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Research Article **Biochemistry**

IMPACT OF FERTILIZER AMMONIUM SULPHATE ON LIVER MARKERS IN *Catla catla*

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ABSTRACT

The biochemical results in *Catla catla* treated with ammonium sulphates showed significant alterations in the content of Total protein, Albumin, Globulin and enzyme activities Aspartate transaminase (AST), Alanine transaminase (ALT), Acid phosphates (ACP) and Alkaline phosphates (ALP). The liver markers as Total protein, Albumin and Globulin were significantly decreased and AST, ALT, ACP and ALP activities of fishes were significantly ($p< 0.05$) increased depending upon the duration of ammonium sulphate exposure as compared with control fish. The maximum liver markers changes were observed in 30 days exposure followed by 20 and 10 days ammonium sulphate exposure. The outcome of the present investigation showed that the fertilizer ammonium sulphate has been proved to be harmful to fishes. Therefore, the information obtained may be useful for management and monitoring of fertilizer contamination in the aquatic environment.

Keywords: *Catla catla*, Liver markers, Ammonium sulphate, Fertilizer

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INTRODUCTION

In most ecosystems animals and plants are exposed to many pollutants. Interaction of pollutants can enhance or decrease the toxicity of the mixture. The mode of action of pollutants can be due either to the chemical structure of the molecules or to alterations in the physiological processes with the organism. The body's response to foreign chemical whether inhaled, absorbed, ingested or injected depends on a host of factors. They include the frequency and duration of exposure, the dose and the age and sex and general health of an exposed individual. The earliest changes in an organism following exposure to a pollutant occur at the cellular level. Some of the important effects are alteration in the structure components of the cell membrane. Inhibition of certain enzyme such as microsomal enzymes, interference in protein, lipid, carbohydrate biosynthesis or

metabolism and alteration in DNA fidelity resulting in mutations and interference with regulation of cell required for cellular processes including catabolism, anabolism and reproduction (Satake *et al*., 1997).

Though the green revolution had pushed up agricultural productivity and made our nation self-reliant, the indiscriminate use of synthetic fertilizers, pesticides etc., have inflicted tremendous damage to the ecosystem (Venkatararaman, 1996). Agricultural fertilizers are widely used in aquaculture to enhance the natural productivity of a pond by stimulating the production of phytoplankton which serve as feed for fishes (Jhingran, 1983). The fertilizers used for the augmentation of productivity of ponds in aquaculture belong to two categories – inorganic and organic. Organic fertilizers include manures of liquid origin, guana, offal, farmyard manure, sewage, plant material, etc. (Jhingran, 1983). Inorganic fertilizers include limestone, urea, ammonium sulphate and phosphate.

Most animals and plants will not survive in water with a pH value below 5 (acid), or above 9 (alkaline). Changes in the pH can also affect the action of other toxins. A number of water pollution toxins such as chromates an chromic acid, beryllium, selenium, cadmium, chlorinated hydracarbons**, ammonium sulphate**, some organophosphorus pesticides, and polyvinyl chloride, have been designated by the WHO. as potential carcinogens, capable of causing cancer in the long term to fish. Inorganic fertilizers are used in association with organic manures to increase the natural productivity of ponds. However, indiscriminate use of inorganic fertilizers including ammonium sulphate, which releases ammonia (both NH_3 and NH_4^+), has created numerous environmental hazards for fish populations (Wicks and Randall, 2002; Erdogan *et al*., 2005; Yadav *et al*., 2007).

The focus of stress research in fish has recently grown to encompass the cellular stress response in addition to that of the whole animal, broadening to understanding of the mechanisms allowing an animal to tolerate stress (Feder and Hofmann, 1999). Oxidative stress, i.e. pathological processes related to overproduction of reactive oxygen species (ROS) in tissues is one of important general toxicity mechanisms of many xenobiotics. Oxidative stress is considered a fundamental factor of ageing processes, leads to DNA damage and is causative agent of many diseases including cancer (Toyokuni *et al*. 1995). The exposure and effects of chemicals in living organisms can be studied by biomarkers, i.e. specific changes (responses) in biological parameters reflecting specific toxicity mechanism such as overproduction of ROS and oxidative stress (Boelsterli 2003). *Catla catla* is one of the most important cultivable fresh water species of tropical countries, the effects of ammonium sulphate in this species is still to be explored. The present study has been undertakes to provide information on the oxidative stress in *Catla catla* exposed to ammonium sulfate.

