

Effect of Inorganic Pollutant (Nitrate) On Biochemical Parameters of the Fish, *Aspidoparia Morar*

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Abstract: An attempt has been made to evaluate the effect of inorganic pollutant (nitrate) on biochemical parameters of the fish *A. morar*. The studied parameters were Glucose, cholesterol, protein, triglycerides, phosphatases and transaminases. LC₅₀ value of fish, *A. morar* for nitrate was found to be 2mg/l. Three sublethal concentrations viz., 25% (0.5mg/l), 50% (1mg/l) and 75% (1.5mg/l) of LC₅₀ value of nitrate were employed for the experiment. The experiment was set up for 9 weeks and effect was observed at weekly intervals. The studies revealed when fish exposed to sublethal concentrations at weekly intervals, exhibited hyperglycemia, hypercholesterolemia, hyperproteinaemia with significant increase in concentrations of triglycerides and phosphatases. The observed alterations were ultimately become the causative for hampering the entire metabolic machinery. The anomalies observed in the above mentioned parameters were found to exhibit more effect as the concentration as well as chronicity of the experiment progresses.

Key Words: Nitrate, Biochemical Parameters, Blood Serum, *Aspidoparia morar*

I. INTRODUCTION

The presence of sublethal concentrations of noxious chemicals in freshwater environments can promote the emergence and development of infectious diseases in fish [1, 2]. It has been suggested that only chronic stress is responsible for impairment of various physiological system of fishes viz., blood, reproduction, osmoregulation etc. besides biochemical profile of the fishes. So in lieu of above fact, investigations were conducted presently to assess the effect of inorganic pollutant, nitrate, on the biochemical parameters of the fish *A. morar*. Various parameters studied include glucose, cholesterol, protein, triglycerides, phosphatases (acid and alkaline phosphatase) and transaminases (glutamate oxalate transaminase and glutamate pyruvate transaminase).

The paper is organized as follows. Section II describes the methodology adopted for the research work. Section III represents the results and discussion of the effect of nitrate on various biochemical parameters of fish in. Section IV reflects conclusion part of the research work.

II. MATERIALS AND METHODS

Specimens of *Aspidoparia morar* for the present studies were procured from River Tawi (J&K, India) of Nikowal region, R.S. Pura and after acclimatization for 15 days, LC₅₀ value of nitrate was determined to be 2mg/l. The experiments were set up employing 25% (0.5mg/l), 50% (1mg/l) and 75% (1.5mg/l) of LC₅₀ value of nitrate. Blood sample was collected by making an incision through the heart directly into diluting pipettes at regular weekly interval. After centrifugation at 3000rpm for 15 minutes, serum was separated by a fine bulb pipette for subsequent use for the estimation of biochemical parameters with the help of automatic biochemistry analyzer (Accurex-AT-112, Accurex-AT-200D). Glucose was determined by Correl and Langley [3], acid and alkaline phosphatase by Hillmann [4], GOT and GPT by Bergmeyer *et al* [5], cholesterol by Stadman [6], triglycerides by Schettler and Nussel [7] and total protein by Lowry *et al* [8].

Statistical analysis

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The data obtained was analyzed statistically by one way analysis of variance (ANOVA) for determining the significance of changes from control.

III. RESULTS AND DISCUSSION

a) Glucose: Glucose is an important diagnostic tool of carbohydrate related disorder as it has proved to be reliable endocrine and physiological indicator of relative severity of stresses in fishes [9]. Effect of nitrate on glucose contents of the fish, *A. morar* are depicted in the Table 1. It is evidently clear from table that there is significant increase throughout the experimental period of 9 weeks. Wedemeyer and Mcleay [10] reported that high levels of blood glucose are caused by disorders in carbohydrate metabolism under physical or chemical stresses. Presently too hyperglycemic response may then also reflect an indication of disrupted carbohydrate metabolism possibly due to enhanced breakdown of liver glycogen. The significant elevation ($p < 0.01$) of blood glucose level in the fish *A. morar* can also be ascribed to the excessive mobilization of glycogen from muscle and hepatic tissue simply to produce more energy to combat stress of nitrate toxicity. It is on record [11] that stressful stimuli elicit rapid secretion of glucocorticoids and catecholamines from adrenal tissue. Both of these hormones are known to produce hyperglycemic response by increased conversion of liver glycogen into glucose to supply the greater energy demand by stress induced increased metabolism [12]. Though these hormones have not been measured presently but the observed hyperglycemic condition may plausibly be ascribed to the nitrate induced hypersecretion of these hormones which cause glycolysis in the liver and muscle in nitrate exposed fishes. Contrary to present discussion Omoregie *et al.* [13] suggested that the progressive accumulation of plasma glucose depicts stress induced hyperglycemia resulting from the incomplete metabolism of blood sugar due to impaired osmoregulation.

