Toxic Impact Of Detergent (Bright) On Growth, External Morphology, Behaviour, Liver Toxicology, Haematological Parameters And Histopathology Of Nile Tilapia (*Oreochromis Niloticus)*

Ayesha Sadiqa[1], Sara Parveen[2], Hafiza Fizzah Riaz[3], Ulfat Ayub[4], Asma Shaheen[1], Sajida Rasheed[1], Masooma Zahra[5], Yasir Nawaz[1*], Javaria Zafar[1], Muhammad Saleem Khan[1]

[1]Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, Pakistan
[2]Department of Zoology, University Of Education Lahore, Pakistan
[3]Department of Zoology, The Islamia University of Bahawalpur, Rahim Yar Khan Campus, Pakistan
[4]Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan
[5]Faculty of Biological Sciences, Superior University Lahore, Pakistan

[*]**Corresponding author:** Yasir Nawaz
royyasirnawaz@gmail.com

Abstract

Introduction: Nile tilapia is cichlid fish. Globally tilapia is second important culture fish after carp. Chemical pollution of aquatic environments increased, seriously harming fisheries and ecosystems alike. **Methods:** Three glass aquarium of 45.72 x 60.96 x 45.72 cm was used. One was control and others were treatment groups. Different concentration of detergent was used 0.2 g/l for low dose and 0.4 g/l for high dose treatment. Experiment was conducted for 28 days. Stock density was 6 fishes per aquarium. The blood samples were preserved in EDTA tubes for haematological analysis. **Results:** Detergents reduce the fish growth. The mean body weight in control was 28.3± 3.72g while in high and low dose was 23.23±5.35g, 18.08±3.99g. Fish body was worst affected by detergents as swelling on lips and gill lamellae. Further, damage of caudal fin, scars on anal fin and swelling in eye region were also observed. Haematological analysis revealed that WBCs, RBCs, PLT, HGB, MCH, HCT increased and MPV, PDW decreased. Analysis of liver toxicology profile showed that level of urea and ALT decrease while level of AST increased. Behaviour showed increase in foraging while decrease in aggressiveness by detergents impact. No shoaling movement was observed. Histopathological revealed gill and liver tissue damage. **Conclusion:** To conclude, the detergents decreased the fish growth. It also affects the external morphology and behavior. Surfactant has been able to influence of histological of gill and liver, where the damage becomes increase with increasing concentration.

Keywords: Nile tilapia, Hematology, Morphology, Behavior, Histopathology

Introduction

Nile tilapia (*Oreochromis niloticus*) is cichlid fish. Nile tilapia is native species from Africa to Egypt but also native to Israel. Globally tilapia is secondly most important culture fish after the carp (1). The nutritional and food security of underdeveloped nations are greatly influenced by fish and fisheries. Aquaculture is the controlled cultivation of organisms from freshwater, brackish water, and salt water. About 50% of food is produced by aquaculture, a fast expanding food-producing region (2).

Detergent is a substance that can be used for cleaning purpose. Surfactant contains both water soluble and in soluble part tail is lipid soluble and head is water soluble. On the basis of charge present on head there are four types of surfactant anionic, non-ionic, cationic and amphoteric. Today, most commonly used surfactant is anionic surfactant (Landeck et al., 2020). Some other harmful chemicals such as insecticides and pesticides, phenol are more easily absorbed by fish due to decrease in surface tension of water (3). After the exposure of detergents the fish show different type of behaviour and stress response such as erect movement, gasping for breathing and frequent surfacing, loss of relax (4). An investigation shows that the growth of fish decrease with increase the concentration of detergents. The actual weight gain is reduced. The consumption of feed also reduced (5, 6).

Detergents that are present in water suck by fish through the skin and gills which affect the lipid, protein and carbohydrates composition. the egg and larvae of fish are more susceptible for aquatic pollution (5). At high concentration the detergents destroy the egg of fish. The embryo whose gastrulation stage is incomplete is more susceptible to surfactant than the embryo with complete gastrulation period (7).The detergents reduce the surface tension of the egg membrane so, enter into the egg easily and disrupt the metabolism of the egg. Detergents inactivate the enzymatic system by inhibiting the egg respiration which leads to oxygen deficiency which is cause of egg damage. Detergents also deform the head, eye and axis development pattern .Detergents also have negative effect on adult fish which stop the formation of blood protein (7).

Haematology deals with the study of blood. Blood consists of 45% blood cells including RBC, WBC, platelets and 55 per cent blood plasma. Blood plasma is a light yellow-colour liquid component of blood in which blood cells are absent,

but contains proteins and other constituents of whole blood in suspension (8). The haematological parameters consist of content of haemoglobin, RBC, WBC, Hct, megakaryocytes. The number of red blood cells, white blood cells and haematocrit decline after the exposure of different types of pollutants (9). The toxic component present in the detergents not only effect the haematological parameters but also destroy the vital organ of the fish such as kinder, liver, gills, epidermis. The destruction of gill cells increase by increasing the concentration of surfactant. At the highest concentration of the surfactant the necrosis, ballooning dilation of lamellae, hyperplasia if lamellae and thickening of epithelium of gills lamellae are found. The ballooning dilation is key factor for the identification of gills damage. The gills are primary organ that are targeted by pollutants (9, 10). At different concentration of detergent the deformation of tissues in liver is present. Liver detoxify the certain harmful substances that enter in the body but its ability of detoxification is limited and depend upon fish species. The accumulation of these harmful substances damage the liver tissues and develop the lesion and start necrosis process some other structural changes also occur such as change the shape of central vein, cellular vacuolation (9). Due to disturbance of liver function the lipid and glycogen is accumulated. At low concentration of detergents the congestion tube of kidney effected but at high concentration the tubules disruption and leucocitary infiltration occur. The histological damage depend upon the time of exposure (11, 12). The purpose of this study was to evaluate the toxic effect of detergent on growth, external morphology, haematology, behaviour and histopathology of Nile tilapia fish captured from Pttoki fish farm.

