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Hemato-Serological Findings as Early signals in Nile Tilapia Oreochromis niloticus Treated with Benzalkonium Chloride

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Abstract

To evaluate the toxicity of benzalkonium chloride in aquaculture, the hemato-serological indices of Nile tilapia Oreochromis niloticus are used as biomarkers. Following exposure to three concentrations of benzalkonium chloride BAC 0.1, 0.25, 0.50, and 1 mg/l (BAC1,2,3 and 4) in aquaria for two durations 21 and 42 days, the microbiological assay in fish aquaria, in addition to blood parameters were assessed. Except for the mean difference between BAC2 and BAC3 ($P > 0.05$) at 42 days, the mean values of the bacterial counts revealed a significant difference between all compared groups ($0.05 \geq P \leq 0.01$). Following exposure to the lower concentrations of BAC (1, 2 and 3), the main blood parameters of Oreochromis niloticus namely red blood cells RBCs, and hemoglobin Hb decreased in fluctuated pattern when compared to control treatment ($0.05 > P < 0.01$), but interestingly peaked at the higher concentration (BAC4), despite there were no significant differences when compared to control (except hemoglobin at 42 days, $P < 0.01$). Contrarily, the white blood cells WBCs rapidly rose at the first concentration BAC1 ($P < 0.01$), particularly at the second duration, compared to the control treatments. The counted WBCs changed after exposure to the following two concentrations BAC2, BAC3, before peaking at the higher experimental concentration BAC4 ($P < 0.01$). The mean cells volume MCV, mean cells haemoglobin MCH and MCHC mean cells haemoglobin concentration exhibited a narrow fluctuation between control and BAC treated fish. Regarding biochemical findings, the average levels of cholesterol and triglycerides showed erratic behavior depending on BAC levels in treatment. The mean alanine transaminase ALT and aspartate aminotransferase AST levels fluctuated between the control and BAC1,2,3 before abruptly increasing at the higher BAC4, with significant differences ($0.05 > P < 0.01$) when control, BAC1,2,3, compared to BAC4, except AST between BAC3 and BAC4 (insignificant- 42 days). Following BAC exposure, other parameters, including protein and urea remained the same as in control fish. The alteration in some hemato-serological markers demonstrated how a compensatory mechanism is generated in response to the stress caused by exposure to biocides.

Keywords: Benzalkonium chloride, Biomarkers, Disinfectant, Oreochromis niloticus, Toxicity.

Introduction

Benzalkonium chloride BAC is a broad-spectrum biocide [1], with the ability to disinfect against fungi, algae, bacteria, and viruses, BAC in lower concentrations 0.002-0.02% is a preservative included in consumer health products including eye drops and intranasal sprays. On the other hand,

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BAC is used for surface disinfection in residential, hospital, and home settings [2].

The primary mechanism of BAC toxicity is the disruption of the integrity and functionality of the cell membrane, which results in cell death [3]. The main sources of BAC released into the environment are sludge and effluent discharged by sewage treatment plants [4,5]. In Minnesota, disinfectant quaternary ammonium compounds in wastewater effluent 0.4 µg/l to 6.6 µg/l and sediments 0.1 µg/l to 4.5 µg/l downstream from wastewater treatment discharges have been observed [6]. These findings are anticipated because the common disposal method for these compounds during their lifetime is down the drain, and although the removal rate in wastewater treatment plants is high, it won't be 100% [7]. Ecotoxicology studies have shown that BAC is very toxic to several aquatic organisms [8]. Additionally, several quaternary ammonium compounds are combined with other chemical contaminants in the aquatic environment rather than occurring as separate compounds and have been shown to have genotoxic effects in the mammalians, plant, and crustacean cells [9, 10]. Experimentally, 16 µg/l BAC resulted in a reduction of the Gram-negative bacterium *Acinetobacter baumannii* [11]. Some earlier investigations were undertaken to determine the toxicity of BAC to various fish species, including, for instance, rainbow trout *Oncorhynchus mykiss* 0.100 to 1.050 mg/l[12], Juvenile *Oreochromis niloticus*, $LC_{50\ /\ 96h}$ = 69.772 ppm [13], *Cyprinus carpio* 1 mg/l for 24, 48, and 96 h [14]. Fish are considered acceptable test organisms because, given their ecological function and trophic location, they serve as significant aqueous pollutant receptors.

