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## Short-Term Effect of Tooth Paste In The Catfish

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### ABSTRACT

The main aim of this study was to evaluate the effect of Colgate Dental Cream on Catfish. Investigation was carried out using Catfish weighing about 475-525 g. Fishes were maintained for 24 h in the water, dissolved with 100 mg of Colgate Dental Cream. Reduced serum total protein, albumin, glucose and urea, concomitant with a reduced concentration chloride ions were observed. Enzymes such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) enzyme activities were changed significantly. In addition, structural architecture of liver, heart, gills, muscle and kidney were also changed. Biochemical and histopathological changes in the catfish were due to the fact that there was an increased demand for energy under stress to cope up with the detrimental effects of the dental cream.

**Keywords:** Colgate dental cream; fish; histopathology; protein; enzymes

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## INTRODUCTION

Environmental pollution is an unfavourable alteration of our surroundings due to direct or indirect activities of man. The high rate of increase of human population, rapid expansion in industrial and urban activities and modernization of agriculture has resulted in the generation of a high volume of waste material causing gradual deterioration of valuable resources of biological productivity<sup>1</sup>. Application of synthetic fertilizers, insecticides and pesticides leads to fast degrading of natural water quality. Aquatic ecosystems that run through agricultural or industrial areas have a high probability of being contaminated by run off and ground water leaching a variety of chemicals.

The many sources of water pollution cause devastating consequences to marine life. Fish and marine mammals at the top of the food chain are exposed to higher levels of toxins due to the fact that they are exposed to toxins directly from the water and toxins from eating other fish exposed to toxins in the water<sup>2</sup>. The river pollution caused by industrial effluents also affects its flora and fauna<sup>3</sup>. Pollutant administered to adult fish at later stages of their sexual maturation have caused spawning failure or lowered spawning success<sup>1</sup>. Hematological characteristics have been widely used in clinical diagnosis of human and domestic animals and their techniques have proved valuable for fishery biologists in assessing the health of fish and monitoring stress response<sup>4</sup>.

Investigation was undertaken to evaluate some hematological changes resulting from the exposure of a freshwater fish, *Heteroclaris* sp. to sublethal concentrations (5.0 and 10.0 mg/l) of zinc in water for a period of fifteen days. Plasma levels of protein and glucose were also lower in the exposed fish when compared to the control<sup>5</sup>. The mechanism by which contaminants can affect fish reproduction are several. Some pollutants are known to influence directly the reproductive hormones in fish or interfere with the egg formation process; they may damage reproductive tissues and impair their functions<sup>6</sup>.

Colgate toothpaste containing active chemical ingredients, which affect the human body. Colgate super shakti dental cream contains calcium carbonate, sarbitol, sodium lauryl sulphate, silica, titanium dioxide, sodium silicate, carrageenan, sodium monofluorophosphate, sodium bicarbonate, sodium saccharin, triclosan. The present study was aimed to evaluate biochemical and histopathological alterations resulting from the exposure of the freshwater fish, *Heteroclaris* sp. to low dose of 100 mg/liter concentrations of Colgate dental cream in the fresh water.

## MATERIALS AND METHODS

### **Exposure of fish to the colgate tooth paste**

Colgate toothpaste waste was completely dissolved in freshwater in a concentration of 100 mg/liter. Fishes were maintained for 24 h in 30 liters of colgate toothpaste dissolved water. The dose 100 mg/liter is selected in order to determine low dose effect in catfish (1mg/10 ml)

### **Collection of blood and tissues**

Fish was taken from the treatment tank after 24 h and blood was collected from the catfish by cardiac puncture and tissues such as muscle, liver, heart and gills were surgically removed. Up to 1.0 ml blood was collected in an Eppendorf tube containing 1.5% EDTA as an anticoagulant.

### **Biochemical parameters**

Blood glucose was estimated by the Asatoor and King. Protein content was measured by Lowry et al. Albumin was measured by Reinhold. Urea content was estimated by Natelson.

### **Enzyme activities**

Alanine transaminase (ALT) and Aspartate transaminase (AST) enzyme activity was measured by Reitmen and Frankel method. Alkaline Phosphatase (ALP) enzyme activity was measured by Bessey .

