Altered biochemical profile in *Oreochromis mossambicus* to phytosynthesised gold nanoparticles (AuNP)

M. Neelu[1] and G. Sharmila[2*]

https://doi.org/10.56343/STET.116.014.004.006
http://stetjournals.com

Article History
Received: 04-05-2021
Revised and Accepted : 05-06-2021
Published: 26-06-2021

Abstract

The impact of chronic toxicity of Gold Nanoparticles (AuNPs) on biochemical parameters in selected tissues of the common edible fish *Oreochromis mossambicus* fingerlings has been studied and the acute lethal and sub-lethal concentration for 120 hours were assessed. For chronic toxicity, two different concentrations 1.6 µL/L and 0.8 µL/L were used to which the fishes were exposed for 30 days. Present study was designed for 30 days of phyto-synthesized Gold Nanoparticles exposure period followed by 30 days of recovery period (no exposure to AuNPs) to understand the toxic effect of toxicant on biochemical parameters of fingerlings of *Oreochromis mossambicus*. During exposure period, the tissues of muscles, liver, gills and kidney showed a significant amount of increase in the biochemical constituents due to the metabolic stress induced by Gold Nanoparticles presumably to compensate the energy loss due to the toxicant. However during recovery period, there was a slight increase in all the parameters which may be due to the removal of AuNPs for regaining the stable state of animal. It is evident from the above results that there is a significant effect of Gold Nanoparticles on the vital organs of tissues in *Oreochromis mossambicus*. It can also be assumed from the above findings that if the recovery period was prolonged, there would be a chance for the exposed fishes to revert the effect caused by Gold Nanoparticles. Therefore, there should be detailed study on the effect of such Nanoparticles which enter into the ecosystem through human activities as it can have adverse effect on the biotic life.

Key words: chronic toxicity, Gold nanoparticles, metabolic stress, oreochromis, recovery period

M. Neelu

email: *viswanathansharmila@gmail.com*

[1]Research Scholar, PG and Research Department of Zoology and Wildlife Biology, A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai - 609 305, Tamil Nadu, India.

[2*]Assistant Professor, PG and Research Department of Zoology and Wildlife Biology, A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai - 609 305, Tamil Nadu, India.

INTRODUCTION

The investigation on risk effects of engineered nanoparticles over ecosystem has recently attracted much attention and it is found that nanoparticles can exert cytotoxicity to animals and plant systems. The small size of nanoparticles may facilitate their entry into living cells. This, together with their enhanced reactivity that allow them to interact more efficiently with biological systems, raises concern about their potential to induce harmful biological effects.

Human activity along coastal areas is increasing, and waste from industrial processes, activities, and natural or man-made hazardous substances ultimately reach the marine environment. The contamination of nanoparticles and metalloids in water leads to accumulation and biomagnification in the food chain. The investigation into the effects on the aquatic environment is of high interest, particularly since the aquatic systems act as a sink for many pollutants, receiving runoff and wastewater from domestic and industrial sources.

Among the various types of nanoparticles, AuNPs are used in numerous applications ranging from biosensors to catalyst, in electronics, new paints, cosmetics and cancer treatments etc., their unique properties, chemical stability, their capacity to exhibit a multiplicity of shapes, particle sizes and surface chemistry ensures that they are a key nanoscale component in many technologies. Gold nanoparticles (AuNPs), in particular, are among the most used. AuNPs has unique optical and electrical properties used in drug delivery, cellular imaging diagnostics and therapeutic agents (Chithrani *et al,*. 2006). The majority of studies propose AuNPs to be internalize within cells by receptor mediated endocytosis (RME),

P - ISSN 0973 - 9157
E - ISSN 2393 - 9249

which is very dependent on particle size (Chithrani and Chan, 2007). Although AuNPs are considered highly biocompatible compared to other nanoparticles (Bar-Ilan *et al,*. 2009), their toxic potential is still largely unknown (Alkilany and Murphy 2010).