Currently, fish serves as a significant and cost-effective source of animal protein for almost a billion people worldwide, making up more than 25% of the total supply [15,16]. Water, especially fresh water, is essential for life, but regrettably, it can also contain biological, organic, and inorganic materials which may harm both human and aquatic life [17,18]. Today more than ever, efforts are being made to manage water quality to sustain aquaculture's productivity and quality, which has benefitted the economies of many countries [19]. Furthermore, over the past few decades, aquaculture has significantly boosted the output of animal proteins in practically every nation on earth [20]. Thereby, fishing pressure and wild stock harvesting from rivers, lakes, oceans, and other open water resources have declined [15]. On the other hand, water quality, environmental stress, the introduction of infectious pathogens, and an increase in the prevalence of fish diseases have all posed several health hazards to aquatic species, particularly fish [21]. On the one extreme, this has increased the use of various chemicals and aqua medicines in aquaculture to enhance and control fish health and water quality [15] and minimize production loss on the other extreme [22]. Synthetic drugs and chemicals have been used to treat or prevent fish infections with varying degrees of success. However, the risk of their excessive use includes immunosuppression, nephrotoxicity, growth inhibition, the development of drug resistance, environmental issues, and meat residues [23]. To prevent parasite and fungus infections, for instance, copper sulphate, formaldehyde, and malachite green were used in aquaculture as exceptionally powerful traditional disinfectants; however, their usage was linked to several biological and physiological problems [24,25]. Tilapias fish is the second-most important cultivated fish worldwide after cyprinids [26]. Consequently, the main goal of the current work is to evaluate the safety of BAC in aquaculture practices in terms of the potential health implications of using the Nile tilapia *Oreochromis niloticus* as an experimental fish.

Materials and Methods

The current experiment was carried out at the Aquaculture Lab, Department of Zoology, Faculty of Science, Al-Azhar University (Assiut Branch), Assiut, Egypt. Benzalkonium chloride 50% (LONZAGARD® BKC cGMP), Lonza was used as test chemical for the current assessment. To determine its effects on blood properties, Nile tilapia (*Oreochromis niloticus*) was exposed to benzalkonium chloride in the current work for 42 days.

Bacteriological assay

After 21, and 42 days, three water samples were collected from each treatment aquarium containing BAC, as well as the control aquarium. The samples

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were collected using clean, sterile, non-reactive caped glass tubes. Samples were sent immediately to the water and wastewater company (Sohag governorate, Egypt) for bacterial enumeration. The bacteria in the water samples were counted using the Pour Plate Method. Heterotrophic plate count (HPC) was used, and the following equation was adopted [27]:

$$\text{Colony forming units CFU} = \frac{\text{Colonies counted}}{\text{The actual volume of sample}}$$

Experimental setup and preparation of work solutions (BAC)

Following a week of acclimatization, tilapias with average body weights of 50±7 g and average lengths of 16±4 cm were selected. 150 fish were divided into 5 groups, 3 aquaria ×10 fish per group and transferred to 15 aquaria, 100×40×50 cm. The experiment was planned to run for six weeks, from 5th August to 16th September 2021. Fish were fed commercial pellets during the tests, and water was replaced every 48 hours to remove fecal and non-utilized feed with water that had the same amount of benzalkonium chloride as the siphoned volume. For BAC, a stock solution of 100 mg/l was diluted from the original solution as follows: An amount of 200µl = 0.2 ml /l was taken in a volumetric flask 1000 ml, then make up to 1 L with distilled water. The work solution based on the pamphlet on BAC chloride toxicity for freshwater fishes 0.223 - 2.4 mg/l, 96h LC_{50} was followed for prolonged (chronic) exposure. The work solutions selected are 0.1, 0.25, 0.50 and 1 mg/which are equivalent 100, 250, 500 and 1000 µl/l, respectively from the stock solution 100 mg/l (preparation based on CAS 8001-54-5; benzalkonium chloride 50% solution, toxicity for freshwater fishes (0.223 - 2.4 mg/L, 96h LC_{50}). In the current study, the control group was singed as C, and the four concentrations (0.1, 0.25, 0.50 and 1 mg/) of BAC were singed as BAC1, BAC2, BAC3, and BAC4, respectively.

Hematological and Biochemical analysis

Blood samples from the experimental setup were taken at equal intervals for hematological and serological assays, after days 21 and 42 days. In the current investigation, three replicates of each variable were collected. Blood was taken from caudal vein [28] and divided into two parts as follows: To determine the full blood count, the first part was transferred to an EDTA anticoagulant tube. The second portion of the blood sample was put into an Eppendorf tube without EDTA, centrifuged for 10 minutes at 3000 rpm, and the supernatant was then analyzed biochemically. Using an automated technical analyzer (Celltac MEK- 6400J/K), the hemoglobin concentration (Hb), hematocrit (Hct), and red blood cells (RBCs) count of whole blood were measured. White blood cells (WBCs) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and were determined based on [29]. The levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Creatinine, Aspartate Aminotransferase (AST), and Triglyceride were determined in serum using the techniques of [30-32].