### **Histopathology**

The sections of muscle, liver, heart and gills were taken of 5 $\mu$  thickness and mounted on slides and stained with hematoxylin and eosin. The stained sections were morphologically evaluated under compound microscope.

### **Statistical Analysis**

All the values are expressed as means  $\pm$  SEM. Statistical analysis was performed using SPSS version 16.0 (Statistical Package). Student's t-test was performed to determine the differences between control and treatments.  $P \leq 0.05$  was considered to be significant.

## RESULTS AND DISCUSSION

The total protein, albumin, glucose and urea content in the blood of catfish were determined for the control (Table 1). Fishes from colgate toothpaste dissolved water, showed changes in the content of protein, albumin, glucose and urea at the end of 24 h (Table 1). A generalized decrease in total protein, albumin, glucose and urea content in colgate toothpaste treated catfish compared control. The total protein, albumin and glucose content were significantly decreased, while the urea content was not significantly decreased compared to control.

**Table- 1: Effect colgate dental cream (CDC) 100 mg/L on Catfish hematology following 24 h of exposure.**

Parameter	Control	CDC-Treated
Total Protein (g/dl)	40.82 ± 0.965	32.17 ± 0.988**
Albumin (g/dl)	5.08 ± 0.14	4.03 ± 0.14***
Glucose (mg/dl)	118.3 ± 3.35	72.03 ± 2.12***
Urea (mg/dl)	46.78 ± 1.14	44.78 ± 1.23

\*\*P<0.01 and \*\*\* P<0.001

Protein (g/dl), albumin (g/dl), globulin (g/dl) content, AST, ALT and ALP enzyme activities in the muscle and liver of the catfish was given in the Table 2. There was a tremendous increase in the ALP, ALT and AST enzyme activities were found in the muscle. In the liver tissue, there was moderate increase of AST activity was noted. Globulin level slightly reduced compared to the control level in the muscle and gills. Reduced level of protein and ALT were found in the muscle and gills. Catfish muscle showed insignificant changes even after exposed to colgate toothpaste for 24 h. In the liver tissue, ALP enzyme activity was reduced.

**Table 2. Colgate dental cream (Strong Teeth) (CDC) 100 mg/L effect on Catfish muscle and liver tissues following after 24 h exposure.**

Parameter	Muscle		Liver	
	Control	CDC-Treated	Control	CDC-Treated
Protein (g/dl)	2.00± 0.1	1.70±0.134	1.75±0.14	2.58±0.107**
Albumin (g/dl)	0.50±0.063	0.50±0.081	0.15±0.34	1.50±0.073***
Globulin (g/dl)	1.47±0.76	1.28±0.094	1.60±0.123	1.08±0.101**
AST (IU/L)	5.18±0.23	19.22±1.03***	17.0±1.63	29.33±0.881***
ALT (IU/L)	2.33±0.17	36.37±1.94***	44.17±1.77	31.17±3.85*
ALP (IU/L)	30.0±2.78	72.83±2.32***	94.67±4.57	43.67±4.75***

\*P<0.05, \*\*P<0.01 and \*\*\*P<0.001

Protein (g/dl), albumin (g/dl), globulin (g/dl) content, AST, ALT and ALP activities in the control and treated catfish heart and gills were given in Table 3. In the heart tissue, AST and ALT enzyme activity was increased five folds and seven folds respectively. On the other hand, there was also a remarkable increase in the ALP activity in the gills of treated catfish compared to controls. Similarly, a marked increase in the level of AST and ALT were also noticed. Protein (g/dl) and albumin (g/dl) content was slightly increased in the heart and gills. Histopathological examination showed structural alterations in muscle, liver, heart and gills. Fragmented and striated muscle cells, accumulation of lipids and melanin in white muscles were observed (Figure 1). Fibrosis, lipid accumulation, hypertrophy, degeneration of blood vessels and hepatocytes, aggregation of myeloid cells, cytoplasmic vacuolization were observed in the liver following colgate toothpaste exposure (Figure 2). Aggregation of cardiac cells, hypertrophy in arteries

were occurred in the liver (Figure 3). Secondary lamellae fusion, distortion of secondary lamellae erosion and disintegration of blood capsules, hyperplasia of the epithelium were observed in the gills (Figure 4).