Fishes are considered to be most sensitive creatures and they may accumulate these nanoparticles and pass on them to humans. Data from ecotoxicity tests in invertebrates, fish and algae have indicated low hazard potential of nanoparticles on aquatic species (Lovern *et al.,* 2007, Oberdörster *et al.,* 2006, Smith *et al.,* 2007). While toxicity mechanisms have not yet been completely elucidated, available ecotoxicology data on the sub-lethal impact of nanoparticles in aquatic invertebrates suggests that oxidative stress, genotoxicity, and effects on the immune system are key features of their toxicity. Most studies assessing the effects of nanoparticles in aquatic invertebrates have focused on freshwater species, in particular the crustacean *Daphnia magna* (*D. magna*) (Scown *et al.,* 2010). However, studies on the sub-lethal effects have revealed oxidative damage in brain of largemouth bass exposed to the carbon-based fullerene C60 (Oberdörster, 2004).

Fishes accumulate pollutants (AuNPs) in their fatty tissues like liver and they may enter in the body of fishes by the gills or through food and skin. The liver, gill, muscle and kidney were selected for analysis in this study due to their role in the bio accumulation and bio magnification (lethal effects). It has been previously reported that AuNPs can have a long blood circulation time and can accumulate in the mammalian liver.

The present study examines the effect of Gold Nano particles on *Oreochromis mossambicus* by estimating its effect on some biochemical parameters of selected organs. Common name of *Oreochromis mossambicus* is Mozambique tilapia. It is an exotic fish from South Africa and spread worldwide through introductions for aqua culture and also easy to keep and breed in captivity, but this species is on IUCN Red List. It has been directly introduced as a fishery resource by Governmental agencies.

The mouth breeding habit of this species allows it to nurture and carry its young ones to long distances to invade habitats far from the original site of introduction. These are easy to raise and harvest and they have a mild, white flesh that is appealing to consumers. Mozambique tilapias are resistant to wide varieties of water quality issues and pollution levels. Due to these abilities they have been used as bio assay organisms to generate metal toxicity data for risk assessments of local freshwater. With this background, the present study was carried out with an objective to examine the effect of Gold Nano particles on the biochemical parameters in selected tissues of *O.mossambicus* fingerlings on exposure to sub lethal concentrations for 30 days and also to investigate the ability of *O.mossambicus* to retrieve from the impacts caused by Gold Nanoparticles by assessing the biochemical parameters after a recovery period of 30 days.

MATERIALS AND METHODS

Collection and acclimatization of the specimen

Live and healthy *O.mossambicus* (n = 80) were collected from a commercial fish farm at Vadakkarai village of Nagapattinam taluk, Tamilnadu, India and brought to the lab in the plastic bags. Then the bags were cut open and fishes were allowed to swim the aquarium water after they were disinfected by treatment of 0.05 % potassium permanganate. They were stocked and maintained in aquarium under a normal temperature of 23±10^{o}C and a pH of 7.8 to acclimatize for a period of 10 days, before starting the experiment. The mean length of the fish used in the experimetns was 5 to 8 cms (range 5.0 to 8.0) and weight 6.75gm (range 3.5 to 7.3), the fishes were fed twice daily, with oilcakes. During the experimental period, the medium was changed every 48 hours and aeration was done continuously up to the completion of the experiment. The feeding was stopped prior to the experimentation to reduce additive and contamination effects of the animal excreta in the test medium, as suggested by Arora et al., (1984). The experiment was conducted in the wet laboratory of the PG & Research Department of Zoology & Wildlife Biology, Mannampandal, during August, 2018 to January, 2019.

Selection of toxicant

Gold Nanoparticles, commonly used in biomedical, pharmaceutical and cosmetic application has been taken for present study. It has been collected from Central Leather Research Institute CLRI at Chennai.

Bio-assay

Based on the preliminary tests, two sub-lethal concentrations 1/5th and 1/10th LC50 for 120 hrs were chosen to assess the chronic toxicity of AuNPs on the biochemical parameters of *Oreochromis mossambicus* exposed for 30 days. In order to perform the experiment, nine troughs (triplicates of control and experimental troughs; each trough having a capacity of 12 ltrs.) were taken and previously acclimatized 6 fishes were transferred to each trough. Of the nine troughs, three were kept as control without any toxicant, three were kept in a concentration and the remaining three were contaminated with another concentration of AuNPs.

