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Impact of Lihocin and *Aeromonas veronii* on Metabolic Biomarkers of fish *Catla catla* against to Immunostimulant Silver Nanoparticles

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Abstract

Nanoparticles are fundamental building blocks of nanotechnology. Advances in nanotechnology have opened new horizons in nanomedicine, allowing ecofriendly synthesis of nanoparticles that can be assembled into complex structures. In the present study the silver nanoparticles are synthesized by *Aloe vera* leaf extract due to the presence of natural phytochemicals which provide natural capping and reducing agents for the synthesis. Pesticidal pollution and infectious diseases continue to be a major problem in aquaculture fields leads to decrease in production. Novel studies and technologies are devoted to understanding the mechanisms of disease for the design of new drugs by silver nanoparticles because of its antibacterial mechanism. Pesticides are one group of toxic compounds that can have a profound effect on water quality and aquatic ecosystems. In the present study Lihocin an organochlorine pesticide and *Aeromonas veronii* showed a gradual increase in LDH and G-6-PDH activity due to non productive binding of Lihocin and *A.veronii* leads to blockage of enzyme synthesis and also showed decreased trend in SDH and MDH due to the impairment of oxidative metabolism and might be due to disruption of mitochondrion integrity augmented by cellular damage under the impending toxicity of pesticide and *A.veronii*. Silver nanoparticles acts as immunostimulant minimized toxicity of pesticide on enzyme biomarkers of fish and also showed antimicrobial mechanism against fish pathogen *A.veronii* and animals tried to recover from toxic stress.

Keywords: Silver nanoparticles, *Catla catla, Aloe vera*, Lihocin, *A.veronii,* Enzyme biomarkers, Antibacterial activity,

INTRODUCTION

Nanotechnology has grown rapidly over the past few years and has even ventured into all scientific fields including clinical medicine. Out of all kinds of nanoparticles, silver nanoparticles (AgNPs) seem to have attracted the most interests in terms of their potential application. AgNps are one of the most attractive nanomaterials for commercialization applications [1]. Thus, development of environmental friendly methods for the synthesis and assembly of nanoparticles is of considerable importance to explore their potential applications [2].

Silver nanoparticles have antibacterial activity against bacteria, fungi and viruses [3,4]. Silver ions (Ag+) are released when interact with microbes and these ions may affect and damage the cell and leads to death of microbes. The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in content with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death [5].

The development of microbial resistance against silver nanoparticles makes them advantageous antibacterial agents. Notably silver nanoparticles, are safe and non-toxic to human and animal cells at low concentrations because the possible toxicity of silver nanoparticles is considered extremely low as compared to other heavy materials present in the environment [6].

Nanotechnology has a wide usage potential in aquaculture and seafood industries [7.8]. Direct use of silver nanoparticles in water to treat a fungal disease has been found toxic to young trout whereas a water filter coated with silver nanoparticles can prevent the fungal infections in the fish culture. It can say here that the health of fish in aquaculture, nanotechnological applications on antibacterial surfaces in the aquaculture system, nanodelivery of veterinary products in fish food using porous nanostructures and nanosensors for detecting pathogens in the fish culture system.

Pesticides are one group of toxic compounds linked to human use that have a profound effect on aquatic life and water quality. Pesticides are substances used to control pests, including insects, water weeds, and plant diseases. They are used to help reduce malnutrition and starvation of humans and animals. The faster a given pesticide breaks down in the environment, the less threat it poses to aquatic life. Insecticides are typically more toxic to aquatic life than herbicides and fungicides [9].

Fish and aquatic animals are exposed to pesticides in three primary ways (a) dermally, direct absorption through the skin by swimming in pesticide-contaminated waters, (b) breathing, by direct uptake of pesticides through the gills during respiration, and (c) orally, by drinking pesticide-contaminated water or feeding on pesticide-contaminated prey. Poisoning by consuming another animal that has been poisoned by a pesticide is termed "secondary poisoning." For example, fish feeding on dying insects poisoned by insecticides may themselves be killed if the insects they consume contain large quantities of pesticides or their toxic byproducts [10].

