

Comparative ameliorating effects of calcium ions and silicon dioxide on serum parameters of *Channa punctatus* (Bloch) in acid aluminum-induced toxicity

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Abstract: The bioavailability and toxicity of aluminum can be significantly amplified in acidic and poorly buffered aquatic environments. High concentrations of both increased H⁺ ions and aluminum are directly toxic to fish. The biochemical variables are used as an index of fish physiology. Significant elevation of blood glucose level noted in acute acid exposure alone as well as in the presence of sublethal (140 mg/L) aluminum for 7 days. There was a significant decline in serum Na⁺ and Cl⁻ ions in both the treated groups. Both serum K⁺ and protein concentrations of fish exposed to a sublethal dose of aluminum revealed elevated values. Blood glucose level, serum K⁺, and protein concentrations significantly decreased the values with the application of ameliorating agents. Application of silicon dioxide and calcium carbonate reduces the toxicity of aluminum on the biochemical parameters of freshwater fish *Channa punctatus* (Bloch).

Keywords: amelioration; calcium carbonate; silicon dioxide; *Channa punctatus*; acid aluminum toxicity

1. Introduction

Pollutants entry and their effects can be studied in aquatic organisms by monitoring them. Fishes are an appropriate indicator of aquatic pollution to study acute and chronic toxicity tests. Water pollution is responsible for damage to both flora and fauna of aquatic ecosystems, and it affects cellular, biological, and physiological processes in them [1]. Toxic elements in fish pose a threat to human health and the environment. Aqueous aluminum has been found as the main toxicant that is responsible for freshwater fish mortality in acidified waters [2]. The aluminum element is present in around 8% of the Earth's crust. It is a silvery white, ductile, and malleable metal. Most of the production of aluminum is from bauxite as a raw material, which contains up to 55% alumina (aluminum oxide). By chemical weathering of rocks, aluminum is found in the clay fraction. Aluminum solubility depends on ambient pH, being at a minimum at around pH 5.5, and it increases on both extremes of the pH scale. Due to acidification, aluminum becomes soluble, and it reaches from the soil to aquatic ecosystems. Soil acidification may cause leaching of Al metal, which changes the quality of water in the drainage area [2]. Fish kills have been reported in those areas where hard rock mining, milling, and smelting activities were responsible for the release of acid and metals from mine wastes into nearby aquatic bodies. In

Jharkhand, laterite soil accounts for about 90% of the soil. Their formation is due to the leaching process, due to heavy rains, and they are red. Iron and aluminum salts predominate in this soil and are acidic in nature, low in phosphate, nitrogen, calcium, magnesium, and silicon. They develop in areas with heavy rainfall and high temperatures. Heavy rainfall increases the leaching of nutrients, which causes the acidification of soil. In Jharkhand, about 61.1 million tons of aluminum ores (bauxite) open up the possibilities of mobilization of aluminum from soil to nearby aquatic ecosystems. Under these circumstances, excess release of aluminum can enhance and cause loss in economic and edible values of fish and aquaculture. Organic matter in dissolved form, silica, and fluoride are said to reduce the toxic effects of Al to aquatic organisms [3, 4]. The present comparative study was designed to evaluate the effect of ameliorating agents to reduce the adverse effects due to aluminum toxicity in freshwater fish *Channa punctatus* (Bloch).

Fish blood biochemical parameters change due to environmental changes. Stressful conditions show the sensitivity of biochemical parameters due to declining water quality, as an indication of tissue damage, which could have resulted from aquatic pollution [5]. The serum total protein constitutes the total of numerous different kinds of proteins. The bulk of the total amount of proteins present in serum is due to albumins and various globulins. Serum protein can be used to evaluate the fish health condition and as a tool to indicate the stressful environment of fish exposed to changes in water pH [6]. Cortisol is a corticosteroid hormone that is secreted by the cortical cells of the zona fasciculata part of the adrenal gland. In all vertebrates and in fish, this glucocorticoid



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plays an important role in the recovery of homeostasis during or after stressful situations. It is often referred to as a “stress hormone” as it is a vital hormone involved in the stress response. It increases blood sugar; blood pressure levels and also has an immunosuppressive action. Glucose serves as the principal fuel of all the tissues and is a reducing monosaccharide.

2. Materials & Methods

Healthy fish *C. punctatus* of size (20.8 ± 0.4 cm) and weight (0.2 ± 0.03 Kg) were collected from the local fish market. In the laboratory, disinfection of fish was done with a 0.01% potassium permanganate solution. Fishes were kept in solution for 1–2 minutes. Acclimation of fishes was for 15 days in glass aquaria (18” X 12” X 12”) in the laboratory conditions (26 ± 2°C). The pH and dissolved oxygen values of the water were 7.0 ± 1 and 7.5 ± 0.5 ppm, respectively. During acclimation, metabolic waste products were discarded by changing water every day. Fishes were fed with *Tubifex tubifex* and *Daphnia*.

