

TOXICITY OF ALUMINIUM SALTS ON LC50, BLOOD GLUCOSE AND BLOOD CALCIUM LEVELS IN *CATLA* *CATLA* (HAMILTON)

*C. Manjulata¹ and D.E. Babu²

¹Department of Zoology, Nachuni Degree Mahavidyalaya, Nachuni, Khorda, Odisha

²Department of Zoology, College of Science and Technology, Andhra University, Visakhapatnam, A.P. India

*Author for Correspondence

ABSTRACT

The effect of three forms of Aluminium salts, $AlCl_3$, $Al_2(SO_4)_3$ and $Al(OH)_3$ on the LC50, blood glucose and serum calcium levels of *Catla catla* were determined and found toxic. The LC50 values were found to be 9.93, 7.74, 5.97 and 4.66 ppm for 24 hrs, 48 hrs, 72 hrs and 96hr respectively for $AlCl_3$. Similarly the LC50 values observed for $Al_2(SO_4)_3$ were 11.65, 9.58, 7.26 and 6.53 ppm for 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively and for $Al(OH)_3$ the LC50 values determined were 10.1, 8.04, 6.92 and 5.08 ppm for 24 hrs, 48 hrs, 72 hrs and 96 hrs respectively. The calcium levels of the blood serum were found initially decreasing after the fish were exposed to the three forms of Aluminium salts for 15 days and the decrease in $AlCl_3$ was more predominant than the other two salts; 39% decrease in plasma calcium was noticed in the fish in 15 days when they were exposed to $AlCl_3$. Similarly 20% and 8% decrease in plasma calcium was noticed in the fish when they were exposed to $Al_2(SO_4)_3$ and $Al(OH)_3$ respectively. A gradual increase in the blood glucose levels were noticed when the fish were exposed to the three forms of Aluminium salts and the levels continue to increase till the 15 day experimental duration. 21% increase was noticed in blood glucose levels after treatment with $AlCl_3$, 9% and 16% with $Al_2(SO_4)_3$ and $Al(OH)_3$ respectively.

Keywords: Aluminium, Toxicity, *Catla*, LC50, Serum calcium, Blood glucose

INTRODUCTION

Aluminium is a natural contaminant of many soils, minerals and generally present in ground and surface water in very low concentration. Rain and water run offs are mainly responsible for increasing concentration of Aluminium salts in soil and aquatic bodies, concentration of Aluminium also increases due to mineral weathering (Schofield and Trojnar, 1980). Even though, Aluminium is not a heavy metal, it is proved toxic to human and other fauna, but detailed information is needed about the toxicity of Aluminium salts. It has been determined that fish tend to be more sensitive to Aluminium toxicity than aquatic invertebrates (Sparling *et al.*, 1997). Aluminium salts enter the food chain through different routes; Aluminum chloride and Aluminium sulphate are natural ingredients of food substances including soft drinks, baking powder, self-rising flour etc; drugs, such as antacids, analgesics, and anti-diarrheals contain additives such as Aluminium hydroxide. Aluminium is toxic to fish in acidic, un-buffered waters starting at a concentration of 0.1 mg/l. Aluminium ions influence cell permeability regulation by altering calcium ion concentration. Free Calcium levels are important in various metabolic reactions including neuromuscular excitability, cardiac and other muscle contractions and bone metabolism.

Taouil *et al.*, (2013) studies have revealed glucose intolerance in association with an increase in both total serum calcium and PTH. According to the studies of Koo *et al.*, (1992), increased Aluminium uptake causes encephalopathy and osteomalacia in humans. *Catla catla* is a most preferential fish for human consumption; contaminated fish with high concentrations of Aluminium is likely to cause deleterious effect on human health. In lower concentration Aluminium toxicity leads to bioaccumulation and ultimately enters the food web of human beings (Abedi *et al.*, 2012).