Materials and Methods

Collection of samples

The Fishes were obtained from aquaculture ponds in Pattoki. The specimens were weight an average between 20- 26 g and measure 11 cm in length. The animals were kept in plastic bags with fresh water in which oxygen has permeated, ensuring that no death occur during transportation. The specimens were kept in iris water glass tanks with aerators and aquarium heaters to maintain oxygen and temperature levels. Fish were acclimatized for seven days in glass tanks before the trial begins. By using the electrical conductivity meter and pH meter water quality parameters was monitored.

Experimental Design

The animals were classed in a semi-stable 14 days after stabilization, in three experimental water glass tanks (6 fish each tank) with triangular dimensions of 45.72 x 60.96 x 45.72 cm. Commercial food was provided to the fish twice a day. Trial was conducted in three aquariums one is control group and other two are treatment group. In treatment groups one group was provided with 0.2g (low dose) dose of detergent and other was provided with 0.4g (high dose) dose of detergent. Every day before treatment, the water was changed. Every tank produced about 80% of water with animal waste and removed with the help of suction pump. In the glass water tanks, fresh water was added (13).

Chemicals

Bright (powder detergent) was used as experimental chemical. It was weighted accurately as per requirement and dissolved in water to introduce in aquarium. Bright washing powder was laundry detergent brand by Colgate Palmolive company formerly known as National Detergent Limited which is subsidiary of American multinational company Colgate Palmolive and Pakistani Lakson group. It was launched in 1977 and located in Karachi Pakistan.

The animals were taken one by one to a small water container at the conclusion of the 28 day. Blood samples were obtained from a farm vein in EDTA vials by use of BD syringes in order to evaluate genetics using a comet assay (13).

Behaviour Study

The fish behaviour was observed after the exposure of dose in treatment groups and control group on daily basis for ten minutes with the help of stop watch. Six behaviour patterns were observed such as schooling, shoaling, vertical and circular movement, foraging and aggressive behaviour.

Growth calculation

The growth of fish was measured through FCR value. The Fish Growth and Feed Conversion Rate (FCR) was used to calculate the growth of Nile tilapia fingerlings fed on agro-industrial feed. The Fish Growth and Feed Conversion Rate (FCR) is a significant method to calculate artificial feed acceptability.

$$\text{FCR} = \frac{\text{Ttoa dry feed take in (g)}}{\text{Wet weight gain in (g)}}$$

$$\text{RGR (relative growth rate)}\% = \frac{\text{Wf (final weight)} - \text{W1(initial weight)}}{\text{T(Time)}} \text{x } 100$$

Morphometric measurements

Body length of the fishes was measured one by one with the help of measuring tape before and after the trial both in control and treatment groups (14).

Haematological analysis

The samples of blood were used to study haemoglobin, red blood cells, white blood cells, Mean cell haemoglobin, platelet counting and for cell haemoglobin concentration. Due to poor solubility in water of clove oil, it will be dissolved

in ethanol firstly and then fingerlings from the tank will be aesthetics for five minutes with 60mg/L concentrations. Blood sample was collected from each sample through with posterior vein applying heparinized needle and after applying this protocol blood samples were transferred to the Molecular Lab for analysing. Capillary tubes was used to count RBCs and WBCs (15).

Histopathology

After the treatment of detergents for 28 days the tilapia were picked from control and treatment groups and dissected. After the dissection the gills, liver, kidney were removed and preserved in 10% formalin in a test tube. Samples were subjected to fix for about 48 hours before the tissue processing. Samples were washed in water to eliminate extra fixative. They were dehydrated through graded series of alcohol, processed into wax, sectioned with a rotary microtome, mounted on glass slides, de-waxed and stained with hematoxylin and eosin stain for microscopic examination at magnification 160X. Photomicrography of sections was mounted on glass slides and pictures taken with digital motic Ettah, Bassey (5).

Statistical Analysis

The data was subjected to statistical analysis using Minitab version 17. Tukey's honesty significance test, one way ANOVA was performed to check differences among means. P value 0.05 or less than 0.05 indicated that results were significant but higher than 0.05 the results are not significant.

Results

This study was planned to investigate the impact of detergents on haematology, growth, behaviour, histopathology and external morphology, liver toxicology of Nile tilapia *(Oreochromis niloticus)*.

Haematological Analysis

Mean of HGB in controlled group was 6.6±0.29 g/dl while in experimental group T1 was 8.2±0.29 g/dl and in T2 was 10.37±1.17 g/dl, while its P value was 0.0055. Mean of WBCs in controlled group was 51.07±0.82 10^3/ul, while in treatment group T1 was 84.7±3.57 10^3/ul and in T2 was 102±9.2 10^3/ul. The P value of WBCs was 0.0003. Average of LYM for control, T1, T2 was 97.53±1.74 %, 94.23±3.26% and 95.3±1.92% respectively with 0.4277 P value. Mean of MON in control was 0.8±0.08 % while for T1 was 1.4±0.37 % and for T2 was 3.23±1.39 %. P value for MON was 0.0608. Mean of GRA for control was 0.8±0.08 % while for T1 was 0.67±0.21 % and T2 was 0.8±0.37 %. The P value of GRA was 0.8331.