Statistical analysis

To express the data, the mean and standard deviation of variables were tabulated. To statistically evaluate the impacts of the tested chemical on blood variables between the experimental fish groups, a one-way analysis of variance (ANOVA) test was applied. For data analysis, the SPSS, V: 25 software was used. To compare mean differences, LSD multiple range test was employed. Probability values between 0.05 and 0.01 were evaluated as significant. Statistically non-significant, significant, and highly significant outputs were accompanied by symbols NS, * and ** respectively.

Results and Discussion

Bacteriology assay in aquaria

A remarkable decrease ($P \leq 0.05$), in bacteriology resulted at both experimental durations, Tables 1, 2. In control aquaria, the counted colony forming units were 5023.3±135.4 CFU and 5624.3±270.1 CFU after 21 and 42 days, respectively. Following that, their numbers rapidly reduced as BAC levels rose. Except for the mean difference between BAC2 and BAC3 (insignificant, $P = 0.47$) at 42 days, Table 2 has shown the presence of highly significant differences between the recorded mean values, between any compared

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concentrations including the control group ($0.05 \geq P \leq 0.01$).

Table 1. Means of bacteriological burdens recorded at the two experimental durations from control and BAC- containing aquaria of *Oreochromis niloticus*.

CFU	C	BAC1	BAC2	BAC3	BAC4
21days	5023.3± 135.4	3511.3±187.6	3031.6±44.2	2838±61.5	1957.6±24.5
42 days	5624.3±270.1	3195.6±61.9	2703.6±38.7	2652.6±92.6	1806.3±65.0

CFU= colony forming units; C= control; BAC1, BAC2, BAC3, BAC4=0.1,0.25,0.5,1 mg/l benzalkonium chloride, respectively.

Table 2. LSD multiple comparisons for bacteriological colonies between different treatments.

(I - J) Treatment (mg/l)		Mean Difference (I-J)	*P - value*
After 21 days exposure			
C	BAC1	1512.00**	*.00*
	BAC2	1991.66**	*.00*
	BAC3	2185.33**	*.00*
	BAC4	3047.66**	*.00*
BAC1	BAC2	479.66*	*.05*
	BAC3	673.33**	*.00*
	BAC4	1535.66**	*.00*
BAC2	BAC3	193.66**	*.01*
	BAC4	1056.00**	*.00*
BAC3	BAC4	862.33**	*.00*
After 42 days exposure			
C	BAC1	2428.66**	*.01*
	BAC2	2920.66**	*.01*
	BAC3	2971.66**	*.01*
	BAC4	3818.00**	*.00*
BAC1	BAC2	492.00**	*.00*
	BAC3	543.00**	*.00*
	BAC4	1389.33**	*.00*
BAC2	BAC3	51.00NS	*.47*
	BAC4	897.33**	*.00*
BAC3	BAC4	846.33**	*.00*

CFU= colony forming units; C= control;(BAC1, BAC2, BAC3, BAC4) = (0.1,0.25,0.5,1 mg/l benzalkonium chloride, respectively).
LSD= least significant differences.
* ***The mean difference is significant at level 0.05.***
** ***The mean difference is significant at level 0.01.***
NS ***The mean difference is nonsignificant.***

Hematological parameters

The data in Table 3 summarizes the average blood indices measured during the experiment involving Nile tilapia *Oreochromis niloticus* exposure to benzalkonium chloride. When compared to the control group, the mean RBCs and hemoglobin exhibited decreased variation ($0.05> P <0.01$) following the exposure to BAC1,2 and 3, then peaked at the higher concentration BAC4 at both sampling times 21 and 42 days compared to control groups, despite the presence of no statistical differences ($P >0.05$) as indicated in Table 4. In relation to BAC concentration, MCV varied; it peaked at BAC4, compared to control fish for both 21 and 42 days, respectively ($P <0.01$) as shown in table 4. However, there was an erratic fluctuation between the two treatments, namely control and BAC4. At the lower BAC1,2,3 concentrations, MCH increased considerably with comparable differences, particularly at 21 days ($0.05> P \leq 0.01$), while at the higher BAC4 concentration it decreases once again at both time periods, Table 3. MCHC exhibited a dramatic decrease at both periods between the BAC1,2,3 compared to control means ($P <0.01$). WBCs varied between the control and the three concentrations BAC1,2,3, before rising

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sharply at BAC4 (P <0.01) at both sampling intervals 21 and 42 days. All scored hematological parameters were concentration-dependent rather than time-dependent. Whoever, WBCs were shown to be influenced by both BAC concentration and exposure time. The mean values MCV and MCH were noticed to be out of balance during the BAC exposure period, Tables 3, 4.

Table 3. Means of hematological parameters recorded at the two experimental durations from control and BAC- containing aquaria of *Oreochromis niloticus*.

