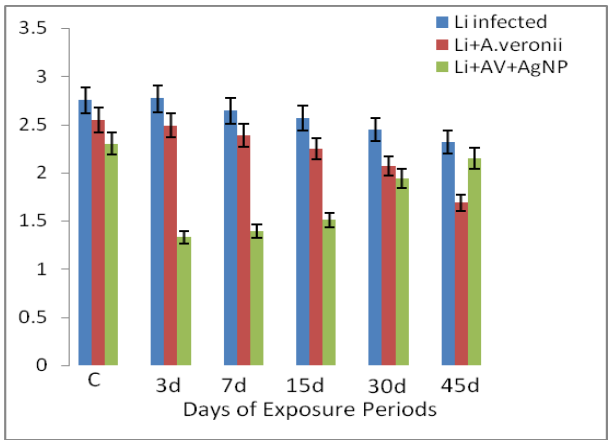


**Table-5: Variations in Protein levels of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

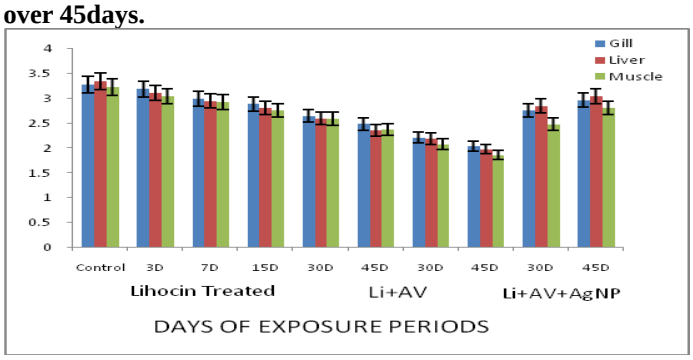



**Table-6: Variations in Protease activity levels of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

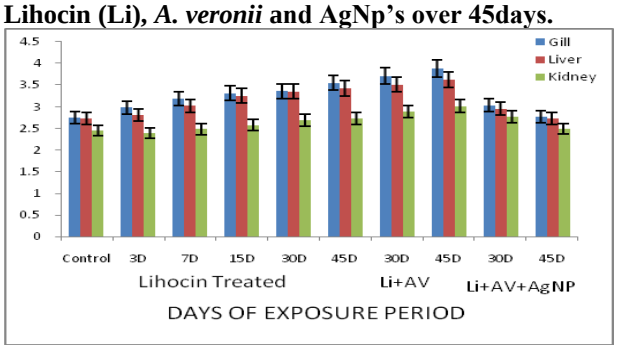



**Table-7: Variations in Total Free Amino Acids of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

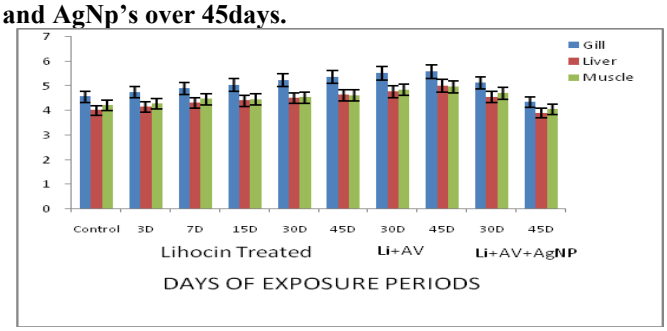



**Table-8: Variations in Alanine Amino Transferase Activity Levels of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

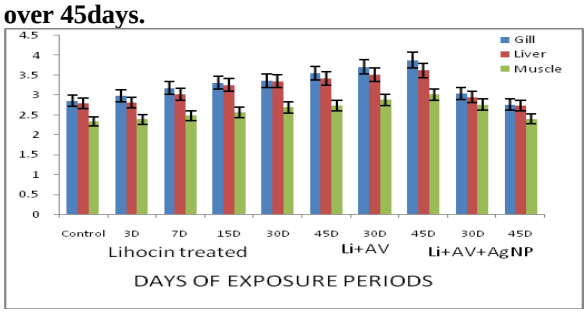
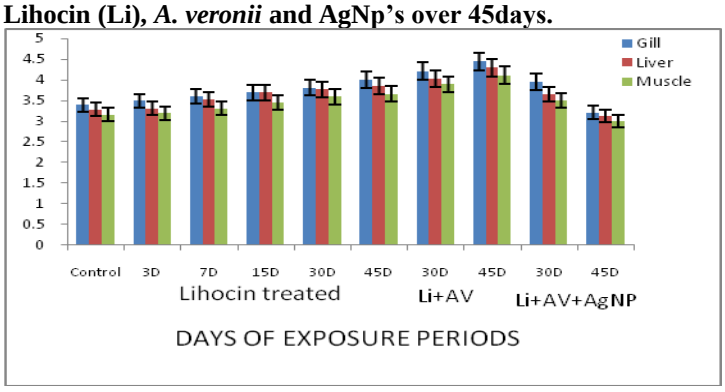



**Table-9: Variations in Aspartate Amino Transferase Activity Levels of *Catla catla* exposed to sub lethal concentration of Lihocin (Li), *A. veronii* and AgNp's over 45days.**

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