MATERIALS AND METHODS

Collections and Acclimatization of Experimental Animal

Ninety juveniles of the fresh water fish *Catla catla* (Catla) were collected from the local fish pond at Thittai, Thanjavur district, Tamil Nadu. They were approximately weighed 4.27 ± 0.03 gram. These fishes were brought to the laboratory and acclimatized for 15 days glass aquaria containing aged tap water. Aged tap water (water stored for 24 hours) was used throughout the study to minimize mortality of the fishes during acclimatization; the aquarium water was maintained under standard conditions (Oxygen level of 6.00 – 6.50mg/L, pH 7.2-7.2 and temperature 27 –29 º C).

Experimental setup

The experiments were carried out with the help of small square type glass troughts of 10-liter capacity, which were covered with in iron wire gauge to avoid the jumping of the fish from the trough. To provide proper supply of oxygen an aerator was used. The test media was changed daily with fresh addition of the toxicant and sporolac.

Experimental Design

For sublethal toxicity tests 80 fishes were selected and divided into four groups (one control and three experimental) with 20 fish in each aquarium filled with water. The desired concentration (1/10 of 96h LC50 – 148 mg/l) of the toxicant was added directly in order to maintain constant concentration of the toxicant (Sheik Mohamed Salahudeen *et al*., 2014). The experiment was conducted for 30 days and sampled at 10 days interval and no mortality was observed during the above treatment period. After 10, 20 and 30 days, blood was collected and the fish were sacrificed. The tissues were removed and washed with saline and blotted. The tissues were homogenized using a glass homogenizer with chilled phosphate buffer (pH 7.4). Group I: Normal Control; Group II:Ammonium sulphate (148 mg/l) exposed fish (10 days); Group III :Ammonium sulphate (148 mg/l) exposed fish (20 days); Group IV: Ammonium sulphate (148 mg/l) exposed fish (30 days).

Serological analysis

End of the experimental periods, both experimental and control fish were anesthetized with 10 ppm Benzocain for 3 min. Following anesthesia, blood samples obtained from the caudal circulation with and without heparinised 2 ml disposable syringe fitted with a 21 gauge hypodermic needle. The blood was allowed to clot for 1 h at room temperature and overnight at 4^{o}C. Serum was isolated by centrifugation at 6000 rpm for 10 min and stored in aliquots at -20^{o}C until use..

Biochemical estimation

The serum GOT was estimated by the method of Reitman and Frankel, (1957). The serum GPT was estimated by the method of Reitman and Frankel, *(*1957). The serum alkaline phosphatase activity was estimated by the method of King and King's (1954).

RESULTS AND DISCUSSION

The biochemical results in *catla catla* treated with ammonium sulphates showed significant alterations in the content of Total protein, Albumin, Globulin and enzyme activities Aspartate transaminase (AST), Alanine transaminase (ALT), Acid phosphates (ACP) and Alkaline phosphates (ALP).

Table 1 represents the liver markers (Total protein, Albumin, Globulin and enzyme activities AST, ALT, ACP and ALP) changes in *Catla catla* of Control and experimental fish. It is evident that the total protein, albumin, globulin and AST, ALT, ACP and ALP activities of Control was 3.74± 0.18, 1.25±0.06, 2.49±0.12, 20.11±1.00, 18.94±0.94, 3.62±0.18 and 12.12±0.60 respectively. The total protein content of fish was decreased to 3.53±0.17 for 10 days exposure, 3.26± 0.16 for 20 days exposure and 2.86±0.14 for 30 days of exposure of ammonium sulphate when compared the control fish. The albumin content of fish was decreased to 1.04± 0.05 for 10 days exposure, 0.95± 0.04 for 20 days exposure and 0.76± 0.03 for 30 days of exposure of ammonium sulphate when compared the control fish. The globulin content of fish was decreased to 2.49± 0.11 for 10 days exposure, 2.31± 0.11 for 20 days exposure and 2.10± 0.10 for 30 days of exposure of ammonium sulphate when compared the control fish.