At the end of 30 days of exposure period, three fishes from each trough were sacrificed for bio chemical assay while the remaining three fishes were transferred to water without the toxicant for a period of 30 days (recovery period). At the end of recovery period the same set of biochemical parameters were assessed.

Biochemical study

By following standard procedures, the total soluble carbohydrates, protein and lipid content of the muscle, kidney, gills and liver of the normal as well as the gold Nano particles treated fishes were estimated at the end of exposure and recovery period. The total soluble carbohydrate content was determined according to the method of Dubois *et al.,* (2002), protein content by Lowry *et al.,* (1951) and the lipid content according to the method of Osborne and Voogt (1978).

Statistical study

The results of static bioassay were analyzed using Probit analysis (Finney, 1971). Biochemical parameters were represented as mean ± standard error of mean and the differences in the biochemical parameters among the fish group exposed to sub lethal concentrations of gold Nano particles were subjected to one way ANOVA (analysis of variance). Post hoc test were carried out using Duncan multiple comparison test procedure. Significance was tested at $P < 0.05$.

RESULTS AND DISCUSSION

The acute toxicity of AuNPs to *O. mossambicus* increased with particle concentration, demonstrating a dose dependency. The observations were done manually and recorded. By using probit analysis, the 50% of the fish mortality were assessed.

The Table – 1 depicts the variations in carbohydrate content in the tissues – muscle, liver, kidney, gills of the fish treated with $1/5^{th}$ LC_{50} of 120 hours (T2; 1.6µL/L) and $1/10^{th}$ of the LC_{50} of 120 hours (T3; 0.8µL/L) . During exposure period, the total soluble carbohydrates in all the tissues of T2 (1.6 µL/L) and T3 (0.8 µL/L) increased when compared to the control, but more significant increase was observed in liver and gills. During the recovery period, it increased

Table 1. Variations in carbohydrate content in selected tissues of *O. mossambicus* during exposure and recovery periods (n=54)

TISSUE	T1 (Exposure Control)	T2 (1.6 µl/l)	T3 (0.8 µl/l)	T4 (Recovery Control)	T5 (Recovery 1.6 µl/l)	T6 (Recovery 0.8 µl/l)
MUSCLE	20.3 ± 1.07	22.1 ± 0.27*	23.7 ± 0.31*	22.4 ± 0.91	23.7 ± 1.01*	23.9 ± 1.09*
		-8.86%	-16.74%		-5.80%	-6.69%
LIVER	5.17 ± 1.04	6.02 ± 1.19*	6.87 ± 0.19*	7.01 ± 0.20	7.89 ± 0.29**	8.01 ± 0.30**
		-16.44%	-32.88%		-12.55%	-14.26%
KIDNEY	6.10 ± 1.23	6.52 ± 1.27**	7.01 ± 0.19*	3.12 ± 1.01	4.56 ± 0.19**	5.12 ± 0.20*
		-6.88%	-14.91%		-46.15%	-64.10%
GILLS	4.09 ± 1.02	5.08 ± 1.15*	5.20 ± 0.15**	5.12 ± 1.13	5.78 ± 0.21**	6.10 ± 0.31*
		-24.20%	-27.13%		-12.89%	-19.14%

Each value represents Mean ± S.E. * - indicates $P < 0.01$ ** - indicates $P < 0.05$. Values in Parentheses indicates percentage change over control

Table 2. Variations in protein content in selected tissues of *O. mossambicus* during exposure and recovery periods (n=54)

TISSUE	T1 (Exposure Control)	T2 (1.6 µl/l)	T3 (0.8 µl/l)	T4 (Recovery Control)	T5 (Recovery 1.6 µl/l)	T6 (Recovery 0.8 µl/l)
MUSCLE	21.9 ± 1.22	22.7 ± 1.36**	24.1 ± 1.43*	23.6 ± 1.1	24.9 ± 0.16**	25.9 ± 0.17**
		-3.65%	-10.04%		-5.50%	-9.74%
LIVER	6.25 ± 0.17	6.97 ± 0.21*	7.09 ± 0.29**	7.12 ± 0.30	8.09 ± 0.41**	9.13 ± 0.51**
		-11.52%	-13.44%		-13.62%	-28.23%
KIDNEY	7.14 ± 1.08	8.12 ± 0.18*	9.12 ± 0.21*	6.12 ± 0.20	7.14 ± 0.29*	7.99 ± 0.30*
		-13.72%	-27.73%		-16.67%	-30.56%
GILLS	11.4 ± 1.08	12.8 ± 0.20*	12.9 ± 0.20**	8.45 ± 0.38	9.01 ± 0.40*	11.1 ± 0.51**
		-12.28%	-13.15%		-6.62%	-31.36%