Basing on the fact that scientific information available on toxicity of Aluminium on aquatic fauna is lacking and results are awaited, the following experiments are planned on the Indian major carp, *Catla*

catla to evaluate the LC50, serum calcium and blood glucose levels after exposing the fish to three salts of Aluminium, AlCl₃, Al₂(SO₄)₃ and Al(OH)₃; serum calcium and glucose are taken as major biochemical variants in animals affecting various physiological activities.

MATERIALS AND METHODS

Juvenile fish of *Catla catla* were collected from the fish hatchery at Balabhadrapuram, East Godavari Dist, AP, India (16° 57' 30" N, 82° 0' 20" E) and the fish were initially acclimatized to laboratory conditions in large glass aquaria for a period of two weeks. Thirty to forty individuals from each group in the size range 6±0.5cm with a bodyweight of 15-20 gm were used for the experiment. The three groups of fish were kept in batches in 20 lit glass tanks filled with de-chlorinated tap water under constant aeration. The fish were fed with fish pellet with 36% protein twice a day. The toxicity determination was carried out in a round bottom glass jar of one litre capacity. Healthy fish, which were of the same age group and same body weight of 15-20gms, were used in the experiment. For estimating the degree of toxicity of Aluminium chloride, Aluminium hydroxide and Aluminium sulphate, a batch of 10 test fish were released at a time into each container for each chemical. Experiments for each dose of the chemical were repeated ten times to get average mortality rate from a sample test of 100 specimens. A control experiment with 10 fish was set simultaneously without toxicant. The three test solutions were renewed every 24hr to maintain the dissolved oxygen concentration at optimum level. All the experiments were conducted at room temperature at 27°C using Probit Analysis (Finney, 1971) for determining the LC50 values. Three experiments were performed to determine the LC50 values of 95% upper and lower confidence limits by adopting Hamilton *et al.*'s method LC50 (1977).

Blood samples were collected from experimental fish after 24hr, 48hr, 5 days, 10 days and 15 days of exposure at 1.0 and 2.0 ppm concentration of the salts, blood was drawn by puncturing the caudal blood vessels. Sera were separated by centrifugation and analyzed for calcium following the methods of Webster (1992). Blood glucose levels were measured using one touch Ultra TM blood glucose monitoring system using fast draw design test strips. Human blood sample (self) was used as control for blood glucose determination. pH was determined in digital pH meter and dissolved oxygen was determined following modified Winkler's method.

RESULTS

The experimental results on water quality parameters are shown in Table 1. It was evidenced that the dissolved oxygen values were reduced in the aquaria when AlCl₃ and Al₂(SO₄)₃ were added to the medium, simultaneously the waters became acidic in these salts.

Table 1: Physico-chemical characteristics of the normal and water and water with dissolved Aluminium salts

Parameters	Normal water	Water dissolved with AlCl ₃	Water dissolved with Al ₂ (SO ₄) ₃	Water dissolved with Al(OH) ₃
Odour	Odourless	Unpleasant	Unpleasant	sore
Temperature °C	27	28	27.3	26.8
pH	7.2	6.7	6.8	7.3
DO, mg/L	4.8	3.6	4.3	4.1
Total hardness, mg/L	238	234	240	244

The effects of Aluminium salts on % mortality is also estimated. When the fish were exposed to AlCl₃, mortality started at concentration 1 ppm after 96 hrs of exposure, in Al₂(SO₄)₃ mortality started at a concentration of 3 ppm and in Al(OH)₃ mortality commenced at 2 ppm. 100% mortality was observed at 13 ppm for to AlCl₃, 15 ppm for Al₂(SO₄)₃ and 14 ppm for Al(OH)₃. The LC50 values of 24 hrs, 48 hrs, 72 hrs and 96hr for the three Aluminium salts were also estimated to conduct sub lethal

toxicological experiments. The concentration of $\text{Al}_2(\text{SO}_4)_3$ required for LC50 is more when compared to AlCl_3 and $\text{Al}(\text{OH})_3$ during 96 hours of exposure.