The AST activity of fish was significantly increased to 32.14±1.60 for 10 days exposure, 39.13± 1.95 for 20 days exposure and 46.12± 2.15 for 30 days of exposure of ammonium sulphate when compared the control fish. The ALT activity of fish was significantly increased to 29.47± 1.47for 10 days exposure, 39.22±1.96 for 20 days exposure, 48.65±2.43 for 30 days of exposure of ammonium sulphate when compared the control fish. The ACP activity of fish was significantly increased to 4.53±0.22 for 10 days exposure, 5.89±0.29 for 20 days exposure, 6.63± 0.31for 30 days of exposure of ammonium sulphate when compared the control fish. The ALP activity of fish was significantly increased to 13.27±0.66 for 10 days exposure, 14.73± 0.73 for 20 days exposure, 16.28± 0.76 for 30 days of exposure of ammonium sulphate when compared the control fish (Figure 1 and 2).

The biochemical results in freshwater carps *Catla catla* treated with ammonium sulphate showed significant alterations in the content of total protein, albumin, globulin and enzyme activities AST, ALT, ACP and ALP. Statistical analysis showed the significant variation in the treatments for the *Catla catla* during the experimental period. However the noticeable alterations in protein, albumin and globulin were observed in ammonium sulphates treated group when compared to control.

Assessment of protein content and enzymes activities can be considered as a diagnostic tool to determine the physiological status of cells or tissues (Manoj, 1999). The effects of toxicants on decreased content of protein in fish have been observed by a number of investigators; Jung *et al*. (2003) in *Paralichthys olivaceus* after exposed to formalin; Jee *et al*. (2005) in *Sebastes schlegeli* after cypermethrin exposure; Datta *et al*. (2007) in *Clarias batrach*us after exposure to arsenic; El-Sayed *et al*. (2007) in *Oreochromis niloticu* safter deltamethrin exposure; Min and Kang (2008) in *Oreochromis niloticus* after benomyl exposure and Kopp *et al*. (2011) in *Cyprinus carpio* exposed to microcystins.

Enzyme activities are considered as sensitive biochemical indicators before hazardous effects occur in fish and are important parameters for testing water and the presence of toxicants (El-Demerdash and Elagamy, 1999). Such a biochemical approach has been advocated to provide an early warning of potentially damaging changes in stressed fish (Casillas *et al*., 1983). Enzymatic activities also provide quick screening methods for assessing the health of fish and can be used to determine the incipient lethal concentration of a toxicant. Some of the enzymes are perceived good bioindicators for animals chronically exposed to contaminants such as metals and other xenobiotics (Mazorra *et al*., 2002). Phosphatases are important and critical enzymes in biological processes; they are responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions (Yousef *et al*., 2007). Any interference in these

enzymes leads to biochemical impairment and lesions of the tissue and cellular function (Enan *et al*., 1982; Khan *et al*., 2001). Therefore, by estimating enzyme activities in an organism, we can easily identify a disturbance in its metabolism. Many chemicals at relatively low doses affect the metabolism of biota by altering healthy enzyme activity (Hochster *et al*., 1972). The responses of various xenobiotic metabolizing enzymes in the fish model are rapidly evolving as important biomarkers for monitoring unacceptable levels of environmental contaminants such as GST, acetyl choline esterase and acid and alkaline phosphatase (Labrot *et al*., 1996).

Acid and alkaline phosphatase (ACP and ALP respectively) catalyse the hydrolysis of various phosphate-containing compounds and act as transphosphorylases at acid and alkaline pHs, respectively. Acid and alkaline phosphatases are known to be involved in a number of cellular functions such as synthesis, transport, and metabolic regulation such as molecule permeability, detoxification, carbohydrate metabolism, protein synthesis, growth and cell differentiation synthesis of certain enzymes, secretory activity, and transport to phosphorylated intermediates across the cell membranes, steroidogenesis and the biosynthesis of energetic macromolecules for various essential functions.