Each value represents Mean ± S.E. * - indicates $P < 0.01$ ** - indicates $P < 0.05$. Values in Parentheses indicates percentage change over control

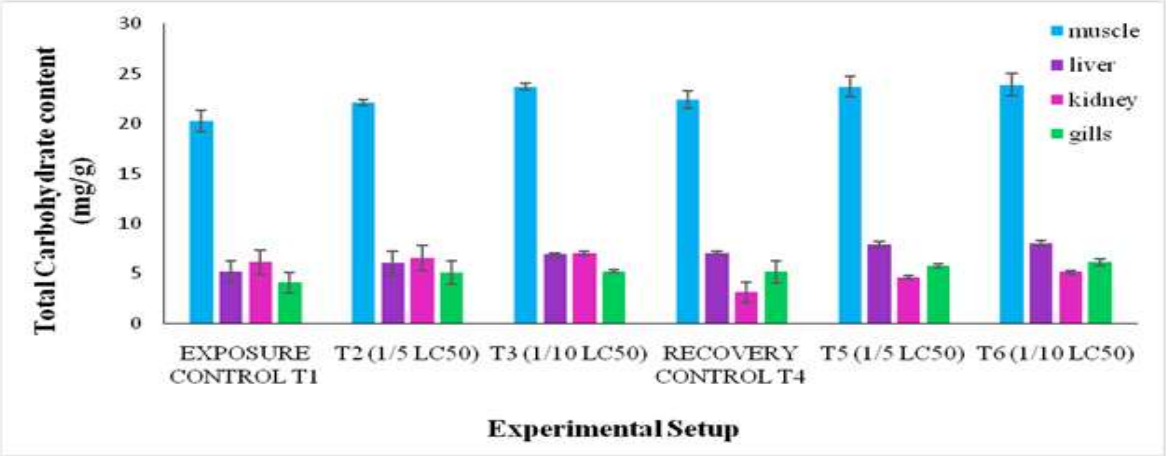

Fig. 1. Effect of AuNPs on the total carbohydrate content in selected tissues during exposure and recovery periods (n=54)

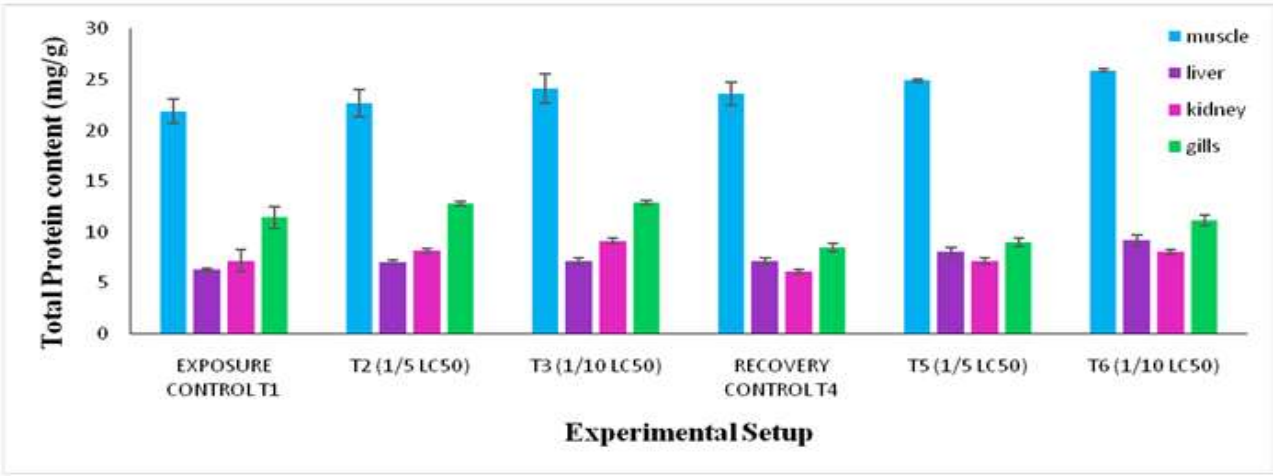

Fig. 2. Effect of AuNPs on the total protein content in selected tissues during exposure and recovery periods (n=54)

