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# Effect of Ammonia Toxicity on the biochemical and enzymatic activity of Indian major carp *Labeo rohita* Ham, 1822

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**Abstract**
Fingerlings of *Labeo rohita* were exposed to acute toxic dose (12.5 ppm) of unionized ammonia for analyzing changes in biochemical parameters viz. total serum proteins, glucose, blood urea content and also to evaluate response of various enzymes like ALP, LDH, AChE, AST and ALT during exposure to 96 hr. acute toxicity assay. The serum protein content exhibited a decreasing trend in exposed fishes in contrary to glucose and urea levels which showed gradual increase in exposed fishes when compared to control fish. A similar trend was also noticed in serum enzyme responses of exposed groups, when compared to control fish. Enzymes like AST, ALT, LDH and AChE exhibited enhanced response in exposed group of *L. rohita* fingerlings at all intervals over control fish, except ALP which showed a decreasing trend.

**Keywords:** *labeo rohita*, ammonia, alkaline phosphatase, lactic acid dehydrogenase, acetyle cholenesterase, asparate amino transferase, alanine aminotransferase

## 1. Introduction

Ammonia is found to be a major toxicant to fishes and other aquatic life, as it enters natural water system from several sources including industrial waste, sewage effluents, coal gasification, liquefaction conversion process plants and agricultural discharges including feed lot run off. It is also a metabolic waste product of fishes. Ammonia toxicity in vertebrates causes convulsions, coma and death, possibly because of the fact that elevated $NH4^+$ displaces K and depolarizes neurons, causing activation of NMDA type glutamate receptor, which leads to an influx of excessive $Ca^{2+}$ and subsequent cell death in the central nervous system [1]. Ammonia is toxic to a variety of aquatic organism including fish [2]. Un-ionized form of ammonia is the most toxic form to aquatic organisms as it can readily diffuse through cell membranes and is a highly soluble liquid. It can cause impairment to cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish. Ammonia is also the principal nitrogenous waste and involves the measurement of contaminant levels to characterize the hazards imposed on the aquatic environment. Ammonia when present in higher concentrations is toxic to living animals and produces several biochemical and physiological changes at cellular level. It has been observed that toxicological studies of acute exposure, changes in concentration and enzymatic activities often directly reflect the degree of cell damage in specific organs [3, 25]. Therefore, monitoring of enzymatic activities in tissues and blood serum stress-related homeostatic adjustments can be used for early warning signals of stress in fish. Although it is difficult to assess the specific exposure, the overall health of an organism can be assessed for timely correction measures for the monitoring the health of fish [4]. Measurement of acetyl cholinesterase (AChE) activity in fish brain is a specific tool to identify pesticide stress [5]. Increase in transaminase activity leading to elevation of alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities occurs due to liver damage [6], which is also related to kidney and gill damage [7]. Lactate dehydrogenase (LDH) has been used for demonstrating tissue damage in fish [8]. Das *et al*. reported increased LDH activity in carps exposed to ammonia and attributed this to the prevalence of hypoxic conditions in the organism. Nitrite exposure causes gill damage [9] and impairment of respiratory system, and thus may lead to hypoxia and an increase in LDH activity in tissues. Stress-induced alteration in alkaline phosphatase (ALP) and acid phosphatase (ACP) activities in tissues and serum have been reported in fish [10]. Such changes have been explained by pathological processes such as liver impairment, kidney dysfunctions and bone disease [11].

## 2. Materials and methods

Fish were collected from various culture ponds located in and around Visakhapatnam and Vizianagaram districts to conduct the present experimental study involving toxicity studies of unionized ammonia on *L. rohita* fingerlings. Fifty fish measuring 25-30 grams in weight and 10-13 cm in total length were maintained in a large tank and got acclimatized to laboratory conditions for one week before commencement of the experiment. Stock solution of ammonia at a concentration of 1mg/ml was prepared and stored in a dark and cool place. Later subsequent concentrations whenever required for the experimental study were prepared freshly from the stock solution. Based on the value of range finding test, the dose for $LC_{50}$ experimental toxicity trial was determined. The data on percent fish mortality obtained during the 96 hour lethal concentration trials were analyzed by employing Probit analysis [12]. A separate batch of 50 fish were maintained without adding un-ionized ammonia as controls. Total protein,