Acid phosphatases act as marker enzymes for the detection of lysosomes in cell fractions and can be altered by the presence of xenobiotics (Cajaraville *et al*., 2000), whilst alkaline phosphatases are intrinsic plasma membrane enzymes found on the membranes of almost all animal cells. Alkaline and acid phosphatase (ALP, ACP) are considered useful biomarkers to determine the pollution level (Basaglia, 2000) and used as a biomarker for a number of diseases (Samman *et al*., 1996). Induction of these biomarkers is a good approach to measure potential impacts of pollutants on environmental organisms (El-Shehawi *et al*., 2007). ACP is hydrolytic lysosomal enzymes released by the lysosomes for the hydrolysis of foreign material; hence it has a role in certain detoxification functions. As the classic macrophage lysosomal marker ACP plays an important part in cellular metabolism, catalyzing hydrolyses of phosphoproteins and the transfer of phosphate

ACP and ALP enzymatic activities have been studied in several organisms and the influence of heavy metals has been reported. Many authors observed a series of phosphatases (ACP and ALP) enzyme responses in different piscine systems when exposed to pollutants. Jiraungkoorskul *et al*. (2003) in nile *tilapia*, *Oreochromis niloticus* after glyphosate herbicide exposure; Gill *et al*. (1990) in *Rosy barb*, *Puntius conchonius* were exposed to mercuric chloride; Molina *et al*. (2005) and Atencio *et al*. (2008) in *tilapia* fish, *Oreochromis* sp. exposed to microcystins. In fish, Cd can exert a wide range of changes in some plasma stress parameters (i.e. cortisol and glucose) (Fu *et al*., 1990; Chowdhury *et al*., 2004) and alterations in the activity of many important enzymes including acid and alkaline phosphatase (Vaglio and Landriscina, 1999; Lionetto *et al*., 2000).

Current accepted opinion of cadmium action as well as other metals is related mainly to their influence on protein molecules, particularly enzymes. They have a strong affinity to bond with the amino acid moieties of proteins and may cause changes in enzyme structures. The most obvious consequences of these changes are the inhibition of enzymes (Drastichova *et al*., 2004).

The liver markers as Total protein, Albumin and Globulin were significantly decreased and AST, ALT, ACP and ALP activities of fishes were significantly ($p< 0.05$) increased depending upon the duration of ammonium sulphate exposure as compared with control fish. The maximum liver markers changes were observed in 30 days exposure followed by 20 and 10 days ammonium sulphate exposure. The outcome of the present investigation showed that the fertilizer ammonium sulphate has been proved to be harmful to fishes. Therefore, the information obtained may be useful for management and monitoring of fertilizer contamination in the aquatic environment. The fish exposed to these fertilizers recover quickly when they were moved to unpolluted freshwater. It is inferred that application of fertilizers to a larger extent may have toxic potential to the adjacent shallow water bodies, posing a threat to their inhabiting freshwater fish fauna.

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Table 1 represents the liver markers (Total protein, Albumin, Globulin and enzyme activities AST, ALT, ACP and ALP) changes in *Catla catla* of Control and experimental fish.

Groups	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)	SGOT (IU/l)	SGPT (IU/l)	ACP (IU/l)	ALP (IU/l)
Control	3.74± 0.18 [a]	1.25±0.06 [a]	2.49±0.12 [a]	20.11±1.00 [a]	18.94±0.94 [a]	3.62±0.18 [a]	12.12±0.60 [a]
Ammonium sulphate exposure (10 days)	3.53±0.17 [b]	1.04± 0.05 [b]	2.49± 0.11 [b]	32.14±1.60 [b]	29.47± 1.47 [b]	4.53±0.22 [b]	13.27±0.66 [b]
Ammonium sulphate exposure (20 days)	3.26± 0.16 [b]	0.95± 0.04 [b]	2.31± 0.11 [b]	39.13± 1.95 [b]	39.22±1.96 [b]	5.89±0.29 [b]	14.73± 0.73 [b]
Ammonium sulphate exposure (30 days)	2.86±0.14 [c]	0.76± 0.03 [c]	2.10± 0.10 [c]	46.12± 2.15 [c]	48.65±2.43 [c]	6.63± 0.31 [c]	16.28± 0.76 [c]