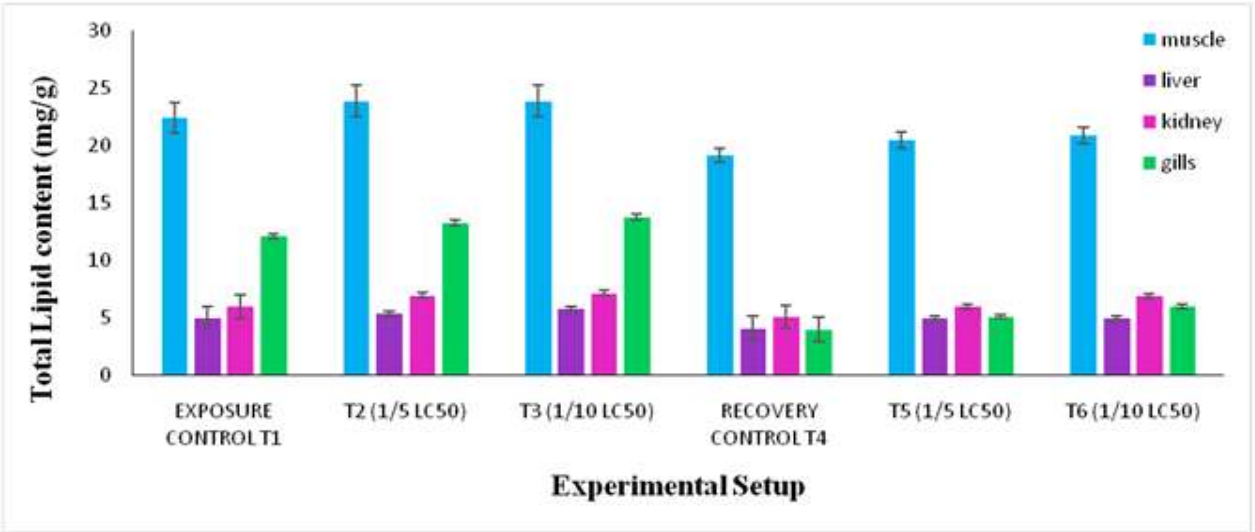

Fig. 3. Effect of AuNPs on the total content of Lipid in selected tissues during exposure and recovery periods (n=54)

Table 3. Variations in lipid content in selected tissues of *O. mossambicus* during exposure and recovery periods (n=54)

TISSUE	T1 (Exposure Control)	T2 (1.6 µl/l)	T3 (0.8 µl/l)	T4 (Recovery Control)	T5 (Recovery 1.6 µl/l)	T6 (Recovery 0.8 µl/l)
MUSCLE	22.4 ± 1.31	23.9 ± 1.38**	23.9 ±1.39**	19.2 ± 0.61	20.5 ± 0.69*	20.9 ± 0.70*
		-6.69%	-6.69%		-6.77%	-8.85%
LIVER	5.01 ± 1.01	5.38 ± 0.19*	5.79 ± 0.20*	4.10 ± 1.02	4.99 ± 0.18*	5.01 ± 1.20**
		-7.38%	-15.56%		-21.70%	-22.19%
KIDNEY	6.00 ± 1.00	6.98 ± 0.19*	7.18 ± 0.21*	5.12 ± 0.99	6.00 ± 0.19*	6.94 ± 0.21*
		-16.33%	-19.67%		-17.18%	-35.54%
GILLS	12.1 ± 0.20	13.3 ± 0.21*	13.8 ± 0.22*	4.01 ± 1.07	5.12 ± 0.19*	5.98 ± 0.20**
		-9.91%	-14.04%		-27.68%	-49.12%

Each value represents Mean ± S.E. * - indicates $P < 0.01$ ** - indicates $P < 0.05$. Values in Parentheses indicates percentage change over control

significantly in kidney of both T2 (1.6 µL/L) and T3 (0.8 µL/L) when compared to the recovery control (Fig. 1).