blood glucose and blood urea content were measured spectrophotometric ally as per the diagnostic protocol given in the diagnostic kits (BMK Laboratories, Thane, India). Similarly enzyme activities viz. alkaline phosphatase (ALP), lactate dehydrogenase (LDH), acetyl cholinesterase (AChE), aspartate amino transferase (AST), and alanine aminotransferase (ALT) in serum were estimated using diagnostic kits from span diagnostics. Blood samples were collected at regular intervals after 12hrs, 24hrs, 48hrs, 72hrs and 96 hrs., post exposure period for analysis. Blood was drawn from the fish by cardiac puncture with 1ml disposable syringe rinsed with anticoagulant (EDTA) and was stored in refrigerator till further use. For serum proteins, blood glucose, blood urea and enzymatic estimation, blood was transferred to micro-centrifuge tubes immediately and allowed to clot at room temperature for 30-40min. After clotting, serum was transferred to micro-centrifuge tubes with a micro pipette and centrifuged at 3000 rpm for 10 min. The clear serum was then transferred to a fresh 2-ml micro centrifuge tube and frozen in sealed condition at -4$^{o}$ c until further analysis.

Statistical analysis was performed using one-way Analysis of variance with the “General Linear Model” procedure. Duncan’s multiple-range test for variables was used to check the data for significant difference.

## 3. Results

The following changes were observed in biochemical and enzymatic activities of *L. rohita* during exposure to 96 hr. acute dose of 12.5 ppm. Serum protein content exhibited a decreasing trend in exposed groups. The total protein content in the control fish was found to be 16.17 mg/dl (10.13-18.61) where as at post exposure intervals, it was found to be 16.05 mg/dl (12.6-20.3) at 12hrs, 14.21 mg/dl (10.4-18.1) at 24hrs, 9.99 mg/dl (4.5-12.3) at 48 hrs, 8.6 mg/dl (4.1-9.3) at 72hrs, 7.19 mg/dl (6.3-9.8) at 96hrs, but a sudden decreasing was noted at 48hrs record in 9.99 mg/dl (4.5-12.3) (Fig.1). Glucose levels in control fish were found to be 13.11 mg/dl (7.3-14.8), but in exposed fish a gradual increase was noted at 12hrs to 96hours of post exposure, recording 15.532 mg/dl (14.24-18.63) at 12 hrs, 23.609 mg/dl (21.18-28.8) at 24hrs, 24.73 mg/dl (22.5-29.1) at 48hrs; 30.20 mg/dl (28.7-34.2) at 72hrs and 32.07 mg/dl (31.2-34.1) at 96hrs (Fig.1). The urea in blood exhibited an increasing trend in all exposed groups. Control fish showed urea levels at 14.494 mg/dl (11.4-15.0). On the other hand at different post exposure intervals the values were found to be15.54 mg/dl (12.6-18.4) at 12hrs, 15.83 mg/dl (10.7-17.1) at 24hrs, 15.91 mg/dl (10.62-20.62) at 48hrs, 18.53 mg/dl (12.7-20.2) at 72 hrs and 20.048 mg/dl (16.5-22.3) at 96 hours respectively (Fig.1).