Organochlorine insecticidal pollution and Bacterial fish diseases are the major threats to the sustainable development of aquaculture causing loss of millions of dollars annually [11]. Lihocin 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 [12]. Lihocin entered in to the aquatic environment cause serious threatening to various aquatic organisms and also cause severe metabolic abnormalities in non target species like fish and freshwater mussels.

Energy, a vital force in a biological system, occupies a key position in the metabolic machinery. The free energy liberated by the breakdown of organic constituents, mainly carbohydrate, as a rule, transforms itself into the phosphate bond energy (ATP) before its utilization in any work [13]. The ATP synthesis in any biological system mainly depends on the phosphorylation of ADP associated with glycolysis and biological oxidation, involving the citric acid cycle and electron transport system [14]. Any change in these cycles of a cell leads to alterations in the energy budget of that cell in particular and that organ as a whole [15]. Pollutants affect not only the rate of carbon flow in a given metabolic pathway, but also the contribution of different metabolic pathway to the total metabolism of an animal.

In some of these studies exposure to pesticides caused either a significant increase or decrease or more effect in the enzyme activities. Lactate dehydrogenase (LDH or LD) is an enzyme found in nearly all living cells (animals, plants, and prokaryotes). LDH catalyzes the conversion of lactate to pyruvic acid and back, as it converts NAD^+ to NADH and back. A dehydrogenase is an enzyme that transfers a hydride from one molecule to another [16].

Succinate dehydrogenase or succinate - coenzyme Q reductase (SDH) or respiratory Complex II is an enzyme complex, found in many bacterial cells and in the inner mitochondrial membrane of mammalian mitochondria [17]. It is the only enzyme that participates in both the citric acid cycle and the electron transport chain [18].

Malate dehydrogenase (MDH) is an enzyme that reversibly catalyzes the oxidation of malate to oxaloacetate using the reduction of NAD^+ to NADH. This reaction is part of many metabolic pathways, including the citric acid cycle [19].

Glucose - 6 - phosphate dehydrogenase is the first enzyme in the pentose phosphate pathway. The main physiological function of G6PD is the production of NADPH and ribose - 5 - phosphate, which are essential for reductive biosynthesis and nucleic acid synthesis [20].

Disease has become a critical factor hampering the development of Indian Major Carp culture in India. Among the various pathogens, the Gram -negative bacteria represent the greatest potential threat to aquaculture. *A.veronii* is a rod shaped motile, gram negative, facultative anaerobe found in fresh water and in association with animals. It can be a pathogen of humans and a beneficial symbiont of leeches [21]. The *A. veronii* bacteria can be found in a number of habitats, including humans, mosquitoes and leeches. It is primarily found in the digestive tract of the leech where it maintains a symbiotic relationship with its host. *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 septicemia [22].

Therefore in the present study, an attempt has been made to explore the effects of Lihocin, *Aeromonas veronii* and AgNps on metabolic biomarkers of the freshwater fish *Catla catla* which is an edible fish of the local area and commercially important fish.

MATERIALS AND METHODS

Experimental Animal

Live specimens of *Catla catla* of (30.0 ± 1.6g) were collected from AP Govt. Fish Breeding and Hatchery Centre, Kalyani dam, near Tirupati, Chittoor district and immediately transferred to transparent polypropylene tank of 500L capacity filled with filtered, well aerated and dechlorinated bore well water. The fish were fed with a commercial pelletized formulated fish feed twice a day. The water quality is maintained constantly throughout the experimental period in control medium and also in pesticide treated aquatic medium.

Collection of tissues

After the experimental period the fish were killed by damaging the brain and denerving the spinal cord between the head and trunk region using a sharp needle and the tissues viz., gill, liver, kidney and muscle were removed from its body. They were washed in ice-cold 0.33M sucrose and blotted dry and the desired amounts of tissue were weighed and used. The tissues are homogenized in 6 volumes of homogenizing buffer (50mM Tris- HCl mixed with 1.15% KCl and pH adjusted to 7.4) using Teflon homogenizer. The resulting homogenate was centrifuged at 16,000 g for 15min in a centrifuge at -4 °C. The supernatant was decanted and stored at -20 °C in a deep freezer for until enzymatic analysis.