Similarly, the protein content in all the selected tissues viz. muscle, kidney, gills and liver increased slightly when compared to the control (Table - 2). During the exposure period, the protein content in kidney of T3 (0.8 µL/L) alone showed a significant increase when compared to T1 (Exposure control). However, during the recovery period, it is obvious that there is a significant increase in the protein content of T6 (Recovery 0.8 µL/L) over T4 (Recovery control) in the tissues of liver, kidney and gills (Fig. 2).

The total lipid content also showed an increasing trend in all the tissues (Table - 3). During the exposure period, only a slight increase in lipid content was observed over T1 (Exposure control), except in kidney and gills of both T2 (1.6 µL/L) and T3 (0.8 µL/L), which is insignificant (Fig. 3). But during the recovery period it showed a significant increase in liver, kidney and gills of both T5 (Recovery 1.6 µL/L) and T6 (Recovery 0.8 µL/L) over T4 (Recovery control).

Carbohydrates are the primary as well as an immediate energy source (Umminger, 1977). A decline in the carbohydrate levels of gill, muscle, and liver of *Oreochromis mossambicus* treated with zinc oxide nanoparticles was observed (Rajan *et al*., 2016).

Although the above findings suggest that the carbohydrate content has decreased on exposure to toxic substances, in our study, we have found a significant increase in carbohydrate content. It is observed that the glucose content of muscle, gill, liver, heart and kidney showed an increase but the increase was not uniform in all the tissues through their experimental results. An increased level of carbohydrate was estimated in muscle, gill and liver tissues of vitamin C doped MgS nanoparticle treated fishes *Oreochromis mossambicus*. (Karuppannan *et al.,* 2018). Furthermore these heavy metals cause elevation or decline in carbohydrate profile which serve as suitable biomarkers of fish health (Rajan *et al.,* 2016). Therefore from the findings of the above authors it is evident that during exposure period, the higher increase in carbohydrate content of liver and gills of treated fishes can be accounted to the compensation of energy loss during detoxification of blood (liver) and increased opercular movement (gills) due to the stress induced by Gold Nanoparticles and the overall increase may be because of the growth of the fingerlings of *Oreochromis mossambicus*.

During the recovery period, the carbohydrate level in almost all the tissues of Gold Nanoparticles pretreated fish returned to normal level except kidney. Surprisingly, we found tremendous increase in the carbohydrate content of kidney when compared to control. It may be due to the major role of kidney as detoxification organ. Since the kidney detoxifies the Gold Nanoparticles such an alteration in carbohydrate content may be found.

In the present study, protein content in different tissues of the exposed fish was observed to increase significantly over T1 (Exposure control) in kidney than that of other tissues. Biomolecules, particularly proteins, are likely to face oxidative damage because of transition metallic ions. Peroxidation or ROS generation may induce oxidative stress leading to variation in these biomolecules. Protein biomarkers are critical indicators of physiological disturbances (Zakia Kanwal *et al.,* 2019).

The increase in energy demand, as well as the altered enzyme activities, will result in the decrease of protein content. Even though many authors have found a decrease in protein content, there's still a possibility of increase which is supported by the following findings. An increased level of protein were estimated in muscle, gill and liver tissues of vitamin C doped MgS nanoparticle treated fishes *Oreochromis mossambicus* (Karuppannan *et al*., 2018). Elevation in total protein and globulin has previously been noted in *Channa punctatus* living in Ni-, Co-, and Cr-polluted water (Zakia Kanwal *et al*., 2019). Dobsikova *et al*., (2006) noticed an elevation in total protein content in common carp with 7 h transportation stress. An increase in these proteins in NP-treated groups can be assigned to the increased protein synthesis to fulfill the requirements of high energy due to NPs mediated strain and to meet the immunotoxic challenge (Zakia Kanwal *et al*., 2019). Present results during the recovery period reveal that the protein level was gradually regained in T5 (Recovery 1.6 µL/L). But the protein content of T6 (Recovery 0.8 µL/L) in liver, kidney and gills have increased significantly. This observation suggests the treated fishes exposed to higher concentration of Gold Nanoparticle must have undergone high damage and hence to repair, there is an increase in the protein content.