**Table-3. Colgate dental cream (CDC) 100 mg/L effect on Catfish heart and gill tissues following 24 h of exposure.**

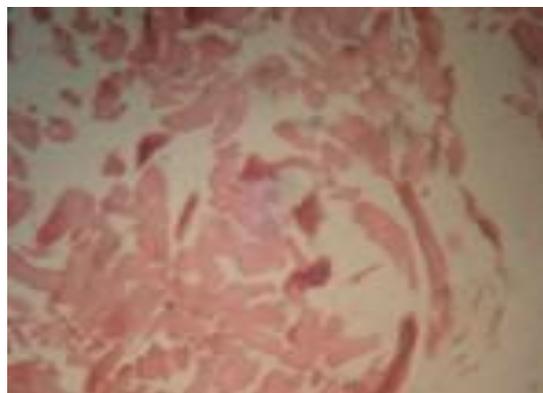
Parameter	Heart		Gill	
	Control	CDC-Treated	Control	CDC-Treated
Protein (g/dl)	1.62±0.186	1.70±0.073	1.50±0.093	1.75±0.099
Albumin (g/dl)	0.30±0.057	0.73±0.071***	0.57±0.055	1.10±0.081***
Globulin (g/dl)	1.42±0.101	0.98±0.132*	0.88±0.07	0.60±0.057*
AST (IU/L)	7.10±0.542	33.00±3.67***	17.67±1.97	29.17±3.49*
ALT (IU/L)	3.00±0.123	22.00±1.34***	26.83±2.35	41.43±2.47**
ALP (IU/L)	45.17±2.76	54.50±3.99	38.83±1.77	74.50±1.40***

\*P<0.05, \*\*P<0.01 and \*\*\*P<0.001

Fishes are in indirect contact with their surrounding environment and any change in the environment will be reflected as changes in their physiological processes and survival. Fishes possess shorter development time compared to mammalian species<sup>7</sup>. Fish are one of the most widely distributed organisms in an aquatic environment and being susceptible to environmental contamination may reflect the extent of the biological effects of environmental pollution in the waters. Monitoring of blood parameters, both cellular and non cellular may have considerable diagnostic value in assessing early warning signs of pesticide poisoning<sup>8</sup>. It is well known that water pollution is an issue beginning to receive more attention recently. Discharging into the rivers, discharging of sewage of fish growing fields are major sources of pollution.



**Control:** Normal architecture



**Treated:** Fragmented and striated muscle cells, accumulation of lipids and melanin in white muscles

**Figure 1: Histopathological examination of muscle. Fragmented and striated muscle cells, accumulation of lipids and melanin in white muscles were observed after CDC exposure.**



**Control:** Normal architecture



**Treated:** Fibrosis, lipid accumulation, hypertrophy, degeneration of blood vessels and hepatocytes, aggregation of myeloid cells, cytoplasmic vacuolization

**Figure 2: Histopathological examination of liver. Fibrosis, lipid accumulation, hypertrophy, degeneration of blood vessels and hepatocytes, aggregation of myeloid cells, cytoplasmic vacuolization were observed after CDC exposure.**



**Control:** Normal architecture



**Treated:** Aggregation of cardiac cells, hypertrophy in arteries

**Figure 3: Histopathological examination of heart. Aggregation of cardiac cells, hypertrophy in arteries was observed after CDC exposure.**



**Control:** Normal architecture

**Treated:** Secondary lamellae fusion, distortion of secondary lamellae erosion and disintegration of blood corpules, hyperplasia of epithelium

**Figure 4: Histopathological examination of gills. Secondary lamellae fusion, distortion of secondary lamellae erosion and disintegration of blood corpules, hyperplasia of epithelium were observed after CDC exposure.**

Contamination of the aquatic environment by heavy metals whether as a consequence of acute and chronic events constitutes additional source of stress for aquatic organisms. Sublethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of aquatic organisms. Omoregie et al<sup>9</sup> reported that toxicants and pollutants have significant effects, which can result in several physiological dysfunctions in fish. Dysfunction in the fish induces changes in blood parameters possible as a result of blood water content.