Experimental strain

Aeromonas veronii, strain, (Virulent Strain) was obtained from MTCC, Chandigarh, India. From this parent culture, sub cultures of *A.veronii* were prepared and doses were made under aseptic conditions. Culture and doses of *Aeromonas veronii* was done following the method of Pelczar (1993). The bacterial suspension was prepared to $1x10^9$ Colony Forg Units as determined using a Neubauer haemocytometer. The selection of bacterial dose was based on earlier report [23].

Preparation of plant extract

Fresh leaves of *Aloe vera* were collected from the garden of the Department of Botany, Sri Venkateswara University, Tirupati. The leaves were washed with distilled water, and after grinding, 10 g leaves was mixed with 100 ml distilled water and heated for 15 min. Then the extract was filtered through Whatman filter paper, collected and stored in refrigerator.

Preparation of Synthesized Silver nanoparticles

Initially 0.787 g silver nitrate was dissolved in 100 ml distilled water.10% of *Aloe vera* leaf extract was mixed with silver nitrate solution in 1:9 proportions and kept at room temperature for 72 hrs for the development of reddish brown colour was observed and the synthesised silver nanoparticles taken for the study.

Succinate dehydrogenase activity (SDH) in the organs was estimated using the colorimetric method of Nachlas *et al.,* (1960) [24]. Lactate dehydrogenase activity (LDH) in the organs was estimated using the method of Srikantan and Krishnamoorthi (1955) [25] as modified by Govindappa and Swami (1965) [26]. Malate dehydrogenase activity (MDH) in the organs was estimated by the method of Nachlas *et al.,* (1960) [24] with slight modification as suggested by Prameelamma and Swami (1975) [27]. G-6-PDH activity in the cytosolic fraction was assayed according to the method described by Bergmeyer and Bernt (1965) [28].

STATISTICAL ANALYSIS

The data obtained from this study were subjected to various statistical tools. The differences in the means (±SEM) between groups were assessed using students't'- test, Pearson's correlation and Levene's tests for equality of variance (SAS, 1988). A P-value of $P < 0.05$ was taken as significant.

RESULTS AND DISCUSSION

It is evident from the results presented in **Table - 1** it was observed that the LDH activity is high in control liver (1.94 ±0.06). The LDH activity is gradually increased in all exposure periods and in all tissues of fish with increasing the exposure period, related to the respective controls.

Table-1: Variations in Lactate dehydrogenase (LDH) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated with sub-lethal concentration of Lihocin, *A.veronii* and AgNps.

Tissue	C+Li						C+ Li + AV			C + Li + AV + AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill %change	1.67±0.16 --------	1.71±0.16 (2.39)	1.85±0.17 (10.77)	2.05±0.17 (22.75)	2.26±0.18 (35.32)	2.39±0.19 (43.11)	2.56±0.20 (53.29)	2.78±0.22 (66.46)	3.08±0.24 (84.43)	2.62±0.21 (56.88)	2.41±0.19 (44.31)	1.95±0.17 (16.96)
Liver %change	1.94±0.06 --------	1.98±0.08 (2.06)	2.15±0.09 (10.82)	2.45±0.11 (26.28)	2.63±0.13 (35.56)	2.89±0.15 (48. 96)	2.99±0.16 (54.12)	3.36±0.18 (73.19)	3.59±0.20 (85.05)	3.05±0.13 (57.21)	2.86±0.11 (47.42)	2.18±0.08 (12.37)
Kidney %change	1.46±0.12 --------	1.51±0.11 (3.42)	1.59±0.16 (8.90)	1.75±0.18 (19.86)	1.91±0.08 (30.82)	2.05±0.17 (40.41)	2.21±0.16 (51.36)	2.40±0.14 (64.38)	2.59±0.16 (77.39)	2.39±0.13 (63.69)	2.10±0.12 (43.83)	1.63±0.09 (11.64)
Muscle %change	1.59±0.08 --------	1.65±0.08 (3.77)	1.71±0.09 (7.54)	1.88±0.09 (18.23)	2.03±0.11 (27.67)	2.14±0.13 (34.59)	2.38±0.15 (49.68)	2.55±0.15 (60.37)	2.74±0.17 (72.32)	2.54±0.13 (59.74)	2.31±0.11 (45.28)	1.85±0.11 (16.35)