b) Cholesterol: Changes in cholesterol content of the presently studied fish, *A. morar* exposed to different sublethal concentrations of nitrate are depicted in Table 1. The treated fishes observed an increase in the cholesterol content throughout the experimental exposure from 2nd day-9th week. Hypercholesterolemia observed presently may seemingly be due to impairment of liver and inhibition of enzymes which convert cholesterol into bile acids. The fact that impairment in the liver tissue is responsible for the increment in the cholesterol content of the treated fishes is authenticated from the histological preparations of the liver which upon treatment with nitrate have been observed to witness necrosis and degenerative changes (Figs.2-3) compared to control fish (Fig. 1). Similar viewpoint that impairment of liver can lead to the release of cholesterol into the blood and hence hypercholesterolemia under the influence of different pollutants has earlier also been put forth by Muazzez *et al.* [14] and Parvathi *et al.* [15]. Gradual increment in the cholesterol content of the presently studied fish in response to the stress created by nitrate toxicity is also indicative of the fact that excessive energy reserves are being compensated by increased cholesterol content which as stated by Lee *et al.* [16] are required by the organism to cope up the effect of the stress caused by various xenobiotics. Cholesterol contents in blood invariably is linked to lipid metabolism. Rise in cholesterol level is an indication and probably suggests that a general increase in lipid mobilization must have taken place simply to fulfil the increasing demand for energy to cope up the stress of nitrate toxicity. Due to higher rate of mobilization of lipids it appears that lipoprotein lipase activity gets disrupted which then might have lead to increased concentration of the cholesterol content in the blood serum of the fish when exposed to pollutional stress [17]. Observed hypercholesterolemia in the fish *A. morar* under the stress of nitrate toxicity, according to present author hence appear to be the combined effect of i) damage and dysfunctioning of the liver tissue and b) Increased rate of lipid mobilization under stress of nitrate toxicity.

c) Protein: Protein is an important constituent of all the cells and tissues as it play vital role in the physiology of living organisms. Since fishes have very little carbohydrates, proteins invariably are used in fish to meet energy demands also [18]. Effect of nitrate on the protein content of the fish, *A. morar* as depicted in Table 1 clearly reveals its significant increase ($p < 0.01$) in all the sets of the experiment from 2nd day (as no change could be observed on day one) upto 9th week compared to that of control fishes (Table 1). Present results are in conformity to the results Mc Donald *et al.* [19], Labelo *et al.* [20] and Kori-Siakpere *et al.* [17] who too observed hyperproteinemia in fishes exposed to different stressors. Blood serum proteins which constitute a fairly reliable biochemical index also reflects the condition of the organism and the

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changes happening to it under the influence of external and internal factors [21]. It is also a known fact that blood is the main transport vehicle for carrying proteins to different organs for its

further processing. Among different tissues where actually catabolism of proteins takes place in liver. Since under the stress of nitrate toxicity, liver tissue has been observed to undergo various degenerative changes (Fig. 3), it seems that under such conditions the whole mechanism for the transport of proteins from blood to liver itself gets disrupted. Due to impairment in the liver tissue, proteins which actually had to reach liver, however, remain in the blood only. It hence appears to be the probable reason of hyperproteinemia observed in *A. morar* when subjected to nitrate toxicity. Hyperproteinemia observed presently, as stated by Mc Donald *et. Al* [19] may also lead to increased osmotic pressure and osmolality of the blood serum due to entry of proteins from the damaged hepatic tissue into the general circulation. Additionally impairment in liver tissue itself can also lead to leaching of proteins from the damaged cellular architecture into the blood circulation thereby causing increased concentration of proteins in the blood serum of the fish.

d) Triglycerides: Triglycerides are the important diagnostic for treatment of disease involving lipid metabolism [18]. Presently, gradual increase in triglycerides contents of the fishes were observed at regular intervals in all of the three sublethal concentrations of nitrate (Table 1) employed presently. The increase has been found to be statistically significant ($p < 0.01$) which can be explained on the pretext that under the stressful condition fishes may mobilize more and more triglycerides from liver tissue to meet increased demand for the energy. This appears to be one of the strategies these fishes employ to cope up the detrimental conditions imposed by the stress of nitrate toxicity and to sustain toxicity related increased physical activity, biotransformation and excretion of xenobiotics. Since triglycerides serve as an important source of energy, by its increase, fish under stress of any pollutant can to some extent they combat its severity. As, homeostasis of lipids is one of the principal function of liver tissue [22], gradual increase in triglycerides content of presently studied fishes may possibly reflect liver dysfunction due to nitrate toxicity. Impairment of liver tissue (authenticated by its histological preparations, Figs. 2-3) under the stressful conditions may then cause an imbalance between rate of synthesis of triglycerides and their utilization. Liver, being the primary site for the catabolism of triglycerides, its impairment may inhibit its breakdown into simpler forms, due to obstruction in the pathway of catabolism of triglycerides and hence the resultant increase in its serum level.