It was reported in the current study that during Gold Nanoparticle exposure period, the tissues of exposed fishes showed a slight increase in the lipid content over T1 (Exposure control). A significant increase can be observed in kidney and gills of T2 (1.6 µL/L) and in liver, kidney and gills of T3 (0.8 µL/L).

Improper utilization of lipids by the target tissues such as liver may be the reason for the increase. It is suggested that the increase in the total lipid content of liver and gills proves that the administration of nanoparticles induced damages in the structural organization of these tissue Furthermore these heavy metals cause elevation or decline in lipid profile, which serve as suitable biomarkers of fish health (Rajan *et al*., 2016). Other workers also recorded significant elevations in these parameters. Elevation or depletion in lipid profile is either due to disturbance in the metabolism of lipids or may be due to impaired clearance from plasma which favour liver dysfunction (Rajan, *et al*., 2016). Very limited work had been carried out both in field and laboratory based studies in fish pertaining to the effect of heavy metals on lipid profile (Rajan *et al*., 2016).

The observation of increased lipid profile in this present study is in agreement with the above findings of the authors.

During the recovery period, more prominent increase in the lipid content over T4 (Recovery control) was observed in gills, kidney and liver of the previously exposed fishes. Due to the toxic impact of Gold Nanoparticles, an increase in lipid content might have been found.

REFERENCES

Alkilany, A. M. and C. J. Murphy. 2010. Toxicity and cellular uptake of gold nanoparticles: what we have learned so far?, *J. Nanopart. Res.* 12(7): 2313-2333. https://doi.org/10.1007/s11051-010-9911-8

Bar-Ilan, O., R. M. Albrecht, V. E. Fako and D. Y. Furgeson 2009. Toxicity Assessments of Multisized Gold and Silver Nanoparticles in Zebrafish Embryos. *Small* 5(16): 1897-1910. https://doi.org/10.1002/smll.200801716

Chithrani, B. D. and W. C. Chan 2007. Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Lett.* 7(6): 1542-1550. https://doi.org/10.1021/nl070363y

Karuppannan, P., Saravanan, K and M.P. Santhi, 2018. Synthesis, characterization of Vitamin C Doped MgS Nanoparticles and Toxicity Assessment against Freshwater Fish *O. mossambicus*. *Int. J. Pharm. Sci. Rev. Res*., 22: 154-159.

Lovern S.B., Strickler J.R . and R. Klaper. 2007. Behavioural and physiological changes in Daphnia magna when exposed to nanoparticle suspensions (titanium dioxide, nano – C- 60) and C(60). *Pharmacol. Rev*., 53(2): 283-318. https://doi.org/10.1021/es062146p

Oberdörster, G. E. 2004. Manufactured nanomaterials (fullerenes, C60) induce oxidative stress in the brain of Largemouth bass, *Environ. Health Persp*. 12: 1058 – 1062. https://doi.org/10.1289/ehp.7021

Rajan, M.R., Archana, J., Ramesh, R and V. Keerthana. 2016. Toxicity of Zinc Oxide Nanoparticles in Tilapia *Oreochromis Mossambicus*. *PIJR*. 5(10):220-224

Scown, T.M., Van Aerle and C.R. Tyler. 2010. Review: Do engineered nanoparticles pose a significant threat to the aquatic environment. *Crit. Rev. Toxicol*., 40(7): 653 – 670. https://doi.org/10.3109/10408444.2010.494174

Smith, C.J., Shaw B.J., and Handy R.D. 2007. Toxicity of single walled carbon nanotubes to rainbow trout, respiratory, toxicity, organ pathologies and other physiological effects. *Aqua. Toxicol*. 82: 94 – 109. https://doi.org/10.1016/j.aquatox.2007.02.003

Zakia Kanwal, Muhammad Akram Raza, Farkhanda Manzoor, Saira Riaz, Ghazala Jabeen, Shafiq Fatima and Shahzad Naseem. 2019. A comparative assessment of nanotoxicity induced by metal (silver, nickel) and metal oxide (cobalt, chromium) nanoparticles in Labeo rohita. MDPI. *Nanomater.* 9(2):309:1-20. https://doi.org/10.3390/nano9020309