Parameters (unit)		C	BAC1	BAC2	BAC3	BAC4
RBCs ($\times10^6$/µl)	21 days	2.2±0.3	1.7±0.1	1.8±0.07	1.8±0.1	2.5±0.07
	42 days	2.3±0.1	1.8±0.09	1.6±0.1	1.5±0.3	2.3±0.07
Hb (g/dl)	21 days	7.8±0.2	6.7±0.7	6.7±0.3	7.5±0.8	9.8±0.2
	42 days	8.8±0.2	8.0±0.3	7.1±1.3	6.9±1.03	10±0.2
MCV (fL)	21 days	120±1.7	156.6±20.5	173.6±6.7	155±4.4	166.6±5.4
	42 days	117.6±1.8	142±10.1	156.3±7.5	187.6±4.5	163.3±20
MCH (pg)	21 days	34.6±1.9	37.9±1.8	37.9±1.3	41±1.3	24.06±0.9
	42 days	37.0±2.4	42.3±4.6	40.8±2.5	44.3±2.05	42.2±1.7
MCHC (g/dL)	21 days	33.3±2.4	24.5±3.0	21.7±0.7	26.5±0.08	24.4±0.9
	42 days	36.4±0.9	27.5±3.04	23.1±1.5	23.6±1.6	26.1±2.7
Hct (%)	21 days	34.2±2.01	27.8±5.5	30.8±2.3	28.1±2.9	40.9±1.1
	42 days	35.5±2.1	28.8±5.4	32.0±7.8	30.1±6.6	38.9±4.9
WBCs ($\times10^3$/µl)	21 days	27.6±1.1	50.5±13.9	45.2±11.6	59.3±7.7	107±7.9
	42 days	36.6±4.7	71.0±1.6	94.7±9.5	48.4±9.5	106.8±12.9

C= control;(BAC1, BAC2, BAC3, BAC4) = (0.1,0.25,0.5,1 mg/l benzalkonium chloride, respectively).

Table 4. LSD multiple comparisons for hematological parameters between different treatments and times.

Parameters		C & BAC 1	C & BAC 2	C & BAC 3	C & BAC 4	BAC 1 & BAC 2	BAC 1 & BAC 3	BAC 1 & BAC 4	BAC 2 & BAC 3	BAC 2 & BAC 4	BAC 3 & BAC 4
RBCs	21 d	.02*	.03*	.04*	.08^{NS}	.80^{NS}	.65^{NS}	.00**	.83^{NS}	.00**	.00**
	42 d	.03*	.00**	.00**	.63^{NS}	.28^{NS}	.13^{NS}	.01**	.61^{NS}	.00**	.00**
Hb	21 d	.03*	.03*	.55^{NS}	.00**	1.0^{NS}	.10^{NS}	.00**	.10^{NS}	.00**	.00**
	42 d	.26^{NS}	.03*	.02*	.12^{NS}	.25^{NS}	.14^{NS}	.01**	.69^{NS}	.00**	.00**
MCV	21 d	.00**	.00**	.00**	.00**	.09^{NS}	.86^{NS}	.30^{NS}	.07^{NS}	.47^{NS}	.23^{NS}
	42 d	.03*	.00**	.00**	.00**	.18^{NS}	.00**	.05*	.01**	.50^{NS}	.03*
MCH	21 d	.03*	.08^{NS}	.00**	.00**	.55^{NS}	.04*	.01**	.01**	.00**	.43^{NS}
	42 d	.08^{NS}	.19^{NS}	.02*	.08^{NS}	.59^{NS}	.48^{NS}	.96^{NS}	.23^{NS}	.62^{NS}	.45^{NS}
MCHC	21 d	.00**	.00**	.00**	.00**	.12^{NS}	.26^{NS}	.58^{NS}	.017**	.04*	.54^{NS}
	42 d	.00**	.00**	.00**	.00**	.04*	.07^{NS}	.49^{NS}	.79^{NS}	.14^{NS}	.22^{NS}
Hct	21 d	.05*	.26^{NS}	.06^{NS}	.04*	.32^{NS}	.91^{NS}	.00**	.37^{NS}	.00**	.00**
	42 d	.23^{NS}	.52^{NS}	.32^{NS}	.52^{NS}	.55^{NS}	.81^{NS}	.08^{NS}	.71^{NS}	.21^{NS}	.12^{NS}
WBCs	21 d	.09^{NS}	.19^{NS}	.03*	.00**	.68^{NS}	.49^{NS}	.00**	.28^{NS}	.00**	.00**
	42 d	.00**	.00**	.16^{NS}	.00**	.01**	.01**	.00**	.00**	.15^{NS}	.00**

C= control;(BAC1, BAC2, BAC3, BAC4) = (0.1,0.25,0.5,1 mg/l benzalkonium chloride, respectively).
* The mean difference is significant at level 0.05.
** The mean difference is significant at level 0.01.
^{NS} The mean difference is nonsignificant.