Fish were separated randomly into five groups. Group I fish were kept as controls in dechlorinated water, at approximately constant temperature (26 ± 2°C) and pH (7.2 ± 0.5). Group II was exposed to acidified water at pH < 5 with stock solutions of 1 M H₂SO₄ for a period of 7 days. Group III was exposed to acidified water along with a 1/5th sublethal dose of 140mg/l aluminum sulfate Al₂(SO₄)₃.16H₂O (Qualigens) for 7 days. Group IV fishes were treated with acid plus Al with 100 mg of calcium carbonate (precipitated, Merck) for another 7 days to ascertain the ameliorating effect of calcium in aluminum toxicity. Group V fishes were treated with acid plus Al with 150 mg of silicon powder (Loba Chemie) for the next 7 days to determine the recovery with silicon in aluminum toxicity.

The blood from control and aluminum-treated fishes and ameliorating agents-treated fishes was collected from the cardinal vein in plain vials. It was allowed to clot. Serum was separated from the clot by centrifugation within half an hour. The blood serum glucose level was estimated by the enzymatic method, GOD-POD. Serum triglycerides and serum total cholesterol were estimated by the enzymatic method using a colorimeter. Total protein in blood serum was estimated with the help of the standard kit ‘Crest Biosystems’. Serum total cholesterol was determined by using a colorimeter. Estimation of serum ions like sodium was performed by the ‘Na⁺ colorimetric method’ prescribed by the standard kit (ELITE 2 Kit) of ‘Crest Biosystems’. Serum ions like potassium were measured from blood serum following the method ‘K⁺ colorimetric method’ as suggested by the standardized kit ‘Elyte 2 Kit’ of ‘Crest Biosystems’.

Statistical analysis

Standard deviation, standard error, and Student's t-test were used for analysis of statistical data [7]. Paired sample 2-tailed ‘t-test’ was done to assess the significance level of changes in mean values of biochemical parameters. Data were analyzed with the help of the ‘Microsoft Excel’ software.

3. Results & Discussions

Many significant changes in biochemical parameters were recorded on exposure to the acid alone group as well as acid plus aluminum, in a simulated system of ambient soft water at a pH of 5.0. No mortalities were recorded in the control group or in any of the treated groups. The mean values of the biochemical indices recorded on the test fishes of all the groups have been shown in Table 1 and Figure 1 along with the paired sample t-test, the significance level and critical values.

The various changes were observed on exposure to different experimental conditions. The mean blood glucose level under acute acid exposure at pH 5.0 elevated 5.28% with a recorded value of 64.34 ± 1.22 mg/100mL. Acid with a sublethal concentration of aluminum induced a highly significant rise in serum glucose level of 90.42 ± 2.65 mg/100 mL with +47.96% alteration by the 7th day. Exposure to calcium carbonate and silicon to acid, plus a sublethal concentration of aluminum 140 mg/L, produced a significant decrease in the glucose level. The values recorded after fourteen days of exposure were 64.02 ± 1.32 mg/100 mL and 63.47 ± 1.28 mg/100 mL with -29.19% and -29.80% depression.

Fish treated with acid alone resulted in a decline of -3.25% in serum protein concentration, having a value of 3.20 ± 0.09 g/100 mL, while fish exposed to a sublethal dose of aluminum at pH 5.0 revealed an elevation of 14.06% with a recorded value of 3.78 ± 0.08g/100mL. There was a depression in serum protein concentration by 13.06% with a value of 3.28 ± 0.05g/100mL with the application of calcium carbonate in acid plus aluminum at pH 5.0. Silicon also significantly reduces the percentage of serum protein by 14.81% having a value of 3.22 ± 0.07 g/100 mL.

Serum Na⁺ concentration showed a decline of 5.43% in acid alone exposure fish, having a value up expos 144.46 ± 1.05 mMol/L. The fish group exposed to water of pH 5.0 containing 140 mg/L of aluminum revealed ionic disturbance, and the Na⁺ level declined significantly by 3.78% with a recorded value of 146.98 ± 1.18 mMol/L. Treatment of fish with the ameliorating agent calcium carbonate resulted in an elevation of 1.60% having a value

Table 1. Biochemical parameters of *Channa punctatus* under control conditions, acid alone, and acid with aluminum exposure for the acute exposure period of 7 days & with ameliorating agents calcium ions and silicon dioxide, under acid with aluminum exposure for the acute exposure period of 7 days.