Mean of RBC for control was 2.04±0.02 10^6/ul while for T1 2.28±0.38 10^6/ul and T2 was 2.86±0.33 10^6/ul. P value of RBC was 0.0700. Average of HCT for control, T1 and T2 were 28.97±1.19 %, 32.33±2.64 % and 45.73±5.56 % respectively while P value for HCT was 0.0080. Mean of MCV for control was 142.37±2.05 um^3 while for T1 was 155.67±13.02 um^3 and T2 was166.8±3.13 um^3. P value of MCV was 0.0549. Mean value of MCH was 31.97±1.32 % for control group and 35.97±3.35 % was for T1 and 36.5±0.91 % was for T2.

While P value for MCH was 0.1482. Mean of MCHC was 22.63±0.21 g/dl for control while for T1 was 21.8±1.58 g/dl and for T2 was 22.1±0.22 g/dl. P value was 0.6779 for MCHC. Mean of RDW for control was 9.47±0.45 % while for T1 was 20.33±0.58 % and T2 was 13.37±0.62 %. P value of RDW was 0.0001. Average of RDW-SD was 74.87±1.2 um^3 for control while for T1 and T2 were 229.07±6.42 um^3, 113.17±13.97 um^3. P value of RDW-SD was 0.0001. Mean of PLT was 127.37±1.65 10^3/ul for control while for T1 was 196.33±4.26 10^3/ul and T2 was206.27±36.01 10^3/ul. P value was 0.0182 for PLT. Mean of MPV for control was 7.47±0.37 um^3 while for T1 was 6.17±0.31 um^3 and T2 was 6.9±0.14 um^3. P value for MPV was 0.0119. Mean of PCT for control was 0.1±0 % while for T1 was 0.19±0.07 % and T2 was 0.14±0.03 %. P value for PCT was 0.2030. Similarly, average of PDW for control, T1 and T2 were 10.23±0.25 %, 7.33±0.33 % and 9.33±0.29 % respectively while P value of PDW was 0.0002. This is shown in table 1.

Table 1: The hematological profile of control, T1 and T2 groups due to detergent (Bright)

Parameters	Control	Low dose	High dose	F value	P value
	Mean±SD	Mean±SD	Mean±SD		
HGB (HB%)	6.6±0.29	8.2±0.29	10.37±1.17	13.96	0.0055**
WBC(TLC)	51.07±0.82	84.7±3.57	102±9.2	41.06	0.0003***
LYM%	97.53±1.74	94.23±3.26	95.3±1.92	0.9818	0.4277
MON%	0.8±0.08	1.4±0.37	3.23±1.39	4.631	0.0608
GRA%	0.8±0.08	0.67±0.21	0.8±0.37	0.1882	0.8331
RBC	2.04±0.02	2.28±0.38	2.86±0.33	4.28	0.07
HCT	28.97±1.19	32.33±2.64	45.73±5.56	12	0.0080**
MCV	142.37±2.05	155.67±13.02	166.8±3.13	4.891	0.0549
MCH	31.97±1.32	35.97±3.35	36.5±0.91	2.668	0.1482
MCHC	22.63±0.21	21.8±1.58	22.1±0.22	0.415	0.6779

RDW	9.47±0.45	20.33±0.58	13.37±0.62	196.2	0.0001****
RDW-SD	74.87±1.2	229.07±6.42	113.17±13.97	162.6	0.0001****
PLT	127.37±1.65	196.33±4.26	206.27±36.01	8.41	0.0182*
MPV	7.47±0.37	6.17±0.31	6.9±0.14	10.15	0.0119*
PCT	0.1±0	0.19±0.07	0.14±0.03	2.104	0.203
PDW	10.23±0.25	7.33±0.33	9.33±0.29	52.18	0.0002***

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01); T1 = 0.2 g/l, T2 = 0.4 g/l

Effects of detergent (Bright) on Liver Toxicology

Mean of urea in controlled group was 11 while in experimental group T1 was 9.67 and in T2 group was 10. Mean of Crt in control group and T2 group was 0 while in T1 group was 0.23. Mean value of ALT in control group was 43 while in T1 group was 11.47 and in T2 group was 72. Mean value of AST in control group was 261 while in T1 group was 219 and in T2 group was 315. Mean value of SR in control group was 86 while in T1 group was 14.33 and in T2 group was 25. This is shown in table 2.

Table 2: Comparison of toxicological profile in control, T1 and T2 group

Parameters	**Control**	**Low dose**	**High dose**	**F value**	**P value**
	Mean± SD	**Mean± SD**	**Mean± SD**		
Urea	11±2	9.67±1.25	10±1	0.3043	0.7484
Crt	0±0	0.23±0.05	0±0	2.714	0.1447
ALT	43±4	11.47±2.86	72±6	85.05	0.0001****
AST	261±97	219.33±8.99	315±39	1.244	0.5331
SR	86±3	14.33±2.49	25±6	181	0.0001****

NS= Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01); T1 = 0.2 g/l, T2 = 0.4 g/l

Comparison of different toxicological parameters in different groups

Level of urea in control group and T2 (0.4 g/l) group was more as compared to T1 (0.2 g/l) while level of Crt in control group and T2 (0.4 g/l) group was less than T1 (0.2 g/l) group, level of ALT in control group and T1 (0.2 g/l) was less than T2 (0.2 g/l) group, level of AST in control group and T1 (0.2 g/l) was less than T2 (0.4 g/l) and level of SR in control group and T2 (0.4 g/l) was more than T1 (0.2 g/l) group.

