



## TOXICOLOGICAL EFFECTS OF DETERGENT ON HISTOLOGICAL AND BIOCHEMICAL PARAMETERS OF GILLS OF *CYPRINUS CARPIO*

### Toxicology

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### ABSTRACT

Detergents are widely used in both the industrial and domestic premises in the form of soaps and washing powder or liquids to wash various articles. Afterwash of the detergents are either drained directly into the aquatic environment or they find their way into it by natural sewage. It causes a drop in the water oxygen level causing oxidative stress and also get accumulated in the tissues and different organs of the aquatic organisms leading to various histopathological alterations in the normal fish physiology. The present investigation has been performed to see the effects of detergent Surf excel Quick wash on the gills of *Cyprinus carpio*. The fish were divided into 4 groups containing 9 fish in each group. Group-1 fish were designated as control. Group 2, 3 and 4 fishes were treated with 0.0075g/l, 0.0125g/l and 0.0175g/l concentrations of detergent respectively for 15 days. Various histological and biochemical changes were observed in the gills after exposure to different detergent concentrations.

### KEYWORDS

*Cyprinus carpio*, detergent, oxidative stress, gills.

### 1. INTRODUCTION

Major part of water available on Earth is marine and cannot be used directly by humans. Only fresh water that can be used for drinking purposes arises from ground water. Quality of drinking water is deteriorating continuously due to contamination caused by human wastes, chemical pollutants from industries and agricultural inputs. Most of the health problems reported nowadays are directly or indirectly related to the polluted water. Detergents are used for cleaning purposes and are obtained from synthetic organic chemicals. The ability of detergent to form foam when they are used with acid or hard water and low economic cost makes it preferable over soaps (Okpokwasili and Nwabuzor, 1988).

Detergents are used on large scale and due to improper sewage management, they impart toxic effects on aquatic animals such as fishes and microbes (Feisthauer *et al.*, 2004). Detergents are alkaline in nature and this alkalinity is responsible for its effectiveness as cleaning agent. Being non-biodegradable, detergents are expected to have same alkalinity after a long period of time when they are added to a water body (Mer *et al.*, 2018). Detergents are widely used in both industrial and domestic premises as soaps and detergents for washing purposes (Jawahar *et al.*, 2015). Most of the fish dies when concentration of detergent in water reaches 15ppm and 5ppm concentration of detergent is enough to kill fish eggs (Zimring and Rathje, 2012).

Gills are filamentous, thread like proteinaceous structures performing transfer of ions and water as well as gaseous exchange (Hoar and Randall, 1984). Fish can easily uptake the molecules of detergent via epithelial lining of gills and they get circulated to different parts of body (Chandanshive, 2015).

### 2. MATERIALS AND METHODS

Present study was conducted on gills of common carp *i.e.* *Cyprinus carpio*, which were collected from fish farm Jagatkhana, Nalagarh, District Solan, Himachal Pradesh. The experimental setup was done in fish farm Jagatkhana for 15 days per detergent concentration. All the fish were kept in water tank of approximately 2000L capacity under natural conditions and was free of any other pollutants. The detergent was purchased from local market of Shimla under trade name 'Surf Excel Quick Wash'. Required quantity of detergent was weighed and dissolved directly in tank in which fish were kept. All experimental procedures were conducted after the approval of Department of Fisheries, Himachal Pradesh, India.

#### 2.1 Grouping Of Animals

Experiment was conducted in still water.

The fish were divided into 4 groups containing 9 fish in each group –

1. Group-1 fish were designated as control.
2. Group-2 fish were treated with 0.0075g/l concentration of detergent for 15 days.
3. Group-3 fish were treated with 0.0125g/l concentration of

detergent for 15 days.

4. Group-4 fish were treated with 0.0175g/l concentration of detergent for 15 days.

The fish of each group were dissected after 5, 10, 15 days for each concentration. The gills were dissected from normal and treated fish and further procedures were carried out.

#### 2.2 Histological Study

Gills of the fish were excised immediately after sacrificing the fish. After dissection, tissues were immersed in normal saline solution. Tissues were fixed in Bouin's fixative for 24 hours and then washed in running tap water until the entire yellow color disappeared. Tissues were dehydrated serially in different grades of alcohol (30%, 50%, 70%, 90%, 100%) and cleared in xylene. Tissues were then embedded in paraffin wax (58-60°C). Sections of about 5-6µm thickness were cut on the rotary microtome and subjected to hematoxylin-eosin staining.