To sum up, it can therefore be inferred that besides increase in cholesterol and proteins, triglycerides content of the fishes simply indicate that fish needs excessive amount of energy to cope up the stress of nitrate toxicity.

e) Phosphatases and Transaminases: Data on effect of nitrate on enzymatic activities of *A. morar* is depicted in Table 1. Data clearly reveal that exposure of nitrate caused significant elevations ($p < 0.01$) in the activities of serum phosphatases and transaminases during the experimental exposure of 9 weeks in all of the treated sets. Like other biochemical indices, increase in enzymatic activities (phosphatases and transaminases) appears to be gradual at weekly intervals during entire experimental period. Several reports [23, 24] have earlier also revealed that these blood enzymes increase in their concentrations when treated with various environmental pollutants. Presently observed increase in enzymatic activities according to the present author may be due to a) leakage of these enzymes from hepatic cells into blood and b) their increased synthesis by liver under stress of nitrate toxicity. Since liver is the primary organ for detoxification as well as major site of toxicants reaction [25], significant increase in both of presently studied enzymes simply suggests that nitrate might have affect the liver cells and so does the enzymes elaborated by it. Both phosphatases and transaminases are considered to be important in assessing the biochemical status of liver [26]. Their presence in blood serum is indicative that liver tissue has either get injured and or has undergone dysfunction. Monitoring of liver enzymes and their leakage into the blood has hence proved to be a very useful tool in toxicity related effects on liver [27]. These enzymes usually percolated into the blood serum (phosphatases and transaminases) only when hepatic cells are damaged [28]. In light of above discussion presently observed increase in enzymatic activities of *A. morar* under the stress of nitrate toxicity appears to be indicative of the fact that impairment in liver tissue due to nitrate treatment must have resulted in the release of enzymes into the general circulation and hence an increase its concentration.

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IV. CONCLUSION

Present studies thus clearly evinces that nitrate toxicity definitely alter the entire biochemical profile in *A. morar* by changing the levels of glucose, cholesterol, protein, triglycerides besides enzymatic activities in the blood serum. Such changes at biochemical levels might result in impairment of energy requiring vital processes and hence can deteriorate the health status of the fish population.