The amplified activities of AST and ALT in liver and muscle of *C. gariepinus* due to arsenic publicity indicating enhanced tissue proteolysis. For this reason the total protein content recorded reduced level. The tissue proteolysis was previously reported for different fish species subjected to pollution<sup>10,11,12,13</sup>. This could probably enlighten the use of protein as an alternative source of energy due to high energy demand that induced by pollutants<sup>13</sup>. Similarly, Datta et al<sup>14</sup> reported that exposure of *Clarias batrachus* to arsenic induces diminution of total hepatocyte protein content. Blood proteins were found to decrease with Colgate dental cream (Strong teeth) exposure in the present study. This could be attributed to renal excretion or impaired protein synthesis or due to a liver disorder<sup>15</sup>. On the other hand, the observed decrease of plasma protein could also result from the breakdown of protein into amino acids first and possibly into nitrogen and other elementary molecules. A similar reduction in protein has also been reported in *Saccobranhus* fossils following exposure to chlordane<sup>16</sup>. *Cyprinus Carpio* were exposed to two non-essential (Hg and Pb) and two essential (Cu and Ni) heavy metal salts at lethal and sub-lethal concentrations. Serum protein and globulin level showed an initial sharp increase from 2 to 20 h, followed by a decline that extend over a period of 72 h. Serum albumin showed an initial immediate decline from 2 to 4 h, followed by an intermittent period of recovery and decline that extend over a period of 72 h<sup>17</sup>.

A reduction of blood glucose was observed in this study. It was found to be significant at low concentrations of Colgate dental cream. Changes in carbohydrate metabolism occur in fish exposed to various sublethal concentrations of pollutants. Blood glucose has been employed as an indicator to environmental stress<sup>18</sup>. The increase in blood glucose is usually correlated with

the mobilization of glycogen and development of a status of hyperglycaemia. The hyperglycaemia response varies with the nutritional status of the fish<sup>19</sup>. It is not known whether zinc exposure affects glucose reserve directly or indirectly via other internal factors. The most likely source of glucose loss is through the kidneys, which could indicate a suppression of energy dependent glucose retention in kidney tubules. Decreased glucose absorption has been reported in *Pontius conchnius* exposed to mercury nitrate<sup>20</sup> and in *C. Isheriensis* (Sydenham) exposed to waterborne lead<sup>15</sup>. Similarly, hematological changes were resulted from the exposure of a freshwater fish, *Heteroclarias* sp. to sublethal concentrations (5.0 and 10.0 mg L<sup>-1</sup>) of zinc in water for a period of fifteen (15) days. Plasma levels of protein and glucose were also lower in the exposed fish when compared to the control<sup>5</sup>.

Changes to urea content are the consequence of metabolic changes in fish cells brought about by Colgate dental cream. Therefore the increase in protein synthesis and cellular increase in transamination activity contributed to a tissue overload of ammonia that required elimination through urea formation as part of the tissue function. Freshwater fish, *Oreochromis niloticus*, were exposed to low levels (0.05 mg/L) of metals (silver [Ag], cadmium [Cd], copper [Cu], chromium [Cr], zinc [Zn]) to investigate responses of serum biochemical parameters over different exposure periods (0, 5, 10, 20, 30 d). Total protein, triglyceride concentrations decreased in Cu-exposed ones. The concentration of blood urea nitrogen decreased in Cr-exposed one (Oner *et al.* 2008).

## CONCLUSION

Our results concluded that Colgate dental cream causes pollution in water bodies is very harmful to fish because of toxic pollutants present in the toothpaste, polluted water reduced survival, blood parameter levels in catfish. Pesticides from fields also polluted rivers and lakes and this polluted water is harmful for fishes to survive and reproduce. Colgate toothpaste containing harmful chemicals such as calcium carbonate, sorbitol, sodium lauryl sulphate, silica, titanium dioxide, sodium silicate, carrageenan, sodium monofluorophosphate, sodium bicarbonate, sodium saccharin, triclosan. The partial or combined effect of these chemicals altered hematological, biochemical and structural architecture of catfish

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