Effects of detergent (Bright) on Tilapia's behaviour (Feeding & Movement)

During observation, the foraging behavior of fish in T1 (0.2 g/l) and T2 (0.4 g/l) group was decrease as compared to control group with P value 0.0001. The vertical movement of fish in T2 (0.4 g/l) was increased than T1 (0.2 g/l) and control group with P value 0.0989. The schooling movement of fish was increased in T2 (0.4 g/l) group as compared to control and T1 (0.2 g/l) group with P value 0.2310. Circular movement of fish was increased in T1 (0.2 g/l) group as compared to T2 (0.4 g/l) and control group with P value 0.9443. The aggressive behavior of fish was increased in T2 (0.4 g/l) group as compared to T1 (0.2 g/l) and control group with P value 0.0001. No schooling behavior was observed in control, T1 (0.2 g/l) and T2 (0.4 g/l) groups. This can be seen in table 3.

Table 3: Behaviour patterns of Nile tilapia in different groups

Observation	**Control**	**Low dose**	**High dose**	**F value**	**P value**
	Mean± SD	**Mean± SD**	**Mean± SD**		
Foraging	50.73±26.05	10.8±8.2	14.2±8.22	25.16	0.0001****
Vertical	5.07±2.17	4.13±2.78	6.4±3.04	2.446	0.0989
Schooling	0.8±0.65	0.73±0.68	1.13±0.6	1.517	0.231
Circular	0.33±0.6	0.4±0.61	0.33±0.58	0.05738	0.9443
Aggressive	5.33±3.28	14.53±6.28	18.13±8.38	9.771	0.0001****

NS= Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01); T1 = 0.2 g/l, T2 = 0.4 g/l

Effect of detergent on growth of fish

The mean and SD value of body weight in control group was 28.3± 3.72 g with P value 0.0001. The mean and SD value of body length in control group was 12.33±0.57 cm with P value 0.0001. The mean and SD value of body weight in treatment 1 (0.2 g/l) group was 18.08±3.99 g with P value 0.0002. The mean and SD value of body length in T1 was 12.13±1.22 cm with P value 0.0001.The mean and SD value of body weight in treatment 2 (0.4 g/l) was 23.23±5.35 g with P value with P value 0.0002. The mean and SD value of body length was 12.57±0.77 cm with P value 0.0001. This is indicated in table 4.

Table 4: Measurement of body length and weight of fish in different groups

Parameters	Body weight (g)	Body length (cm)	Parameters	Body weight (g)	Body length (cm)	Parameters	Body weight (g)	Body length (cm)
Control			**Treatment 1 group (Low dose)**			**Treatment 2 group (High dose)**		
Mean	28.28	12.33	**Mean**	18.08	12.13	**Mean**	23.23	12.6
SD	3.72	0.57	**SD**	3.99	1.22	**SD**	5.35	0.77
Mean± SD	28.3± 3.72	12.33±0 .57	**Mean± SD**	18.08±3.99	12.13±1 .22	**Mean± SD**	23.23±5.35	12.57±0.7 7
T Value	17	48.58	**T Value**	10.14	22.19	**T Value**	9.71	36.51
P Value	0.0001* ***	0.0001* ***	**P Value**	0.0002***	0.0001* ***	**P Value**	0.0002***	0.0001** **

NS= Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01); T1 = 0.2 g/l, T2 = 0.4 g/l

Body weight and length relationship

The body weight of all the sample fishes in T1 (18.08±3.99 g) and (T2 23.23±5.35 g) was measured and compared with control group (28.3± 3.72 g) and a significant difference was observed. Similarly the body length in T1 (12.13±1.22 cm) and T2 (12.57±0.77 cm) was measured and compared with control group (12.33±0.57 cm). Tilapia body weight and length was measured after the treatment of detergent (Bright) and observed that detergent cause the loss of body weight and slightly increase in body length. This is shown in table 5.

Table 5: Relationship of body length and weight of fish in different groups

Parameters	Control	Low	High	F value	P value
	Mean± SD	**Mean± SD**	**Mean± SD**		
Body weight (g)	28.3± 3.72	18.08±3.99	23.23±5.35	6.676	0.0084**
Body length(cm)	12.33±0.57	12.13±1.22	12.57±0.77	0.2928	0.7504

NS= Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01); T1 = 0.2 g/l, T2 = 0.4 g/l

Effects of detergent on external morphology of fish

The external morphology of controlled group were observed normal and compared with experimental group T1 (Low dose) and T2 (High dose). The body of the fish was affected significantly by the action of detergent in both experimental groups. The swelling on the lips and gills lamellae were observed which is exposed to 0.2 g/l and 0.4 g/l concentration of detergent (Bright). Some other morphological parameters are also observed such as destruction of caudal fins, swelling on the eyes and scares on the anal fins in treatment groups. This is shown in figure 1.

Figure 1: Destruction of cadual fins (Fig A), damaging of skin (Fig B), swelling on lips (Fig C) and scare on anal fins (Fig D) by impact of detergent (Bright) on Nile tilapia *(Oreochromis noctilous)*

Histopathological Studies

Histology of gill and liver tissues of Nile tilapia culture at different detergent (bright) concentration were also studied.

Gills

Control group gills were observed normal and were compared with low (0.2 g/l) and high (0.4 g/l) concentration treated tilapia's gills. Ballooning dilatation of gills lamellae, inter lamellar hyperplasia, fusion of primary and secondary lamellae, damage of gills lamellae, congestion of blood spaces and slightly epithelium lifting was observed. This can be seen in figure 2.