#### 2.3 Biochemical Study

Protein content was estimated as per the method of Lowry *et al.*, (1951). Superoxide dismutase (SOD) activity was determined by the method of Mishra and Fridovich, (1972). Catalase (CAT) assay was done as per method of Aebi, (1984) and lipid peroxidation was estimated according to the method of Dhindsa *et al.*, (1981).

#### Statistical Analysis

Data was presented as mean ± SEM. Comparison amongst groups was performed using one-way ANOVA test. Statistical significance was set at P\*\*<0.01, P\*<0.05.

### 3. RESULTS

#### 3.1 Effects of detergent on histology of gills

##### Normal gills

Microscopic examination of normal gills after hematoxylin eosin staining revealed the presence of primary and secondary gill lamellae, gill filaments, gill rakers with gill raker epithelium. Pillar cells with some blood spaces, gill arches and mucous cells were noticed. The secondary lamellae also termed as respiratory lamellae, were highly vascularized and covered with a thin layer of epithelial cells. Each secondary lamella had a central vascular layer composed of pillar cells covered by basement membrane and an outer epithelial layer. (Fig. A).

##### 0.0075g/l detergent treated gills

Carp gills showed same blood spaces as that of control. Elongation of primary gill lamellae were observed at some places. Epithelium was normal as that of control. Hyperplasia at certain places was also seen (Fig. B).

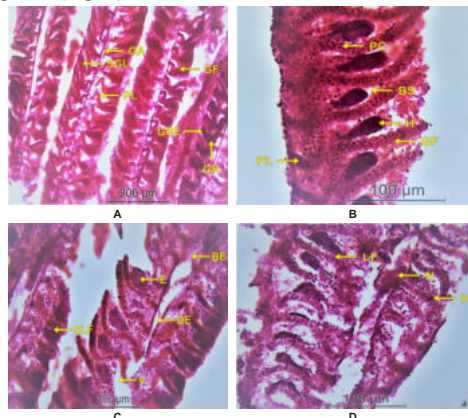
##### 0.0125g/l detergent treated gills

Major changes in gills including hyperplasia, fusion of the secondary lamellae, epithelial lifting and swelling of the capillaries was noticed after 15 days of detergent administration. Disorganization and rupture

of the secondary lamellae, bulging and degeneration of epithelium and vacuolization was also identified (Fig. C).

#### 0.0175g/l detergent treated gills

Hemorrhage and loss of lamellae were seen in gills after 15 days. Various blood vessels frequently packed with red blood cells especially near the gill filaments were noticed. Hypertrophy of the mucous pavement cells, hyperplasia, lamellar fusion and necrosis was also observed. Total curling of secondary gill lamellae was seen at some places (Fig. D).



**Fig. A:** *Cyprinus carpio* gills from the control group revealing gill filaments (GF), gill arches (GA), primary lamellae (PL), secondary gill lamella (SGL), gill rakers (GR) and gill raker epithelium (GRE). **Fig. B:** Section of gills treated with 0.0075g/l concentration of detergent for 15 days showing gill filaments (GF) along with blood spaces (BS), pillar cells (PC), elongated primary gill lamellae (PL), and hyperplasia (H). **Fig. C:** Gills exposed to 0.0125g/l concentration of detergent for 15 days showing edema (E), bulging of epithelium (BE), fusion of secondary lamellae (SLF), degenerated epithelium (DE) and vacuolization (V). **Fig. D:** Gills exposed to 0.0175g/l concentration of detergent for 15 days showing necrosis (N), lamellar fusion (LF) and hyperplasia (H).

#### 3.2 Effects of detergent on biochemical parameters

Protein level in normal gills of *Cyprinus carpio* was 59.91±0.82 mg/g after 15 days. The protein content for 0.0075g/l, 0.0125g/l and 0.0175g/l concentrations of detergent was observed 49.13±0.48, 37.20±0.52 and 26.22±1.11 mg/g.

MDA production was 6.49±0.06 n moles of TBARS formed/g of fresh tissue weight for control gills of *Cyprinus carpio*. An increase in peroxidation level was observed in comparison to control. It was found to be 6.93±0.01, 7.07±0.01 and 7.27±0.02 n moles of TBARS formed/g of fresh tissue weight for 0.0075g/l, 0.0125g/l and 0.0175g/l concentrations of detergent respectively.

The SOD specific activity for control fish was 2.83±0.02 units/mg protein/minute. The SOD activity increased with increase in the concentration of detergent. It was measured 3.13±0.02, 3.50±0.02 and 3.63±0.02 units/mg protein/minute for 0.0075g/l, 0.0125g/l and 0.0175g/l concentrations of detergent.