Serological analysis

In *Oreochromis niloticus*, the difference in the mean values of total protein and urea between the control groups and the BAC-exposed group was not statistically significant (P>0.05), Tables 5,6. The mean glucose level rose in the blood of fish exposed to BAC1,2,3 compared to the control fish group (P<0.01), particularly at the 21 days, then increased proportionally before declining once more at BAC4, at days 21with no significant differences. However, the mean glucose differed significantly at 42 days between BAC2 & BAC4 (P<0.01), and BAC3,

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BAC4 (P=0.01). The levels of cholesterol were measured throughout the two test durations in a zigzag pattern; in comparison to the control group, the levels of cholesterol determined from BAC1 were higher at both test durations 21 and 42 days, when compared to control findings (P<0.01), Tables 5,6. Following that, cholesterol started to decline again at the second exposure BAC2. Finally, cholesterol rose at the third treatment BAC3, and reduced at the higher exposure concentration BAC4. As well as cholesterol, triglyceride mean levels were upper-lower in trend and were time - BAC concentrations dependent. The means ALT and AST levels showed a fluctuating pattern between the control and the concentrations of BAC1,2,3, before drastically peaking at the higher concentration BAC4 at 21 and 42 days, showing different degrees of significance (0.05>P≤0.01). When compared to the starting level of the control group, the creatinine level was found to have increased significantly after the experiment (0.05>P≤0.01).

Table 5. Means of serological parameters recorded at the two experimental durations from control and BAC- containing aquaria of *Oreochromis niloticus*.

Parameters (unit)		C	BAC1	BAC2	BAC3	BAC4
Protein (mg/dl)	21 days	3.1±0.04	3.2±0.1	3.1±0.02	3.1±0.1	3.1±0.01
	42 days	3.02±0.04	3.01±0.1	2.9±0.1	2.9±0.05	3.01±0.09
Glucose (mg/dl)	21 days	92±3.5	115.3±3.1	134±12.4	135.3±4.5	123.6±5.8
	42 days	105.3±4.5	118.3±21.1	111.3±5.08	119.6±4.03	149±7.09
Cholesterol (mg/dl)	21 days	170.3±4.0	192.3±6.7	185±12.0	200±3.5	182.3±7.1
	42 days	152.3±2.7	195±10.7	162.6±11.8	181.3±7.2	192.3±6.7
Triglyceride (mg/dl)	21 days	98.6±3.6	64.6±4.9	131.6±46.5	128.3±7.6	165.3±4.5
	42 days	121±2.3	130.6±18	153.3±13.6	125±4.4	155.3±9.8
Creatinine (mg/dl)	21 days	0.5±0.01	0.5±0.02	0.4±0.03	0.4±0.01	0.5±0.02
	42 days	0.5±0.02	0.5±0.02	0.6±0.01	0.6±0.01	0.7±0.05
Urea (mg/dl)	21 days	14.6±1.3	14.6±1.8	13±1.7	15±2.3	14±0.8
	42 days	15±0.8	13±0.8	14±0.8	14.6±1.3	14.6±2.2
ALT(U/L)	21 days	26.3±2.8	25.6±2.7	25±3.8	25.3±4.03	33.6±3.7
	42 days	39.6±2.2	37.3±6.7	39.6±2.2	38±1.7	62.6±11.1
AST(U/L)	21 days	38.3±1.8	42.6±4.03	49.6±7.6	45.6±4.9	154±6.9
	42 days	125.3±6.5	105±11.3	143.3±6.2	178.6±10.7	189±10.3

C= control;(BAC1, BAC2, BAC3, BAC4) = (0.1,0.25,0.5,1 mg/l benzalkonium chloride, respectively).

Table 6. LSD multiple comparisons for serological parameters between different treatments and time.