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REFERENCES

- [1] S. Raina, "Studies on effect of different environmental stressors on blood and immune organs of *Labeo* species. Ph.D. Thesis Department of Zoology, University of Jammu, Jammu", 2012.
- [2] A. Sachar, and S. Raina, "Haematological alterations induced by lindane in a fish, *Aspidoparia morar*", Global Journal of Biology, Agriculture and Health Sciences, Volume 3, Number 1, pp. 38-42, 2014
- [3] N.V. Correl, and R.W. Langley, "Glycogen determination in liver and muscle by use of anthrone reagent", J. Biol. Chem., Volume 26, pp. 583-593, 1956.
- [4] G.Z. Hillmann, "Continuous photometric measurement of prostrate acid phosphatase and alkaline phosphatase activity", Z. Klin. Chem. Klin. Biochem., Volume 9, pp. 273, 1971.
- [5] H.V. Bergmeyer, M. Horder, and R. Rej, "Approved recommendation on IFCC method for glutamate oxaloacetate transaminase and glutamate pyruvate transaminase", J. Clin. Chem. Clin. Biochem., Volume 24, pp. 481-489, 1986.
- [6] T.C. Stadman, "Preparation and assay of cholesterol and ergosterol. In: Colonick, S.P. and Kaplan, N.O. (Eds.) Method Enzymology", Volume 3p, Number 5, pp. 39, Academic Press, New York, 1957
- [7] G. Schettler, E. Nussel, "Method for triglycerides", Aeb. Med. Soz. Med. Prav. Med., Volume 10, Number 25, 1975.
- [8] O.H. Lowry, N. Rosenbrough, A.L. Farr, and R.J. Randall, "Protein measurement with the folen- phenol reagent", J. Biol. Chem., Volume 193, pp. 265-275, 1951.
- [9] J.L. Soenges, P. Rey, G. Rozas, M.D. Andres, and M. Aldegunde, "Effects of cortisol and thyroid hormone treatment on the glycogen metabolism of selected tissues of domesticated trout *Oncorhynchus mykiss*", Aquac., Volume 101, pp. 317-328, 1992.
- [10] G.A. Wedemeyer, and D.J. Mc leay, "Methods for the tolerance of fishes to environmental stressors, In Stress: stress and Fish [A.D. Pickering (ed.)]", Academic Press, London, New York, pp. 247-275, 1981.
- [11] T. Nakano, and N. Tomlinson, "Catecholamine and carbohydrate concentrations in rainbow trout (*Salmo gairdneri*) in relation to physical disturbance", J. Fish Res. Bd. Can., Volume 24, pp. 1701-1715, 1967.
- [12] D.K. Sriwastava, and V.M.S. Srivastava, "Erythrocytic abnormalities under urea stress in *Cirrhinus mrigala* (Hamilton) fingerlings", Curr. Sci., Volume 54, Number 20, pp.799, 1985.
- [13] E. Omoregie, E.B.C. Ufodike, and R.I. Keke, "Tissue chemistry of *O. niloticus* exposed to sublethal concentrations of gammalin-20 and acetellic 25EC", J. Aquat. Sci., Volume 5, pp. 33-36, 1990.
- [14] O. Muazzez, G. Atli, and M. Canli, "Effects of metal (Ag, Ad, Cr, Cu, Zn) exposures on some enzymatic and non-enzymatic indicators in the liver of *Oreochromis niloticus*", Bulletin of Environmental Contamination, Volume 82, Number 3, pp. 317-321, 2009.
- [15] K. Parvathi, P. Sivakumar, M. Ramesh, and Sarasu, "Sublethal effects of chromium on some biochemical profiles of the fresh water teleost, *Cyprinus carpio*", International Journal of Applied Biology and Pharmaceutical Technology, Volume 2, Number 1, pp. 295-300, 2011.
- [16] R.N. Lee, S.D. Gerking, and B. Jezierska, "Electrolyte balance and energy mobilization in acid stressed rainbow trout *Salmo gairdneri* and their relation to reproductive stress", Environ. Biol. Fish., Volume 8, pp. 115-123, 1983.
- [17] O. Kori-Siakpere, B.R. Ikomi, and M.G. Ogbé, "Biochemical response of the African catfish: *Clarias gariepinus* (Burchell, 1822) to sublethal concentrations of potassium permanganate", Annals of Biological Research, Volume 2, Number 2, pp.1-10, 2011.
- [18] K.M. Adamu, and O. Kori-Siakpere, "Effects of sublethal concentrations of tobacco (*Nicotiana tobaccum*) leafdust on some biochemical parameters of hybrid catfish (*Clarias gariepinus* and *Heterobranchus bidorsalis*)", Brazilian Archives of Biology and Technology, Volume 54, Number 1, pp. 183-196, 2011.
- [19] D.G. Mc Donald, H. Hope, and C.M. Wood, Journal of Experimental Biology, Volume 88, pp. 109-131, 1980.
- [20] S.L. Labelo, D.K. Saunders, and T.I.G. Crawford, "Transsections of the Kansas Academy of Science", Volume 104, pp. 183-194, 2001.
- [21] A.M. Shalaby, Y.A. Khattab, and A.M. Abdel-Rahman, "Effects of garlic *Allium sativum* and chloramphenicol on growth performance, physiological parameters and survival of Nile Tilapia, *Oreochromis niloticus*", J. Venom. Anim. Toxins Incl. Trop. Dis., Volume 12, Number 2, pp.172-201, 2006.
- [22] A.A. Hadi, A.E. Shokr, and S.F. Alwan, "Effect of aluminium on the biochemical parameters of freshwater fish, *Tilapia zilli*. Journal of Science and its Applications", Volume 3, Number 1, pp. 33-41, 2009.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

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[23] V. Karan, S. Vitorovic, *et al.*, "Functional enzyme activity and gill histology of carp after copper sulfate exposure and recovery", *Ecotoxicol. Environ. Saf.*, Volume 40, pp. 49-55, 1998.

[24] S. Agrahari, K.C. Pandey, and K. Gopal, "Biochemical alteration induced by monocrotophos in the blood of fish, *Channa punctatus* (Bloch)", *Pesticide Biochemistry and Physiology*, Volume 88, Number, pp. 268-272, 2007.