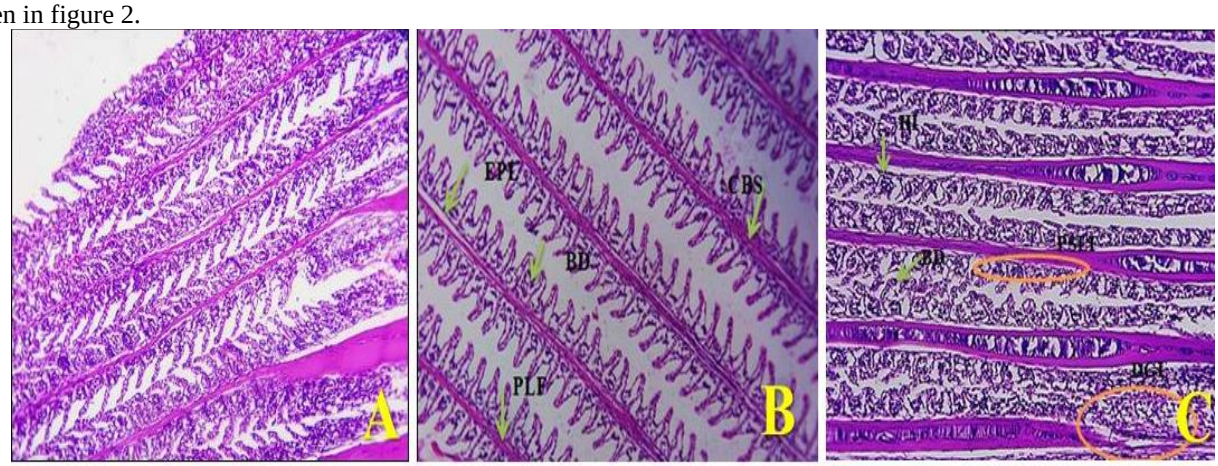

Figure 2: Gills was observed normal in control group (Fig A), Epithelium lifting (EPL), congestion of blood space (CBS), primary lamellar fusion (PLF) and ballooning dilatation (BD) in low dose treatment (Fig B). Inter lamellar hyperplasia (HI), destruction of gills lamellae (DGL) and ballooning dilatation (BD) in high dose treatment (Fig C).

Liver

The liver was observed normal and compare with low and high dose treatment groups. Formation of lipid vacoule, blood lipid vacoule, peripheral nucleus in heptocyes, damage of cytoplasm and necrosis in heptocytes was observed. This can be seen in figure 3.

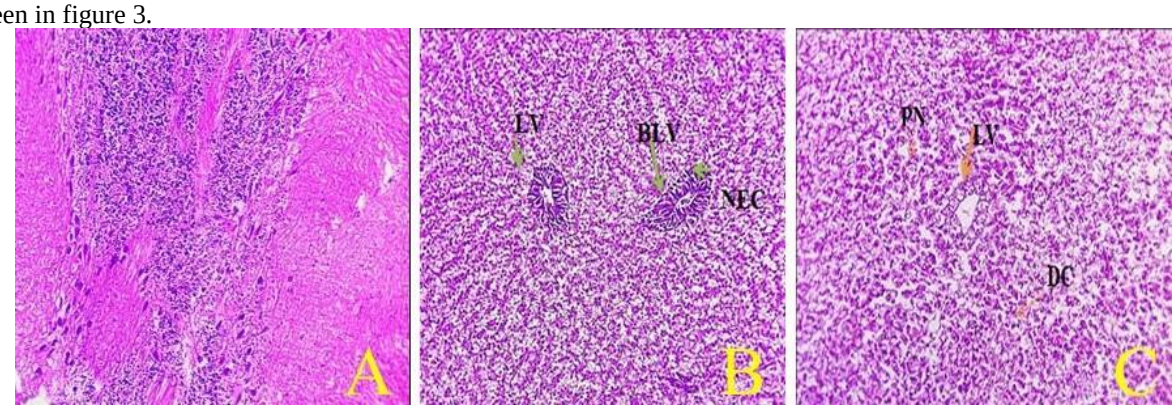

Figure 31: Light micrograph (160X) hepatocytes of Nile tilapia *(Oreochromis noctilous)* observed in the control group (Fig A) had homogeneous cytoplasm and central nucleus, Lipid vacuole (LV), blood lipid vacuole (BLV) and necrosis (NEC) in hepatocytes in low dose treatment (Fig B), Peripheral nucleus (PN) in hepatocytes, destruction of cytoplasm (DC) in high dose treatment (Fig C).

Discussion

The study aimed to determine the effect of detergent (Bright) on growth, histopathology, haematology, external morphology and behavior of Nile tilapia *(Oreochromis niloticus)*.

White blood cells have a significant impact on the immune system. Changes in the number of white blood cells caused by toxicant exposure or stressful conditions (16). Significant elevation in WBCs count was observed by increasing the concentration of detergent. Highest value was observed in high dose (0.4 g/l) 102±9.210^3/ul followed by low dose (0.2 g/l) 84.7±3.5710^3/ul and control group 51.07±0.8210^3/ul detergent induces the defence mechanism of fish in response to stress of pollutants. These results in line with the studied performed on Nile tilapia exposed to linear alkyl benzene sulphonate (16), *Cichlasoma dimerus* exposed to sublethal concentrations of 4-tert-Octylphenol (10). and *Labeo rohita* methyl orange dye solution treated (17).

Blood platelets play a role in both general defence systems and blood coagulation. The formation of thrombocytes is typically inhibited when fish are exposed to various types of water contaminants (18). The numbers of PLT increase in high dose (0.4 g/l) treatment group 206.27±36.0110^3/ul followed by low dose (0.2 g/l) treatment group 196.33±4.2610^3/ul and control group 127.37±1.6510^3/ul. Current study investigate detergent (Bright) induce infection in fish due to infection the number of PLT count increase in blood. These results are not in line with the studied performed on Nile tilapia exposed to linear alkyl benzene sulphonate (16).