Values are mean± SD of 6 individual observations. All values are significant at P< 0.05 by ANOVA.

It is also obvious from the results that the LDH activity is gradually elevated in all exposure periods, of fish treated with *A.veronii* and the maximum increase was recorded on day 45 in all tissues; however the highest percentage elevation was obtained in liver (85.05%). However, the LDH activity is gradually decreased / recovered in directing that the animal tried to come to normal level **(Table - 1**) When the fish treated with silver nanoparticles.

The increase in the activity and the specific activity of LDH in the liver, gill, kidney and Muscle **(Table-1)** in response to an exposure to Lihocin and *A.veronii* indicates the decline in the catalytic efficiency of LDH in Lihocin treated fish. The reduction in the activity and the specific activity may be due to non-productive binding of Lihocin or its metabolites with the enzyme molecules, and / or by blocking of enzyme synthesis. The finding of increase in the activity of LDH in Lihocin treated tissues is agreement with the earlier reports of endosulfan induced reduction in the activities of ATPases, SDH and mixed.

SDH activity was gradually and significantly decreased with increase in the exposure periods. It is observed that the maximum decrease was recorded on day 45 in gill, liver, kidney and muscle tissues of fish *Catla catla* treated with sub lethal concentration of Lihocin, and *A.veronii* for 45days.

Table-2: Variations in Succinate dehydrogenase (SDH) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated with sub-lethal concentration of Lihocin, *A.veronii* and AgNps.

Tissue	C+Li						C+ Li + AV			C + Li + AV + AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill %change	1.98±0.08 --------	1.92±0.06 (-3.03)	1.84±0.03 (-7.07)	1.78±0.04 (-10.10)	1.71±0.06 (-13.63)	1.65±0.11 (-16.67)	1.61±0.014 (-18.68)	1.56±0.018 (-21.21)	1.52±0.015 (-23.23)	1.69±0.018 (-14.64)	1.75±0.016 (-11.61)	1.85±0.017 (-6.56)
Liver %change	2.72±0.07 --------	2.56±0.11 (-5.88)	2.47±0.12 (-9.19)	2.35±0.16 (-13.60)	2.28±0.14 (-16.17)	2.16±0.10 (-20.58)	2.13±0.014 (-21.69)	1.98±0.034 (-27.20)	1.94±0.028 (-28.67)	2.28±0.014 (-16.17)	2.22±0.16 (-18.38)	2.52±0.19 (-7.35)
Kidney %change	2.39±0.12 --------	2.26±0.14 (-5.43)	2.14±0.11 (-10.46)	2.08±0.09 (-12.97)	1.96±0.14 (-17.99)	1.91±0.10 (-20-08)	1.84±0.018 (-23.01)	1.80±0.017 (-24.68)	1.72±0.015 (-28.03)	1.82±0.016 (-23.84)	1.98±0.012 (-17.15)	2.24±0.016 (-6.27)
Muscle %change	1.56±0.15 --------	1.49±0.16 (-4.48)	1.43±0.12 (-8.33)	1.37±0.10 (-12.17)	1.32±0.11 (-15.38)	1.26±0.13 (-19-23)	1.24±0.12 (20.51)	1.18±0.14 (-24.35)	1.14±0.16 (-26.92)	1.22±0.14 (-21.79)	1.36±0.18 (-12.82)	1.48±0.16 (-5.12)

Values are mean± SD of 6 individual observations. All values are significant at P< 0.05 by ANOVA.