Serum Parameters	Control Value	Acid alone Exposure	Acid with Al exposure	Acid with Al + Ca^{2+}	Acid with Al + SiO_2	ANOVA (F)
Glucose (mg/100mL)	61.11 ± 0.86	64.34 ± 1.22 t = 2.11	90.42 ± 2.65 t = 11.63***	64.02 ± 1.32 t = 7.72**	63.47 ± 1.28 t = 7.61**	58.77**
Protein (g/100mL)	3.31 ± 0.10	3.20 ± 0.09 t = 3.76*	3.78 ± 0.08 t = 7.45**	3.28 ± 0.05 t = 4.22*	3.22 ± 0.07 t = 10.57***	7.60*
Cortisol (pg/mL)	585 ± 2.02	455 ± 1.30 t = 63.43***	242 ± 0.94 t = 198.03***	507.8 ± 1.06 t = 177.59***	514 ± 1 t = 154.48***	9667.68**
Triglyceride (mg/dL)	81.2 ± 1.06	85.8 ± 1.06 t = 6.78**	202.4 ± 1.20 t = 68.39***	120.6 ± 0.87 t = 46.16***	94 ± 1.22 t = 72.10***	2102.08**
Cholesterol (mg/dL)	127.8 ± 1.01	130.4 ± 0.92 t = 1.69	209.8 ± 1.56 t = 45.13***	147.2 ± 1.42 t = 30.32***	137.2 ± 1.23 t = 34.76***	729.72**
Na^+ (mMol/L)	152.76 ± 0.7	144.46 ± 1.05 t = 4.83**	146.98 ± 1.18 t = 3.76*	149.34 ± 1.66 t = 2.51	149.84 ± 1.78 t = 2.43	5.44*
K^+ (mMol/L)	4.10 ± 0.22	3.75 ± 0.14 t = 1.24	4.18 ± 0.19 t = .38	3.81 ± 0.24 t = 2.24	3.84 ± 0.22 t = 2.86*	0.81
Cl^- (mMol/L)	135.14 ± 2.97	129.06 ± 1.66 t = 2.29	127.7 ± 1.58 t = 4.30*	131.94 ± 1.94 t = 1.80	131.45 ± 2.2 t = 1.52	1.79

Showing t-values obtained for paired sample t-test [7] along with the significance level. Values reported as Mean SEM. *P < 0.05, ** P < 0.01, *** P < 0.001; ANOVA – (* -Significant; ** - Highly Significant).

of 149.34 ± 1.66 mMol/L, while fish exposed to sublethal aluminum at pH 5.0 with silicon showed a raised value of 149.84 ± 1.78 mMol/L with an elevation of 1.94%.

The mean blood triglyceride level under acute acid exposure at pH 5.0 was elevated by 5.66% with a recorded value of 85.8 ± 1.06 mg/dL. Acid with a sublethal concentration of aluminum induced a highly significant rise in serum triglyceride level of 202.4 ± 1.20 mg/dL with a +149.26% alteration by the 7th day. Exposure to calcium carbonate and silicon to acid, plus a sublethal concentration of aluminum 140mg/l, produced a significant decrease in the triglyceride level. The values recorded after fourteen days of exposure were 120.6 ± 0.87 mg/dL and 94 ± 1.22 mg/dL with -40.41% and -53.55% depression.

There was an elevation of serum cholesterol in the acid alone group at pH 5.0, with significant values of 130.4 ± 0.92 mg/dL, having a 2.03 % alteration. Serum cholesterol level shows a rise in acid with the aluminum group by 64.16% with a recorded value of 209.8 ± 1.56 mg/dL. Fish treated with calcium carbonate resulted in a decline of cholesterol level by 29.83% in a value of 147.2 ± 1.42 mg/dL. While fish exposed to silicon revealed a decline of cholesterol level by 34.60% with a recorded value of 137.2 ± 1.23 mg/dL.

To find out the physiological condition of the fish, plasma chemicals seem to be useful tools [8]. The high magnitude of stress response is due to short-term intense stress, which increases cortisol levels, followed by a decline. This increase in cortisol level is responsible for the secondary response in the level of glucose. Cortisol plays an important role in stress-associated increase in plasma glucose level as cortisol is mobilized from the liver [9].