Red blood cells are the blood components that are most frequently produced. The fish body uses a range of physiological methods of compensation to try to maintain the red blood cell count within the limits of specific physiological requirements. Red blood cell count is a very stable indication Pechiammal and Vasanthi (19). The number of RBCs increase in high dose (0.4 g/l) treatment group 2.86±0.3310^6/ul followed by low dose (0.2 g/l) treatment group 2.28±0.3810^6/ul and control group 2.04±0.0210^6/ul. Body may increase red blood cell production to compensate for any condition those results in low oxygen levels. Detergent reduces the level of dissolved oxygen in the water due to eutrophication. These results disagree with the studied performed on *Labeo rohita* (19), Nile tilapia *(Oreochromis niloticus)* exposed to linear alkyl benzene sulphonate (16).

Increased HCB level was observed by increasing the concentration of detergent(Bright) as the highest level of HCB (10.37±1.17 g/dl) was observed in high dose (0.4 g/l) followed by low dose (0.2 g/l) (8.2±0.29 g/dl) and control group (6.6±0.29 g/dl). Due to increase in number of RBCs the level of HCB also increases. These results was not in line with the studied performed on common carp *(Cyprinus carpio)* exposed to nonylphenol and ethinylestradiol (20), *Labeo rohita* (19).

Statically significant increased HCT level was observed by increasing the concentration of detergent (Bright) as the high level of HCT 45.73±5.56% was observed in high dose (0.4 g/l) treatment group followed by low dose (0.2 g/l) 32.33±2.64% and control group 28.97±1.19%. Due to low level of oxygen in blood and failure of heart functions the number of HCT increase in blood. These results disagreed with the study performed on rainbow trout (*Oncorhynchus mykiss*) (21).

PDW acts as a marker of platelet size heterogeneity and assesses variance in platelet volumes. Additionally, it is a symptom of platelet anisocytosis, which takes place concurrently with platelet activation and leads to the development of pseudopods that increase platelet diameter and apparent volume (22). Statically significant decreased in PDW value was observed in low dose (0.2 g/l) 7.33±0.33% and high dose (0.4 g/l) 9.33±0.29% treatment group followed by control group 10.23±0.25%. The low PDW could be caused by megakaryocytes in the bone marrow malfunctioning. Within this knowledge no research work was conducted previously on this parameter.

Red blood cell distribution width (RDW) is a popular tool for the differential diagnosis of micro- and normocytic anaemia's since it is a reliable predictor of anisocytosis. Erythrocyte size dispersion is reflected by RDW (23). Statically significant increased RDW value was observed in low dose (0.2 g/l) treatment group 20.33±0.58% followed by high dose (0.4 g/l) 13.37±0.62% and control group 9.47±0.45%. A high RDW indicates that smallest and largest red blood cells are significantly different in size. Increase in RDW value was resulted due to deficiency of certain nutrients such as iron, folate and vitamin B12. Within this knowledge no research work was conducted previously on this parameter.

In toxicological research, the naturally occurring enzymes alanine transaminase (ALT) and aspartate transaminase (AST), which are found in the liver and other organs, are particularly useful markers. They are classed as tissue-specific or serum non-functional enzyme and they play a significant part in the metabolism of proteins and amino acids in a variety of human organs. As a result, information and evidence of tissue or organ malfunction may be provided by their increased levels in fish serum (16). Statistically significant increased in value of AST, ALT was observed in high dose (0.4 g/l) treatment group 315±39, 72±6 as followed by low dose (0.2 g/l) 219.33±8.99, 11.47±2.86 and control group 261±97, 43±4. The current study looks into how damaged cells leak enzymes into the extracellular fluid, increasing membrane permeability, and how damaged liver cells produce more of these enzymes. These results are in line with the studied performed on Nile tilapia *(Oreochromis niloticus)* exposed to linear alkyl benzene sulphonate (16), *Clarias gariepinus* exposed to commercial detergent (Ariel) (24).

Animal behaviour is quite changeable and reflects the activities of its existence. It reacts swiftly to minute environmental changes that might not even approach the threshold for the various systems and functions, sensitivity level, and level of sensitivity. Because of this, behavioural markers of the environment's and animals' health are thought to be very sensitive (25).

Foraging behaviour is quite fascinating. This complex type of fish behaviour is made up of a sequence of sequential actions, beginning with the hunt and discovery of the prey and continuing with the prey's grabbing, treatment inside the mouth, assessment of its fitness as food, and decision-making before swallowing or rejecting. A complex, multimodal, and unstable ethological framework underlies foraging behaviour. It is highly vulnerable to chemical pollutants (25). Statistically significant decrease in foraging frequency was observed in low dose (0.2 g/l) 10.8±8.2 as followed by high dose (0.4 g/l) 14.2±8.22 and control group 50.73±26.05. Current study represented that detergent (Bright) decrease the foraging behavior of fishes. Certain contaminants disrupt the central nervous system which control the action or interfere with the way their sensory systems process which lead to disturbance in feeding. These result agreed with the study performed on foraging Behavior and Sensitivity of Fish to Food Stimuli (25).

Statistically significant increase in frequency of aggressive behavior was observed in high dose (0.4 g/l) treatment group 18.13±8.38 as followed by low dose (0.2 g/l) 14.53±6.28 and control group 5.33±3.28. In current study significant elevation in aggressive frequency was observed by increasing concentration of detergent (Bright). Aggression is associated with heightened amygdala activity and lower prefrontal cortex activity according to experts. Aggressive

behaviour can also result from brain lesions, which can arise with neurodegenerative diseases. Within this knowledge no research work was conducted previously on this parameter.