Parameters		C & BAC 1	C & BAC 2	C & BAC 3	C & BAC 4	BAC 1 & BAC 2	BAC 1 & BAC 3	BAC 1 & BAC 4	BAC 2 & BAC 3	BAC 2 & BAC 4	BAC 3 & BAC 4
Protein	21 d	$.20^{NS}$	$.80^{NS}$	$.74^{NS}$	$.90^{NS}$	$.29^{NS}$	$.12^{NS}$	$.24^{NS}$	$.56^{NS}$	$.90^{NS}$	$.65^{NS}$
	42 d	$.89^{NS}$	$.78^{NS}$	$.33^{NS}$	$.94^{NS}$	$.89^{NS}$	$.40^{NS}$	$.94^{NS}$	$.47^{NS}$	$.83^{NS}$	$.36^{NS}$
Glucose	21 d	$.00^{**}$	$.00^{**}$	$.00^{**}$	$.00^{**}$	$.01^{**}$	$.00^{**}$	$.20^{NS}$	$.83^{NS}$	$.12^{NS}$	$.09^{NS}$
	42 d	$.20^{NS}$	$.54^{NS}$	$.16^{NS}$	$.00^{**}$	$.48^{NS}$	$.89^{NS}$	$.01^{**}$	$.40^{NS}$	$.00^{**}$	$.01^{**}$
Cholesterol	21 d	$.00^{**}$	$.05^{*}$	$.00^{**}$	$.10^{NS}$	$.30^{NS}$	$.28^{NS}$	$.16^{NS}$	$.04^{*}$	$.70^{NS}$	$.02^{*}$
	42 d	$.00^{**}$	$.21^{NS}$	$.00^{**}$	$.00^{**}$	$.00^{**}$	$.10^{NS}$	$.73^{NS}$	$.03^{*}$	$.00^{**}$	$.18^{NS}$
Triglyceride	21 d	$.11^{NS}$	$.12^{NS}$	$.15^{NS}$	$.00^{**}$	$.00^{**}$	$.00^{**}$	$.00^{**}$	$.86^{NS}$	$.11^{NS}$	$.08^{NS}$
	42 d	$.36^{NS}$	$.01^{**}$	$.70^{NS}$	$.00^{**}$	$.05^{*}$	$.59^{NS}$	$.03^{*}$	$.02^{*}$	$.85^{NS}$	$.01^{**}$
Creatinine	21 d	$.00^{**}$	$.02^{*}$	$.24^{NS}$	$.01^{**}$	$.00^{**}$	$.00^{**}$	$.76^{NS}$	$.19^{NS}$	$.00^{**}$	$.00^{**}$
	42 d	1.0^{NS}	$.00^{**}$	$.10^{NS}$	$.00^{**}$	$.00^{**}$	$.10^{NS}$	$.00^{**}$	$.02^{*}$	$.08^{NS}$	$.00^{**}$
Urea	21 d	1.0^{NS}	$.31^{NS}$	$.83^{NS}$	$.68^{NS}$	$.31^{NS}$	$.83^{NS}$	$.68^{NS}$	$.23^{NS}$	$.54^{NS}$	$.54^{NS}$
	42 d	$.14^{NS}$	$.44^{NS}$	$.79^{NS}$	$.79^{NS}$	$.44^{NS}$	$.21^{NS}$	$.21^{NS}$	$.60^{NS}$	$.60^{NS}$	1.0^{NS}
ALT	21 d	$.83^{NS}$	$.68^{NS}$	$.76^{NS}$	$.04^{*}$	$.83^{NS}$	$.91^{NS}$	$.03^{*}$	$.91^{NS}$	$.02^{*}$	$.02^{*}$
	42 d	$.68^{NS}$	1.0^{NS}	$.76^{NS}$	$.00^{**}$	$.68^{NS}$	$.90^{NS}$	$.00^{**}$	$.76^{NS}$	$.00^{**}$	$.00^{**}$
AST	21 d	$.40^{NS}$	$.04^{*}$	$.17^{NS}$	$.00^{**}$	$.19^{NS}$	$.56^{NS}$	$.00^{**}$	$.443^{NS}$	$.00^{**}$	$.00^{**}$
	42 d	$.15^{NS}$	$.20^{NS}$	$.00^{**}$	$.00^{**}$	$.01^{**}$	$.00^{**}$	$.00^{**}$	$.02^{*}$	$.00^{**}$	$.45^{NS}$

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C= control;(BAC1, BAC2, BAC3, BAC4) = (0.1,0.25,0.5,1 mg/l benzalkonium chloride, respectively).
*** The mean difference is significant at level 0.05.***
**** The mean difference is significant at level 0.01.***
NS The mean difference is nonsignificant.