The stress due to acidification induces changes in physiology apart from the lethal effect in fish. It has been observed that chum salmon *O. keta* juveniles display avoidance behavior at pH 5.8 under laboratory conditions [10]. Cortisol secretion under stress causes a decline in immunity and reproductive physiology in fish. Under acid stress, juvenile carp *Cyprinus carpio* at pH 4.5 show an increase in the level of plasma cortisol and a decline in immunoglobulin M (IgM) levels [11]. Salmonid fish exposed to sulfuric acid water at pH 4.5-5.0, the development of the embryo is affected, and malformations take place [12]. Even if acid stress is not lethal but indirect effects on the physiological mechanisms of fish have been observed.

The effects of sublethal levels of aluminum chloride have been studied on the biochemical parameters in adult *Tilapia zilli* [13]. Glucose, total protein, cholesterol, cortisol, and alkaline phosphatase values were observed.

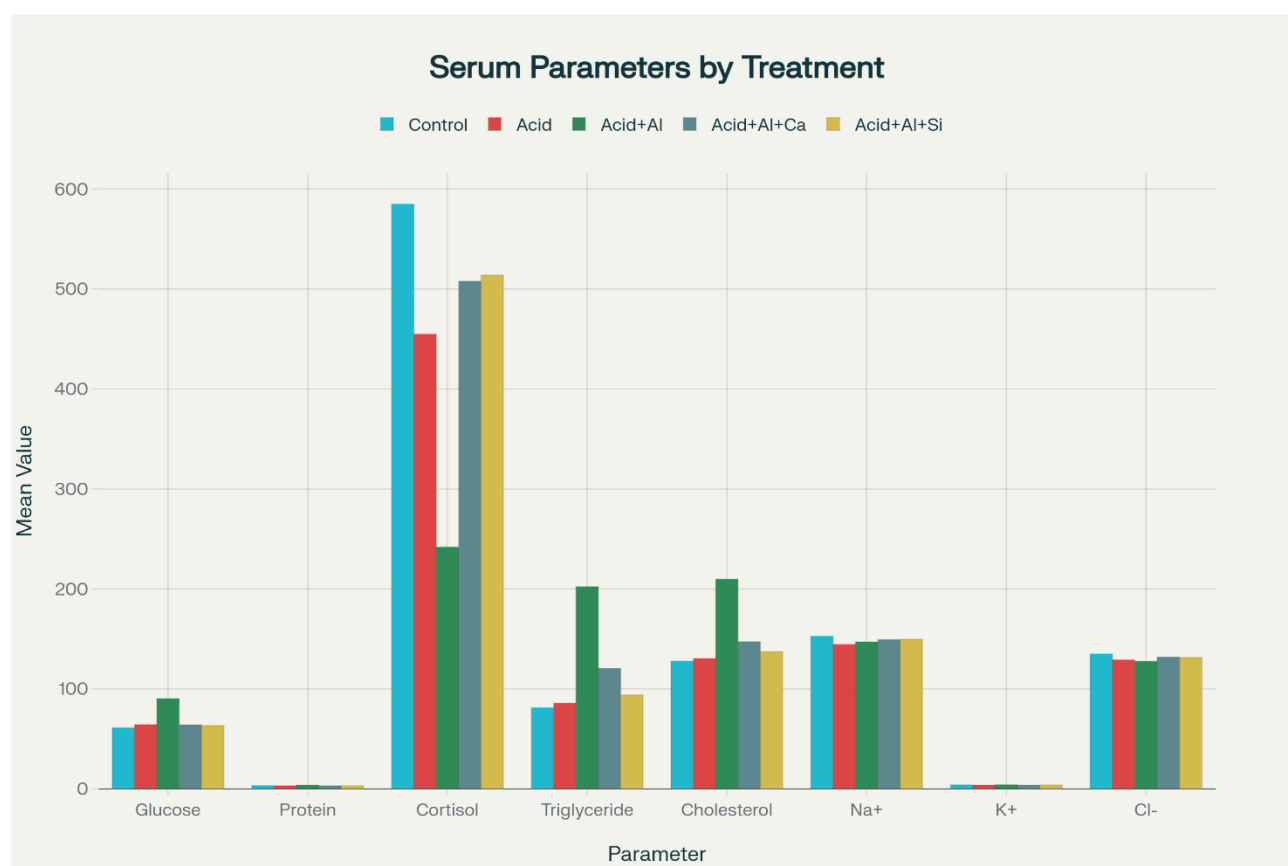


Figure 1. Changes in mean serum levels of glucose, protein, cortisol, triglyceride, cholesterol, sodium (Na⁺), potassium (K⁺), and chloride (Cl⁻) under control conditions and after exposure to acid alone, acid with aluminium, acid with aluminium plus calcium ions, or acid with aluminium plus silicon dioxide. Data are shown as mean $\pm$ standard deviation for each group. Statistical significance was determined using ANOVA; detailed t-values and significance levels are indicated for each comparison. Abbreviations: Ca²⁺ (calcium ions), SiO₂ (silicon dioxide), ANOVA (Analysis of Variance).