Among the control groups the maximum value was recorded in liver tissue of *Catla catla* fish **(Table- 2)**. It is also observed that the SDH activity was also more decreased in all tissues of fish treated with *A.veronii* and

the highest decrease was observed on day 45 in liver tissue (-28.67%) followed by kidney (-28.03%), muscle (-26.92%) and gill (-23.23%) on day 45 day **(Table- 2)**.

The SDH activity, whereas gradually increased / recovered with increasing the exposure periods up to day 45 and maximum increased value was recorded in liver tissue (-7.35%) of fish treated with AgNps **(Table-2)**.The decrease observed in SDH activity may be another evidence for overcoming the toxicity of the pesticide [33] have also observed similar significant decrease in the Succinate dehydrogenase activity of *Tilapia mossambica* exposed to sub lethal concentration of methyl parathion.

It is also evidenced from the results that the MDH activity is more decreased and maximum decrease was recorded on day 45 when treated with *A.veronii*. It is obvious that the highest decrease was recovered in liver (-15.03%) under the *A.veronii* treatment **(Table- 3)**. The MDH activity is gradually increase / recovered in all tissues on fish treated with AgNps **(Table-3)**.
Decrease in MDH values were observed in various tissues of *Catla catla* on exposure to Lihocin and treated with *Aeromonas veronii*. Similar reduction in MDH activity was also observed in *Matrinxabrycon cephalus* after exposure to Folidol 600 [34]. The decrement of MDH activity suggests the impairment of oxidative metabolism. The other reason for decrease of MDH activity might be due to disruption of mitochondrion integrity augmented by cellular damage under the impending toxicity of pesticides [35].

It is obvious from the results that G-6-PDH in gill, liver, kidney and Muscle tissues of *Catla catla* exposed to Lihocin was increased on all sampling days and compared with control **(Table -4)**. The increase was more pronounced was observed on day 45 of exposure periods the maximum elevation of G-6-PDH activity was observed in liver (80.59%) on 45 day of exposure periods **(Table - 4**).

It is also observed that the maximum elevation of G-6-PDH activity was (109.70%) in liver, (86.71%) in gill, (90.63%) in kidney and (83.68%) in Muscle tissue of fish treated with *Aeronmonas veronii* **(Table - 4)**. The G-6-PDH activity is gradually decreased removed in all tissues of fish treated with AgNps and animal tried to overcome stress.

Table- 3: Variations in Malate dehydrogenase (MDH) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated with sub-lethal concentration of Lihocin, *A.veronii* and AgNps.

Tissue	C+Li						C+ Li + AV			C + Li + AV + AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill % change	1.249±0.01 -------	1.243±0.01 (-0.48)	1.235±0.01 (-1.12)	1.218±0.01 (-2.48)	1.189±0.013 (-4.80)	1.172±0.01 (-6.16)	1.155±0.00 (-7.52)	1.138±0.00 (-8.88)	1.119±0.00 (-10.40)	1.146 ±0.00 (-8.24)	1.173 ±0.00 (-6.08)	1.199 ±0.01 (-4.03)
Liver % change	1.898±0.01 -------	1.885±0.01 (-0.68)	1.872±0.01 (-1.36)	1.849±0.01 (-2.58)	1.802±0.014 (-5.05)	1.758±0.01 (-7.37)	1.732±0.00 (-8.74)	1.712±0.00 (-9.79)	1.682±0.00 (-11.38)	1.715 ±0.00 (--9.64)	1.759 ±0.01 (-6.79)	1.859 ±0.01 (-2.05)
Kidney % change	1.056±0.02 -------	1.051±0.02 (-0.47)	1.043±0.02 (-1.23)	1.031±0.02 (-2.36)	1.017±0.023 (-3.96)	0.998±0.02 (-5.49)	0.973±0.01 (-7.85)	0.954±0.01 (-9.65)	0.937±0.01 (-11.26)	0.962 ±0.01 (-8.90)	0.989 ±0.02 (-6.34)	1.020 ±0.02 (-3.40)
Muscle % change	0.796±0.02 -------	0.794±0.01 (-0.25)	0.788±0.01 (-1.00)	0.783±0.01 (-1.63)	0.778±0.013 (-2.26)	0.759±0.01 (-4.64)	0.741±0.01 (-6.90)	0.729±0.00 (-8.41)	0.713±0.00 (-10.42)	0.699 ±0.01 (-13.44)	0.713 ±0.01 (-10.42)	0.745 ±0.01 (-6.63)