Discussion

The ability of BAC to alter cell functions and damage cell membranes has been suggested as the mechanism by which it can be toxic[3]. Petroleum derivatives, insecticides, nanoparticles, cosmetics, pharmaceuticals, and health and personal care products are just a few of the many synthetic and semi-synthetic substances produced every day to meet humanity's insatiable demand for industrialization and development [33,34], but their negative effects are unavoidable. If this strategy is not controlled and managed, it will eventually cause major issues for both people and their environment. For instance, the research by [34] has demonstrated the ability of *Phaseolus vulgaris* L. to absorb three drugs from soil: diclofenac, mefenamic acid, and metronidazole. A significant challenge facing the entire world today is the entry of significant amounts of produced pollutants from point and non-point sources into natural ecosystems. The process of disposal for products containing quaternary ammonium compounds after use is often "down-the-drain" to wastewater treatment systems; as a result, the aquatic environment has the highest likelihood of environmental impact [7]. Fish are consumed throughout the world because they are high in protein and long-chain n-3 polyunsaturated fatty acids [35]. Consequently, maintaining the quality of fish is a big concern, and over time, several preservatives have been used to address this problem. As a disinfectant, formalin at concentrations between 15 - 25 mg/l was effective to preserve fish in the past times. But, the best use of formalin levels has, however, been violated frequently. Although biocides are widely used in many different industries, they are largely uncontrolled and as a result, pose an environmental risk [36], thus their stewardship is essential and critical [37]. Previous works have demonstrated the effects of BAC on both animals and humans. For example, the 96 h LC_{50} for BAC 69.772 ppm revealed negative impacts on the experimental juvenile *Oreochromis niloticus* [13]. On the other hand, human respiratory epithelial cells exposed to BAC at amounts normally found in commercially available nasal solutions were shown to significantly modify their DNA in vitro, raising the possibility that apoptosis and necrosis have occurred [38]. The drastic decrease in bacteriology count resulted here confirming the efficacy of BAC as a disinfectant. Bacteria multiply quickly, infect fish, and increase mortality rates in a few days or weeks [39]. Using a time-to-reduce assay, the disinfecting efficacy of BAC on microbiological colonies in the experimental aquaria elucidated the severe impact of BAC ($0.05 \geq P \leq 0.01$) on the bacterial count. Similar to the current observation, the growth of alga *Pseudokirchneriella subcapitata* was inhibited after exposure to 0.25 mg/l BAC /72 hours [10]. In consistence, study [11] on the bacteria *Acinetobacter baumannii*, BAC, on the one hand, hindered gentamicin killing and, on the other hand, dramatically increased the rate at which resistance mutants appeared. Regarding haematological biomarkers, blood cells serve as excellent biomarkers of biological, physical, or chemical changes in the fish's environment [40, 41]. Alterations in hematological parameters are thus reliable indicators of deterioration in water quality [42,43] caused by xenobiotics. WBCs levels have fluctuated in the current experiment because the immune system was attempting to control stress, particularly as the microbial burden was lowering. Following exposure to the first three BAC1,2,3 concentrations, red blood cells declined, then increased to levels comparable to control fish at BAC4 at both exposure times. This pattern of RBCs fluctuation is in accordance with some similar studies on *Labeo rohita* exposed to fungicide propiconazole [44], and *Oncorhynchus mykiss* exposed to pesticides [45]. Fish exposed to higher BAC4 exhibited higher hematocrit levels than control fish, which may mean that hematocrit is increased simultaneously with increasing RBCs, which may be produced in high numbers to compensate for loss. Furthermore, serological indices such as total protein, glucose, triglycerides, ALT, AST, creatinine, urea, and cholesterol have all been employed as responsive biomarkers to evaluate the influence of stressors in both field and laboratory

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investigations [46-48]. For example, toxic stress is known to cause fish to experience transient changes in blood glucose that may level out at a new homeostatic level [49,50]. The significant rise in glucose levels, especially at the highest BAC4, may imply the process of glycogenolysis to fulfill the energy requirements necessitated by toxic stress or by a reduction in glycogen synthesis as a reaction to BAC-mediated stress. *Oreochromis niloticus*, exposed to alkyl benzene sulfonate caused neurological control posing fish to leap out (hyperactivities) of a treatment aquaria[51]. Similarly, two fish species *Cirrhina mrigala* and *Puntius sophore* showed altered carbohydrate metabolism after being exposed to 0.005 mg/l of alkyl benzene sulphonate [52]. Since the primary function of the liver is to maintain lipid homeostasis, changes in blood levels of cholesterol and triglycerides signify liver dysfunction [53]. Triglyceride and cholesterol levels were found to fluctuate erratically in the current investigation, which is likely due to sterol biosynthesis dysfunction. The experiments on *Oreochromis niloticus* exposed to benzene sulphonate resulted in elevated levels of cholesterol and triglycerides [51], which were attributed to the disruption in fat metabolism, which was consistent with the current explanations. Similar investigations using various fish subjected to different types of environmental toxicants have all got results that are consistent with the current findings. For instance, *Oncorhynchus mykiss*, following exposure to phenol [54], *Oreochromis niloticus* exposed to copper sulphate [47], *Oreochromis niloticus* sampled from a contaminated environment [46,55]. As a stressor, BAC is expected to cause physiological changes associated with the production of free radicals, causing oxidative stress on the biosynthesis within the animal body. BAC was suggested to pass through the blood-placental barrier and enter the mouse neonatal brain [56], generating physiological alterations. The remarkable patterns of ALT and AST found here suggest that they are experiencing stress brought on by BAC exposure, particularly at BAC4 exposure compared to control findings ($0.05>P<0.01$). In accordance with the literature, ALT and AST are found in a variety of tissues and cells, and structural injury to the liver causes them to be discharged into the blood in large quantities [57]. Similar to the current findings, elevated AST and ALT activities were observed in the blood and liver tissues of numerous fish species subjected to varied pesticide doses for various periods of time [58]. Also, the study on *Cyprinus carpio* exposed to BAC at a similar level to the maximum concentration in the current study, resulted in a significant drop in antioxidant enzymes, indicating liver impairment [14]. Our interpretations of AST and ALT are similarly consistent with those of [59], who suggested that the fluctuating levels represent an attempt by fish to achieve balance while being exposed to biochemical and physiological function – impairing chemicals.