The glucose level increased in Atlantic salmon which was exposed to aluminum at pH 5.8 [14]. The same results were observed in Brown trout in the case of exposure to aluminum in acidic fresh waters [15]. In both acid and acid with Al stress, hyperglycemia was observed [16]. Stress induces an increase in plasma catecholamines in fish, which in turn converts glycogen into glucose to meet energy demand in stressful conditions [17].

Mean total protein content of blood under acid Al treatment also increased significantly and is in conformity with the results obtained in *Salmo gairdneri* under aluminum toxicity [18]. Serum protein also reflects the effect of environmental factors on organisms. An increase in protein content reflects the response to stress and an increase in demand for energy. The high protein in serum may be to fulfil the energy requirement in metal stress or due to the direct effect of metal on the cell [19-22].

Significant increase in triglycerides and cholesterol levels was observed in treated fish than the control values. This increase serves as an additional reserve of energy to

mediate metal stress [23]. A change in triglyceride content indicates a liver stress condition, and accumulation of fats may be due to its dysfunction [24].

Serum cortisol value increased significantly in treated fish in the present investigation. Similar results were obtained in rainbow trout [18] in exposure to acid with aluminum and *Clarias batrachus* [25] when exposed to various toxicants. An increase in plasma cortisol level could be used to evaluate the level of stress in fish [26]. Level of cortisol increases under stress conditions [27] as elevated cortisol level activates glycogenolysis and gluconeogenesis processes in fish, which increases glucose levels to meet the need of energy demand.

Research finding shows that aqueous Ca²⁺ helps in the recovery of Al toxicity in fish. Research experiments [28-30] have shown that calcium carbonate helps in the recovery of fish in metal stress. Similarly, the increased dietary calcium reduces uptake of cadmium in rainbow trout and has an ameliorating effect on fish susceptibility to toxic metals [31]. It prevents the entry of metal through

gills. Calcium carbonate shows similar results with arsenic in *C. gariepinus* has been reported to abridge the harmful property [32]. The pH level increases in high concentrations of calcium and non-toxic Ca^{++} ions may have competition for active sites in tissues of fish with toxic metals such as copper and cadmium. In this way, it ameliorates the metal toxicity. Aluminum is less toxic in hard water in the case of the freshwater crustacean, *Hyalella azteca* [33]. $\text{Al}(\text{OH})_4^-$, aluminate is the dominant form, which is less toxic to fish at pH greater than 6.7 [34]. The ameliorating effect of calcium carbonate has been found in an acute toxicity study of aluminum sulfate on *Daphnia magna* [35]. Calcium and magnesium carbonates are found in high concentrations in hard water, which reduces the bioavailability of Al and its toxicity.

Al toxicity is lowered by complexation with silicon and has an ameliorating effect. On Earth, Silicon is present as the second most abundant element. Silicon is released from minerals by weathering processes. In nature, silicon is not found in free form. It is only reactive under conditions of extremely high temperatures in its crystallized form. Silicon solubility is little affected by water and its vapor since it rapidly forms a protective layer of silicon dioxide. Silicon dioxide forms orthosilicic acid when it reacts with water. Aluminum reacts with silicic acid to form hydroxyaluminosilicates (HAS) [36, 37]. Soluble silica reduces the bioavailability of aluminum and lessens its toxicity [38, 39]. It has been seen that monomeric silica has less affinity with aluminum than oligomeric silica [39].

5. Conclusion

The amphoteric property of hydroxyaluminum compounds prevents changes in pH values in the range of 4 and 5. A polymeric form of aluminum is formed in the presence of excess silica, due to which the release of aluminum is less in the environment. Silicon plays a preventive role in stress by aluminum by limiting its availability. Calcium carbonate increases the water hardness and pH, which lowers the solubility of aluminum and its toxicity, and thus has an ameliorating effect on *Channa punctatus* in aluminum toxicity.

The presence of 90% laterite soil along with a significant quantity of bauxite, an important ore of aluminum, in and around Jharkhand, seems to be a problem for the future. It can affect fish and other aquatic organisms and may reduce their edible as well as economic value. Calcium carbonate and silicon show ameliorating effects in recovery from acid aluminum-induced acute toxicity in *Channa punctatus*.

Declarations

Author Contribution: Author conceived of the presented study, performed the literature search, experiments, data analysis and drafted the manuscript.

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