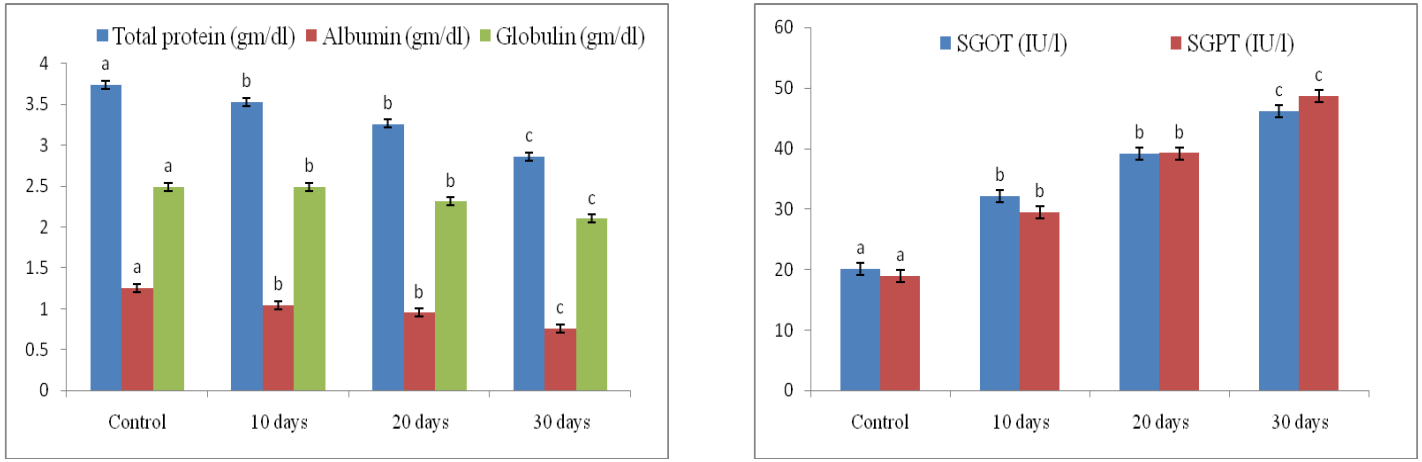
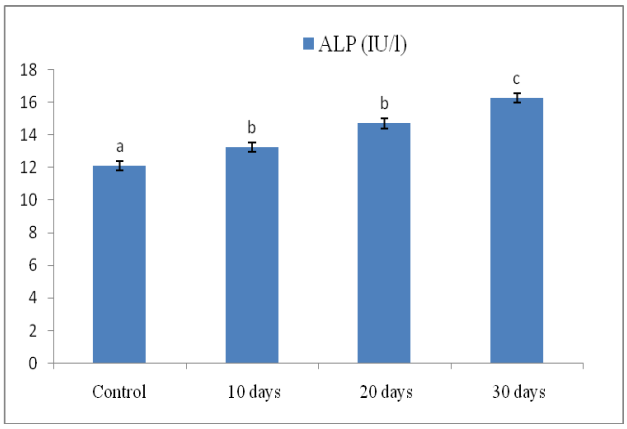


Figure 1 represents the Total protein, Albumin, Globulin content and SGOT (AST) and SGPT (ALT) activities in *Catla catla* of Control and experimental fish.



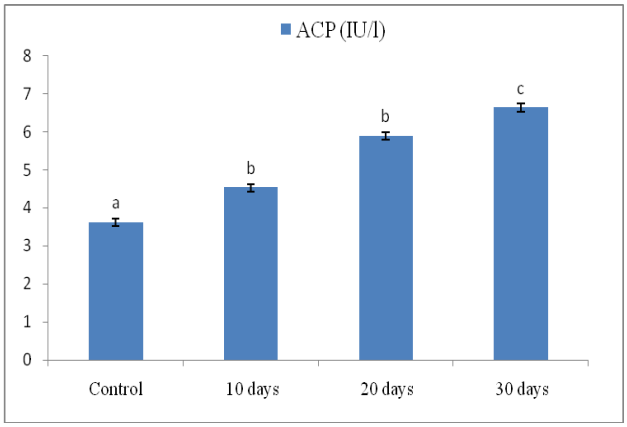


Figure 2 represents the ACP and ALP activities in *Catla catla* of Control and experimental fish.

Values were expressed as Mean ± standard deviation Mean values within the Column followed by different letters (Superscript) are significantly ($p < 0.05$) different from each other were comparison by Duncan's multiple range test (DMRT).

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