Fish grow in a predictable manner in short-lived species found in warmer climates and in an unpredictable manner in long-lived species found in colder climates. Weight and length units are used to measure growth. Other measures, such as glycine uptake by scale or protein retention in tissues, could also be used to quantify growth. The regulation of growth is significantly influenced by nutrition, including dietary amount and quality. The speed of growth is influenced by a variety of environmental conditions, including temperature, oxygen content, and photoperiod (26). Statically significant decrease in body weight was observed in both low dose (0.2 g/l) 18.08±3.99g and high dose (0.4 g/l) 23.23±5.35g as followed by control group 28.3± 3.72g. in current study decrease in the growth responses with increasing concentrations of bright was observed due to poor feeding on the supplied food , increase in metabolism due to detoxification and impaired health which leads to loss of appetite, energy loss due to behavioural activities during exposure This could in turn affect fish survival, reproductive capacity swimming performance and metabolism. These results agree with the study performed on Nile Tilapia (*Oreochromis niloticus*) exposed to Different Concentrations of Detergents Powder (4).

Current study showed that chemical present in the detergent have negative impact on the body of fish such as damage of skin, swelling on lips and eyes, destruction of caudal fins and lesion on anal fins. The fish's skin serves as both a barrier separating it from its surroundings and a method of communication for most interactions with the outside world. It is a sizable organ that extends to the fins and is continuous with the linings of everybody orifice. The fish integument is a multipurpose organ, and its constituent parts may be crucial for motility, breathing, excretion, ion regulation, protection, communication, sensory perception, and thermal regulation (27). Detergent removes the lipid component from the epidermal layer, disrupts the barrier function, damaged the mucous layer, which makes the epidermis more susceptible to bacterial and viral attack. Detergents elevate the pH of the layer, and as the pH rises over time, degradatory proteases become more active while lipid-synthesizing enzymes become less active. Destruction of caudal fins is resulted due to aggressive behavior. These results are in line with the study performed on *Labeo rohita* Sharma and Rani (28).

The histological study of gills at different detergent concentrations revealed that the gill damage increased with increasing detergent concentration. There is no question that detergent played a role in the gill organ cells' deterioration. Histological findings showed that the lamellae at the highest concentrations of detergent exposure exhibited hyperplasia, blood vessel, necrosis, and epithelial lifting. With an increase in detergent concentration, these changes occurred more frequently. A detergent-damaged gill may be indicated by the growing dilatation. This situation can be explained by the fact that many contaminants first target the gills, which carry out important functions like gas exchange and ion osmoregulation and have a high contact area with the external and internal fish environment. The majority of gill injuries are caused by contaminants that harm the lamellar epithelium (29). You can think of epithelial lifting as a forced decrease in gill surface area. In order to sustain a progressive loss of function in the epithelial cells while maintaining the internal osmotic environment. These results are agreed with the study performed on Nile tilapia (29), brown trout (30).

At various detergent concentrations, necrosis, lipid vacuoles, and blood lipid vacuole were seen in liver histology. Distortion of liver tissue had been resulted by detergent. The liver functions as a filter and detoxifies any poisons that enter the body. If the contamination accumulated there, damage would result to the liver. Similar conclusion about the connection between detergent and serious liver lesions like necrotic and degenerative processes have also been made. Anomalies such as an irregularly shaped central vein, cellular vacuolation, peripheral nucleus, disintegration of cytoplasm, and infiltration may be connected to the accumulation of lipids and glycogen due to liver dysfunction as described effects of detergent exposure. As a result of the discovery of vesicular cytoplasm degeneration in fish hepatocytes, it has been hypothesised that either more glycogen was consumed to meet the energy requirements imposed by toxic stress or that glycogen synthesis was inhibited as a potential contributor to glycogen depletion. These results are agreed with the study performed on Nile tilapia (29).

Conclusion

To conclude, the study revealed that the detergents decreased the fish growth. The surfactant did not significantly affect fish grow. Surfactant has been able to influence of histological of gill and liver, where the damage becomes increase with increasing concentration. The careless discharge of soap and detergent effluents might cause tissue and organ damage, which may make fish more susceptible to diseases and ultimately result in the loss of important food species of the aquatic environment. In order to ensure that industrial effluent is properly treated before being released into the environment, it is necessary to implement appropriate effluent treatment technology.

Acknowledgment

Authors are thankful to the Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, Pakistan to complete the work.

References

1. Fuchs RHB, Ribeiro RP, Matsushita M, Tanamati AAC, Bona E, de Souza AHP. Enhancement of the nutritional status of Nile tilapia (Oreochromis niloticus) croquettes by adding flaxseed flour. LWT-Food Science and Technology. 2013;54(2):440-6.