Catalase specific activity for control group fish was 2.97±0.02 unit/mg protein/minute. The enzyme activity increased to 3.28±0.01, 3.39±0.01 and 3.56±0.01 units/mg protein/minute for 0.0075g/l, 0.0125g/l and 0.0175g/l concentrations of detergent respectively.

**Table 1: Changes in protein, lipid content, SOD and CAT specific activity in normal and detergent treated gills of *Cyprinus carpio* for 15 days in different concentrations of detergent.**

Groups	Protein content (mg/g)	MDA content (n moles of TBARS formed/g of fresh tissue weight)	SOD activity (U/mg protein /min)	CAT activity (U/mg protein /min)
Control	59.91±0.82	6.49±0.06	2.83±0.02	2.97±0.02
0.0075g/l	49.13±0.48	6.93±0.01**	3.13±0.02	3.28±0.01**
0.0125g/l	37.20±0.52**	7.07±0.01**	3.50±0.02*	3.39±0.01
0.0175g/l	26.22±1.11**	7.27±0.02	3.63±0.02**	3.56±0.01**

#### 4. DISCUSSION

Detergents create tissue damage and biochemical alterations in the organs of organisms due to their potential toxicity. So, the organisms can respond to it by developing various methods including alterations in the antioxidant status to counteract the stress (Jawahar *et al.*, 2015). The first indication of stress is changes in biochemical contents in the body of an organism. At the time of stress an organism requires adequate amount of energy that can be supplied from reserve biochemical materials (Chandanshive, 2015).

Fish gills are the primary target for toxicants that are dissolved in water (Ogundiran *et al.*, 2010). Various histopathological alterations were observed in gills of *Cyprinus carpio* after treatment with varying concentrations of detergent. Changes observed were curling of secondary lamellae, aneurism, epithelial lifting, hyperplasia, hypertrophy, swelling of gill lamellae with vacuolation and congestion and loss of structural integrity of secondary lamellae. According to Vasanthi and Binukumari (2016), fish exposed to Tide for 10 days exposure period, exhibited marked histopathological changes, such as curling of secondary lamellae. After 20 days exposure, degenerated secondary lamellae were noted. The severity of the damage become more noticeable after 30 days of exposure leading to collapsed secondary lamellae. Swollen gills and fused lamellae, gill bridging, epithelial lifting, hyperplasia, granuloma formation, necrosis and smooth muscle hypertrophy after exposure were observed (Mahjabeen *et al.*, 2016).

Present investigation indicates that protein concentration is affected by detergent Surf Excel. Protein content was decreased with increase in concentration of detergent from 0.0075g/l to 0.0175g/l in gills of fish. This is due to the pollution stress force to the fishes to mobilize protein from muscle to blood, to reimburse to certain acidosis caused by the lactate accumulation (Palanichamy *et al.*, 1995). Proteins being essential substances needed for growth and development of body act as energy sources during stress conditions (Muthukumaravel *et al.*, 2013).

Increase in lipid content was observed during present study in gills of *Cyprinus carpio* after treatment with increasing detergent concentrations. Increase in LPO level has been used as a marker of oxidative damage in organisms (Sayeed *et al.*, 2003). Uner *et al.*, (2005) also reported an increase in tissue MDA content in fishes (*Oreochromis nitoticus*) exposed to Etoxasole.

During present study, increase in SOD and CAT activities were observed. Highest SOD and CAT activity in liver of *Acipenser naccarii* and *Oncorhynchus mykiss* suggest that both enzymes play important role in fighting the generation of superoxide radicals and hydrogen peroxide (Trenzado *et al.*, 2006). There were significantly ( $p < 0.05$ ) higher levels of SOD and LPO but lower CAT, GST and GSH levels in gill, liver and kidney of exposed fish compared to fish from reference reservoir (Mahjabeen *et al.*, 2016). Similar elevation in activity of SOD was also reported in *C. punctatus* following exposure to thermal power plant effluent (Javed *et al.*, 2016)

#### 5. CONCLUSION

Therefore, we can conclude that the detergents promote the utilization of proteins and lipids in fish to cope up the increased energy requirements of enhanced metabolic activity due to oxidative stress. Further studies at cellular level, genotoxicity, neural dysfunction, developmental disorders and morphological abnormalities in response to oxidative stress caused by detergent administration can give a better understanding on immune response of aquatic organisms. It is not possible to stop the use of cleaning products as they are a part of our day today lives. But we can adopt different techniques so that these household wastes do not directly enter the water resources. Thus, better sewage treatment methods should be adopted to overcome the issues related to water pollution and health of aquatic organisms.

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