Three different toxicity curves are plotted for AlCl_3 , $\text{Al}_2(\text{SO}_4)_3$ and $\text{Al}(\text{OH})_3$ taking concentration of test chemicals in ppm on X-axis and duration of exposure in hours on Y-axis. There was a gradual decrease in the slope function corresponding to an increase in the exposure time from 24 to 96 hr. It is clearly evident that slope decline was more with AlCl_3 and $\text{Al}_2(\text{SO}_4)_3$ than $\text{Al}(\text{OH})_3$ along with an increase in exposure time. The values for lethal concentration of the three Aluminium salts for 24 hr, 48 hr, 72 hr and 96 hr were calculated from the regression equation $Y = 1.596x + 10.93$, correlation co-efficient (r) = .997 for AlCl_3 , $Y = 1.741x + 13.08$, correlation co-efficient (r) = .981 for $\text{Al}_2(\text{SO}_4)_3$ and $Y = 1.618x + 11.58$, correlation co-efficient (r) = .993 for $\text{Al}(\text{OH})_3$.

No change in the calcium levels of the blood serum were found when the fish were treated with 1 ppm and 2 ppm concentration of the three Aluminium salts even after 15 days of exposure. It is evident that the serum calcium levels started decreasing from 2.3 ± 0.01 m mol/l and reached 1.4 ± 0.012 m mol/l in the fish treated with AlCl_3 , 1.84 ± 0.01 m mol/l in $\text{Al}_2(\text{SO}_4)_3$ and 2.01 ± 0.016 m mol/l in $\text{Al}(\text{OH})_3$ in 15 days of exposure. There is a decrease of 39% in plasma calcium level was noticed in the fish after 15 days of exposure to AlCl_3 .

Analysis on the blood glucose levels of fish after exposure to AlCl_3 , $\text{Al}_2(\text{SO}_4)_3$ and $\text{Al}(\text{OH})_3$ has clearly indicated a gradual increase till 15 day of continuous exposure; the values never reached the normal level during the experimental duration. No change in the blood glucose levels were observed, when the fish were treated with 1 ppm concentration of AlCl_3 , $\text{Al}_2(\text{SO}_4)_3$ and $\text{Al}(\text{OH})_3$, but at 2 ppm a slight increase in the blood glucose concentration was observed, when the fish with treated with AlCl_3 and $\text{Al}(\text{OH})_3$ after 10 days of exposure. $\text{Al}_2(\text{SO}_4)_3$ could not change the blood glucose levels even after 15 days of exposure at 1 ppm or 2 ppm concentration. Significant changes in the blood glucose levels of the fish at 3ppm concentration of AlCl_3 , $\text{Al}_2(\text{SO}_4)_3$ and $\text{Al}(\text{OH})_3$. The values never reached the normal level during the experimental duration of 15 days. The increase was more pronounced in fish treated with AlCl_3 , which has resulted in enhancing the blood glucose normal value from 62 ± 0.2 mg to 75.2 ± 0.29 , the increase with $\text{Al}_2(\text{SO}_4)_3$ was 67.6 ± 0.238 mg from the initial value of 62 mg and $\text{Al}(\text{OH})_3$ has resulted in enhancing the blood glucose level from 62 mg to 72.2 ± 0.122 mg in 15 days. There was an increase of 21% noticed in blood glucose level after treatment with AlCl_3 , 9% and 16% with $\text{Al}_2(\text{SO}_4)_3$ and $\text{Al}(\text{OH})_3$ respectively.