2. Rath RK. Freshwater aquaculture: Scientific publishers; 2018.
3. Pechiammal K, Vasanthi J. IMPACT OF DETERGENT TIDE ON THE BIOCHEMICAL PARAMETERS OF THE FRESH WATER FISH, LABEO ROHITA. World Journal of Pharmaceutical Research. 2017;6(6):1381-8.
4. Odey AR, Paul JA, Abam AL. Growth Responses of Nile Tilapia (Oreochromis niloticus) Exposed to Different Concentrations of Detergents Powder. Journal of Applied Life Sciences Internasional. 2018;17(3):1-9.
5. Ettah I, Bassey A, Ibor O, Akaninyene J, Christopher N. Toxicological and Histopathological Responses of African Clariid Mud Catfish, Clarias gariepinus (Buchell, 1822) Fingerlings Expose to Detergents (Zip and Omo). Annual Research & Review in Biology. 2017:1-9.
6. Varsha J, Mishra K, Madhuri S, Govind P. Linear alkyl benzene sulphonate, a detergent induced toxicity on the gill of Puntius ticto fish. 2011.
7. Abida FU, Alam P, Retnoaji B, editors. Detergents Effect on Egg Hatchability, Morphometry and Larval Bone Structure of Native Indonesian Fish: Wader Pari (Rasbora lateristriata Bleeker, 1854). E3S Web of Conferences; 2021: EDP Sciences.
8. Moore G, Knight G, Blann AD. Haematology: Oxford University Press; 2016.
9. Setiadi E, editor Impact Of Detergent Waste On Survival, Growth, And Histiphatology On Nile Tilapia (Oreochromis Niloticus) Culture2016.
10. Rey Vázquez G, Lo Nostro F. Changes in hematological parameters of Cichlasoma dimerus (Teleostei, Perciformes) exposed to sublethal concentrations of 4-tert-octylphenol. Archives of environmental contamination and toxicology. 2014;66(3):463-9.
11. Misra V, Kumar V, Pandey SD, Viswanathan P. Biochemical alterations in fish fingerlings (Cyprinus carpio) exposed to sublethal concentration of linear alkyl benzene sulphonate. Archives of environmental contamination and toxicology. 1991;21(4):514-7.
12. Sobrino-Figueroa AS. Evaluation of oxidative stress and genetic damage caused by detergents in the zebrafish Danio rerio (Cyprinidae). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology. 2013;165(4):528-32.
13. Shahzad K, Khan MN, Jabeen F, Chaudhry AS, Ara C, Khan MKA, et al. Study of some toxicological aspects of titanium dioxide nanoparticles through oxidative stress, genotoxicity, and histopathology in tilapia, Oreochromis mossambicus. 2021.
14. KOSAI P, Sathavorasmith P, Jiraungkoorskul K, Jiraungkoorskul W. Morphometric characters of Nile tilapia (Oreochromis niloticus) in Thailand. Walailak Journal of Science and Technology (WJST). 2014;11(10):857-63.
15. Sebastião F, Nomura D, Sakabe R, Pilarski F. Hematology and productive performance of Nile tilapia (Oreochromis niloticus) naturally infected with Flavobacterium columnare. Brazilian Journal of Microbiology. 2011;42:282-9.
16. Gouda AM, Hagras AE, Okbah MA, El-Gammal MI. Influence of the Linear Alkylbenzene Sulfonate (LAS) on hematological and biochemical parameters of Nile Tilapia, Oreochromis niloticus. Saudi Journal of Biological Sciences. 2022;29(2):1006-13.
17. Alaguprathana M, Poonkothai M. Haematological, biochemical, enzymological and histological responses of Labeo rohita exposed to methyl orange dye solution treated with Oedogonium subplagiostomum AP1. Environmental Science and Pollution Research. 2021;28(14):17602-12.
18. Saliu JK, Salami AS. Haematological studies of Oreochromis niloticus exposed to diesel and drilling fluid in Lagos, Nigeria. International Journal of Biodiversity and Conservation. 2010;2(5):130-3.
19. Pechiammal K, Vasanthi J. EFFECT OF DETERGENT TIDE ON THE HAEMATOLOGICAL PARAMETERS OF THE FRESHWATER FISH, LABEO ROHITA. 2017.
20. Schwaiger J, Spieser O, Bauer C, Ferling H, Mallow U, Kalbfus W, et al. Chronic toxicity of nonylphenol and ethinylestradiol: haematological and histopathological effects in juvenile Common carp (Cyprinus carpio). Aquatic toxicology. 2000;51(1):69-78.
21. Ahmadpanah K, Soltani M, Rajabi Islami H, Shamsaie M. Effects of nonylphenol on hematological parameters and immune responses in immature rainbow trout (Oncorhynchus mykiss). Marine and Freshwater Behaviour and Physiology. 2019;52(4):151-65.
22. Izzi B, Gialluisi A, Gianfagna F, Orlandi S, De Curtis A, Magnacca S, et al. Platelet Distribution Width Is Associated with P-Selectin Dependent Platelet Function: Results from the Moli-Family Cohort Study. Cells. 2021;10(10):2737.
23. Fava C, Cattazzo F, Hu Z-D, Lippi G, Montagnana M. The role of red blood cell distribution width (RDW) in cardiovascular risk assessment: useful or hype? Annals of translational medicine. 2019;7(20).
24. Nkpondion N, Ugwumba O, Esenowo I. The toxicity effect of detergent on enzymatic and protein activities of African mud catfish (Clarias gariepinus). J Environ Anal Toxicol. 2016;6(361):2161-0525.
25. Kasumyan A. Effects of chemical pollutants on foraging behavior and sensitivity of fish to food stimuli. Journal of Ichthyology. 2001;41(1):76-87.
26. Dutta H. Growth in fishes. Gerontology. 1994;40(2-4):97-112.
27. Elliott DG. The skin: the many functions of fish integument. 2011.
28. Sharma R, Rani S. EFFECT OF SURFACTANT ON EXTERNAL MORPHOLOGY AND PHYSIOLOGICAL RESPIRATION OF FRESHWATER FISH, LABEO ROHITA''.
29. Setiadi E. Impact Of Detergent Waste On Survival, Growth, And Histiphatology On Nile Tilapia (Oreochromis Niloticus) Culture. 2016.

30. Abel P. Toxic action of several lethal concentrations of an anionic detergent on the gills of the brown trout (Salmo trutta L.). Journal of fish biology. 1976;9(5):441-6.