[25] M.S. Zaki, S. Moustafa, O.M. Fawzi, H.E.C. Bellbasi, S.M. Syame, and I.M. Awad, "Assessment of the hazardous effect of lead pollution on *Tilapia zilli*, including haematological, biochemical and immunological parameters", *Reports and Opinion*, Volume 2, Number 1, pp. 82-87, 2010.

[26] S.R. Verma, S.Rani, and R.C. Dalela, "Isolated and combined effects of pesticides on serum transaminases in *Mystus vittatus* (African catfish)", *Toxicol. Lett.*, Volume 8, pp.67-71, 1981.

[27] M. Salah El Deen, and W.A. Rogeps, "Changes in total protein and transaminase activities of grass carp exposed to diquat", *J. Aquatic Animal Health.*, Volume 5, pp. 280-286, 1993.

[28] E.J. Campbell, C.J. Dickinson, *et al.*, "Clinical physiology", Bulter and Tanner Ltd. London, 1984.

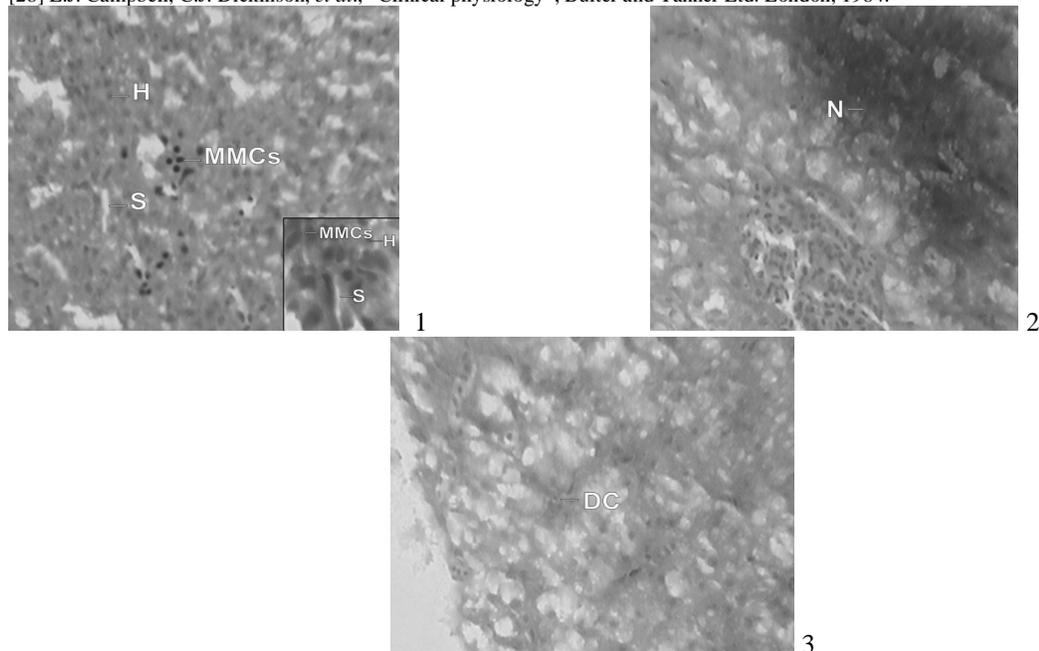


Fig. 1 Microphotograph of Liver tissue from control fish showing Hepatocytes (H), Sinusoids (S) and Melanomacrophage centres (MMCs) (H&E×1000)
Fig. 2-3 Microphotograph of Liver tissue from nitrate treated fish showing Necrosis (N) and Degenerative changes (DC) (H&E×1000) respectively.

Table 1: Showing alterations in Biochemical Parameters of the fish *A. morar* exposed to different sublethal concentrations of nitrate (Mean ± S.E.).

Parameters	Control	25% (0.5mg/l)	50% (1mg/l)	75% (1.5mg/l)
Glucose (mg/dl)	30.0±0.64	54.53±1.88	67.08±0.87	83.69±0.99
Acid phosphatase (KAU)	2.50±0.18	3.99±0.56	5.83±0.66	7.69±0.87

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Alkaline phosphatase (KAU)	2.40±0.33	4.88±0.13	7.14±0.37	11.47±0.99
GOT (U/L)	220.0±1.88	250.23±3.78	279.93±3.11	336.23±1.55
GPT (U/L)	102.0±0.99	105.6±2.33	164.94±1.26	211.02±2.55
Cholesterol (mg/dl)	80.3±0.4	106.6±1.88	132.52±1.08	175.44±1.97
Triglycerides (mg/dl)	22.5±0.16	42.74±0.29	68.55±0.77	101.03±0.56
Total protein (gm/dl)	9.5±0.9	10.82±0.77	12.44±0.45	13.71±0.22