The data on the activity of enzymes viz Alkaline phosphatase (ALP), Lacticacid dehydrogenase (LDH), Acetyle Cholenesterase (AChE), Asparate aminotransferase (AST) and Alanine aminotransferase (ALT) is presented in the table and figs. 2 &3. Significant changes were observed in serum enzyme levels in all exposed groups, when compared to control fish. The Alkaline phosphatase (ALP) content in control group was found to be 27.17 KA units (25.1-31.0), followed by a reduction in their activity in all exposed groups, which recorded 25.67 KA units (23.4-31.2) at 12hrs, 23.12KA units (21.6-26.1) at 24hrs, 19.44 KA units (10.6-18.1) at 48hrs, 17.9 KA units (12.0-18.4) at 72hrs and 16.35KA units(14.5-18.4) at 96 hrs. (fig.2). Asparate aminotransferase (AST) in the exposed group of *L. rohita* fingerlings showed an increasing trend at all intervals over control, except at 12hrs. The values recorded in control fish were found to be 2.174 IU/L (2.1-2.6), whereas at other post exposure intervals they were found to be 2.04 IU/L (1.6-3.01) at 12hrs, 2.3 IU/L (1.4.5-3.3) at 24hrs,2.57IU/L (1.6-3.8) at 48hrs,2.83IU /L (1.8-4.2) at 72hrs and 3.26IU /L (1.1-4.6) at 96hrs. (Fig. 2). Alanine amino transferase (ALT) exhibited gradual increase in all exposure groups, except 12hrs. The control value for ALT was found to be 2.072 IU/L (0.38-3.2) whereas the values at different post exposure intervals were found to be 2.0 IU/L (0.58-3.2) at 12hrs, 2.4IU/L (0.8-3.6) at 24hrs, 2.9 IU/L (0.38-4.3) at 48hrs, 3.27 IU/L (2.4-4.9) at 72hrs and 3.6 IU/L (0.7-4.9) at 96 hrs (fig. 2). The Lactic Acid dehydrogenase (LDH) activity exhibited an increasing trend at all exposed intervals. In the control group LDH activity was 415.8 $ODmin^{-1}g^{-1}$ (408.2-421.2), whereas in exposed groups the values were found to be 422.8 $ODmin^{-1}g^{-1}$ (416.6-431.4) at 12hrs, 463.4 $ODmin^{-1}g^{-1}$ (450.1-481.6) at 24hrs, 517.4 $ODmin^{-1}g^{-1}$ (503.0-523.0) at 48hrs, 638.0 $ODmin^{-1}g^{-1}$ (592-625) at 72 hrs and 639.2 $ODmin^{-1}g^{-1}$ (589-648) 96 hrs. (Fig.3). The Acetyle Cholinesterase (AChE) activity showed an increasing trend at different post intervals, when compared to control. The control value was found to be 278.32 $ug^{-1}$ (258.2-327.2), whereas at different post exposure intervals the values were found to be 281.6$ug^{-1}$ (187.2-298.1) at 12hrs, 304.7$ug^{-1}$(291.8-331.8) at 24hrs, 354.3$ug^{-1}$ (330-364) at 48hrs, 410.03$ug^{-1}$ (394-439) at 72hrs and 508.1$ug^{-1}$ (490-531) at 96hrs (Fig.3).

## 4. Discussion

Acute toxic effect of un-ionized ammonia on fingerlings *L. rohita* showed significant changes in various biochemical and enzymatic activities. The decrease recorded in total protein values from 0 to24 h may be due to the severity of the stress, which causes osmotic imbalance. It has been reported that the damage caused by toxicant to the kidney must have resulted in significant loss of blood protein through renal excretion [13], further augmenting its depletion in the blood [14]. Other reports have also shown a similar effect in the depletion of the plasma protein in fish exposed to various toxicants [15, 16]. These works have suggested that the decreased levels of protein noticed may be a stress-induced deleterious effect on protein synthesis causing its depletion in the serum as happened during the present study. Increase in blood glucose levels has been reported in fish exposed to various toxicants, especially during initial exposure periods. The increase that was recorded in plasma glucose value in the first six hours may be due to the fact that the fish mobilize energy from all available resources to combat the stress [17]. It was attributed that the exposure stress on fish may cause the release of adrenaline and nor-adrenaline to activate the secretion of catecholamine, which increases the conversion of liver glycogen to blood glucose to supply the greater energy demand [18]. Such a similar phenomenon might have been responsible for the increase in the blood glucose levels in the fingerlings of *L. rohita* exposed to acute toxic doses of unionized ammonia in the present case. Fluctuations were observed in the activity of various enzymes tested during the present investigation. The alkaline

phosphatase activity exhibited a decreasing trend in exposed groups over control fish. Inhibition ALP activity in different tissues [13, 19] and in the serum [20] was reported earlier in fish exposed to different toxicants [21]. Lactic acid dehydrogenase (LDH) activity exhibited gradual increase at all exposed groups when compared to control fish. Elevation of LDH level is regarded as an indicator of anaerobic metabolism in pesticide treated fish where aerobic oxidation through the Krebs cycle was adversely affected [22]. Thus, alteration in the LDH activity in liver, kidney and gill can be used as a biomarker indicating stress due to toxicants like ammonia in the aquatic environment. Acetyl Cholinesterase (AChE) activity was found to be high in exposed fish over controls. The initial hyperactivity of fishes exposed to lethal doses may be due to the inhibition of AChE and subsequent accumulation of acetylcholine in the neuro-muscular junction. The fingerlings showed sluggish movement and loss of balance towards the end of 96hrs. Such behavior may be the result of maximum inhibition of AChE in the cerebellum, since the cerebellum control the muscular coordination [23].
Similarly, the observed increase in the level of ALT and AST activity in exposed fingerlings may be due to accumulation of ammonia concentration in the body [24]. Increase in the level of ALT and AST activity during the present study is in agreement with the works carried out by others and may be attributable to the process of either deamination or transamination due to the excess nitrogen in the organism[6]. This elevation may also be attributed to the increase in transaminase activity promoting *in vivo* protein synthesis as suggested by Rouillet, (1964) and may indicate tissue damage as suggested by Rajyasree and Neeraja [6], Jeney [26] *et al* and Oluah [7] (1999).