DISCUSSION

The results of acute toxicity tests conducted on the test specimen *Catla catla* with three different forms of Aluminium salts, AlCl_3 , $\text{Al}_2(\text{SO}_4)_3$ & $\text{Al}(\text{OH})_3$ have clearly indicated their toxic nature leading to alteration in the physio-chemical quality of water and causing mortality of test fish at varying rates. Mortality of fish in Aluminium salts might be due to alteration in blood glucose and blood calcium levels, which hinder the normal metabolism including the heart function. Mahajan and Parurukmani (2012) have reported as LC50 for *Catla catla* as 53.1 PPM at a body size of 3.0 ± 0.5 cm, but in the present investigations, the LC50 values observed were 9.93 ppm, the important point of consideration is about the size of the fish and brand of chemical used; in the present experiments the size of the fish used were 6.0 ± 0.5 cm and the chemical used were E. Merck Ltd, Germany. LC50 values for $\text{Al}_2(\text{SO}_4)_3$ and $\text{Al}(\text{OH})_3$ along with blood glucose and blood calcium are provided for the first time on the fish *Catla catla*. The results have indicated alterations in blood glucose and blood calcium levels, both these have impacts on physiological functioning of the organism. The continuous increase in blood glucose is attributed to the stress induced by the Aluminium salts and decreased levels of blood calcium might be due to hormonal imbalances, a finding, which agrees with those of Compiling studies done by Hartman (2006, Internet) that absorption of calcium at the intestinal level might have been blocked by the action of Aluminium salts hindering the action of cholecalciferols.

The fish showed abnormal behavior when exposed to higher concentration of Aluminium salts, vigorous movements of the fish with increased opercular movement was of significant observation made, excess activity demands more energy accounting for enhanced blood glucose levels. Increase in the concentration of the salts further enhanced their movements. Another important point of discussion is about the development of wounds and black inundations on the skin at LC50

concentration of $AlCl_3$, but the fish developed white patches on the skin when treated with $Al(OH)_3$, followed by mucous secretions indicating the role of the salts on epidermal glands. Feeding activity and opercular movement was reduced gradually before mortality of the fish after exposure to the salts; the effect was more in $AlCl_3$.

REFERENCES

- Abedi M, Bartelheimer M, Poschlod P (2013)**. Aluminium toxic effects on seedling root survival affect plant composition along soil reaction gradients—a case study in dry sandy grasslands. *Journal of Vegetation Science*, **24**(6) 1074-85.
- Finney DJ (1971)**. Probit Analysis, Cambridge university press. 3rd Edition Cambridge, UK: Chapter 4.
- Koo WW, Krug-Wispe SK, Succop P, Bendon R, Kaplan LA (1992)**. Sequential serum aluminum and urine aluminum: creatinine ratio and tissue aluminum loading in infants with fractures/rickets. *Pediatrics*, **89**(5) 877-81.
- Maharajan A, Parurukmani PS (2012)**. Effect of aluminium chloride toxicity against histopathology of gill and liver tissue of Indian major carp, *Catla Catla* (Hamilton). *International Journal of Pharma and Bio Sciences*, **3**(3) 523-30.
- Schofield C, Trojnar JR (1980)**. Aluminum Toxicity to Brook Trout (*Salvelinus fontinalis*) in Acidified Waters. In: Toribara TY, Miller MW, Morrow PE, Eds. (1980), Polluted Rain. Plenum Press, N.Y., pp. 341-363.
- Sparling DW, Lowe TP, Campbell PGC (1997)**. Ecotoxicology of aluminum to fish and wildlife. In: Research Issues in Aluminum Toxicity, pp. 47–68. (Yokel RA, Golub MS, Eds.) Washington, D.C., Taylor & Francis.
- Hamilton MA, Russo RC, Thurston RV (1977)**. Trimmed Spearman-Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environmental Science and Technology*, **11**(7) 714-9.
- Taouil H, Ahmed SI, El Assyry A, Hajjaji N, Srhiri A, Elomari F, Daagare A (2013)**. Manganese, nickel, lead, chromium and cadmium in the watershed Guir, impact on the quality of wells water in Tiykomyne (Eastern Morocco). *International Journal of Agricultural Policy and Research*, **1**(5) 150-5.
- Webster Jr WW (1992)**. A simple microspectrophotometric method for the determination of serum calcium. *American Journal of Clinical Pathology*, **37** 330-3.
- Winkler LW (1888)**. The determination of dissolved oxygen in water. *Berichte de Deutschen Chemischen Gesellschaft*, **21** 2843-2855.