Values are mean± SD of 6 individual observations. All values are significant at P< 0.05 by ANOVA.

Table - 4: Variations in Glucose-6-phosphate dehydrogenase (G6PDH) activity in Gill, Liver, Kidney and Muscle tissues of fish, *Catla catla* treated sub-lethal concentration of Lihocin, *A.veronii* and AgNps.

Tissue	C+Li						C+ Li + AV			C + Li + AV + AgNp		
	Control	3d	7d	15d	30d	45d	15d	30d	45d	15d	30d	45d
Gill	1.28 ±0.23 -----	1.44±0.30 (12.5)	1.68±0.42 (31.25)	1.79±0.38 (39.84)	1.84±0.19 (43.75)	1.98±0.26 (54.68)	2.13±0.31 (66.40)	2.29±0.44 (78.90)	2.39±0.53 (86.71)	1.82±0.48 (42.18)	1.72±0.3 (34.37)	1.48±0.27 (15.62)
Liver	6.08 ±0.12 ---------	6.12±0.08 (0.65)	6.39±0.14 (5.09)	7.62±0.73 (25.32)	8.82±1.06 (45.06)	10.98±0.93 (80.59)	11.26±0.85 (85.19)	11.59±0.73 (90.06)	12.75±0.68 (109.70)	10.49±0.69 (72.53)	9.48±0.77 (55.92)	8.19±0.80 (34.70)
Kidney	4.59 ±0.21 ---------	5.16±0.18 (12.41)	5.38±0.26 (17.21)	6.19±0.29 (34.85)	6.74±0.62 (46.84)	7.86±1.14 (71.24)	8.24±1.27 (79.52)	8.49±1.38 (84.96)	8.75±0.45 (90.63)	9.35±0.41 (103.70)	8.87±0.30 (93.24)	7.49±0.22 (63.18)
Muscle	1.90 ±0.37 ---------	2.19±0.21 (15.26)	2.49±0.41 (31.05)	2.76±0.38 (45.26)	2.82±0.22 (48.42)	2.98±1.06 (56.84)	3.12±1.18 (64.21)	3.36±1.25 (76.84)	3.49±1.41 (83.68)	4.21±1.38 (121.57)	4.05±1.21 (113.15)	3.54±1.15 (86.31)

Values are mean± SD of 6 individual observations. All values are significant at P< 0.05 by ANOVA.

In the present study Lihocin significantly stimulated G-6-PDH activity in the fish indicating mobilization of glucose through pathways other than glycolysis - krebs cycle. G-6-PDH activity is indicative of increase in the HMP shunt pathway under stress conditions. Since G-6-PDH can generate NAD^+ rapidly, there by promoting tryose phosphate oxidation, is found increase during the stress. Similar results are also reported by surendranath *et al.* (1991) [36] and substantiate the present work. In fish exposed to silver nanoparticles showed the opposite trend of results obtained against to the fish treated with Lihocin and *A.veronii* and the animal tried to cope up stress and tried to reach normal level to sustain in the toxic environment.

CONCLUSION

In recent years applications of nanoparticles size compounds to treat bacterial infections attracted the attention of aquaculturists, all over the world. It is well recognized that silver nanoparticles possess potential antibacterial properties against a wide range of bacteria. Silver nanoparticles possess antimicrobial properties and also free radical scavenging properties to eradicate toxic substances from the animal to exist in the toxic environment.

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