## 5. Conclusion

Ammonia in aquatic medium is one of the major factors responsible for significant changes in the fish physiology. The present study provides the first available data on *L. rohita*, stressing the need for basic information required for the establishment of water quality standards for better management of fish cultural ponds. The concentration of un-ionized ammonia in aquaculture ponds should be properly monitored as there are many out sources for un-ionized ammonia to enter culture ponds.

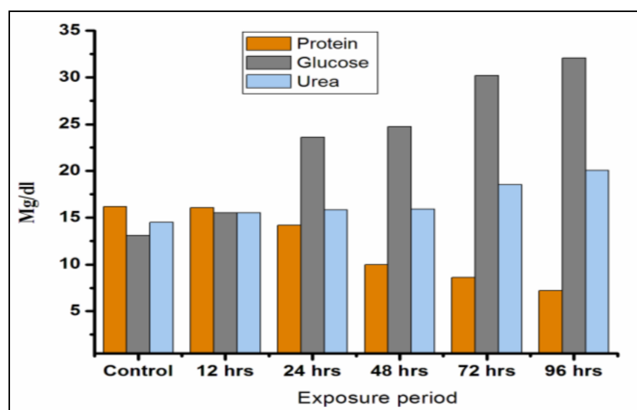


**Fig 1:** Serum protein, glucose and urea levels of *L. rohita* exposed to acute toxic dose of 12.5 ppm un-ionized ammonia, during different post exposure intervals

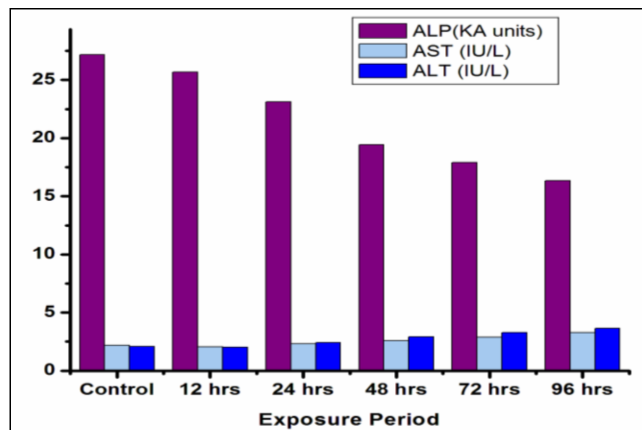


**Fig 2:** ALP, AST and ALT Enzymatic responses of *L. rohita* exposed to acute toxic dose of 12.5 ppm un-ionized ammonia, during different post exposure intervals

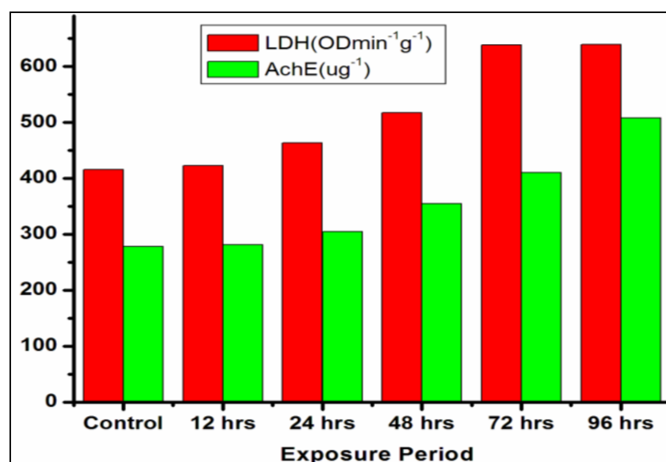


**Fig 3:** LDH and AchE Enzymatic responses of *L. rohita* exposed to acute toxic dose of 12.5 ppm un-ionized ammonia, during different post exposure intervals

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