Conclusion

This assessment is the first to evaluate the impact of widely applicable quaternary ammonium compound (benzalkonium chloride) on Nile tilapia *Oreochromis niloticus* in Egypt. The current findings have revealed that BAC has toxic potential, which resulted in a bacterial count. Furthermore, the main hematological and serological parameters responded to BAC in a concentration-dependent manner. Furthermore, the main hematological and serological parameters responded to BAC in a concentration-dependent manner ($0.05 \geq P \leq 0.01$). Consequently, there is a possibility that BAC could reach the environment and aquatic ecosystems through treated and untreated sewage, including domestic, industrial, and urban runoff because of its extensive use in disinfection. However, the current research suggests that the recommended BAC save concentration for freshwater fish is between 0.1 and 0.5ppm.

Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images that are not ours have been included with the necessary permission for re-publication, which is attached to the manuscript. The author has signed an animal welfare statement.
- Ethical Clearance: The project was approved by the local ethical committee in Department of Zoology, Faculty of Science, Al- Azhar University (Assiut Branch), 71524 Assiut,

Egypt. The authors confirm that, no environmental or health threats have been practiced during the current study.

Author's Contribution Statement

M. A. E.: Prepared fish samples and setup experiments; M. M.: Supervision of the research and revised it; A. B..: Prepared chemical solutions, aquaria and performed the statistics; and R. S.: suggestion the research, writing - review and editing.

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النتائج المصلية الدموية كإشارات مبكرة في البلطي النيلي اوريكروميس نيلوتيكس المعالج بكلوريد البنزالكونيوم

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الخلاصة

الهدف الرئيسي من هذه الدراسة هو تقييم سمية كلوريد البنزالكونيوم في الاستزراع المائي ، باستخدام المؤشرات المصلية الدموية لسمكة البلطي النيلي Oreochromis niloticus كمؤشرات حيوية. بعدما تعرضت الأسماك لثلاث مستويات من كلوريد البنزالكونيوم 0.1 ، 0.25 ، 0.50 ، و 1 ملغم / لتر في الأحواض المائية لفترتين زمنيتين 21 و 42 يومًا ، تم تقييم الفحص الميكروبيولوجي في أحواض الأسماك ، بالإضافة إلى معايير الدم. أشارت نتائج الفحص البكتيري أنه باستثناء متوسط الفرق بين المعاملة الثانية والثالثة من كلوريد البنزالكونيوم (فرق غير معنوي – 42 يوم) فقد أظهرت النتائج فروقاً كبيرة عند مقارنة باقي المتوسطات في جميع المعاملات ($P \leq 0.01 \leq 0.05$). انخفضت مؤشرات الدم الرئيسية ككرات الدم الحمراء والهيموجلوبين بشكل متفاوت عقب التعرض للثلاث تركيزات الأولي عند مقارنتها بمجموعة السيطرة، ثم بلغت ذروتها عند أعلي تركيز من كلوريد البنزالكونيوم رغم عدم وجود فروق احصائية عند مقارنتها بمجموعة السيطرة (باستثناء الهيموجلوبين – 42 يوم) . على العكس من ذلك ، ارتفعت كرات الدم البيضاء بشكل سريع عند التركيز الأول من كلوريد البنزالكونيوم خاصة في الفترة 42 يوما بالمقارنةً نتائج مجموعة السيطرة. تغيرت متوسطات كرات الدم البيضاء بعد التعرض للتركيزين الثاني و الثالث ، قبل أن تبلغ ذروتها عند أعلي تركيز من كلوريد البنزالكونيوم. أظهرت MCV و MCH و MCHC تقلبًا طفيفاً بين مجموعة السيطرة والأسماك المعالجة . فيما يتعلق بالنتائج البيوكيميائية ، أظهر متوسط مستويات الكوليسترول والدهون الثلاثية نمطاً غير منتظم حسب تركيز المعالجة الكيميائية. تقلبت مستويات ALT و AST بين أسماك مجموعة السيطرة و مجموعة المعالجة الكيميائية الثلاث الأولي ، قبل أن تزداد عند التركيز الأعلى مع وجود فروق معنوية(باستثناءAST عند مقارنة التركيزين 3 و4 من كلوريد البنزالكونيوم عقب 42 يوم حيث كانت الفروق للمتوسطات غير معنوية من الناحية الاحصائية) عند مقارنة مجموعة السيطرة , BAC1,2,3 بالمجموعة التي تعرضت ل BAC4. بعد التعرض لكلوريد البنزالكونيوم ، بقيت المعلمات الأخرى ، بما في ذلك البروتين واليوريا كما هي في مجموعة السيطرة .أشار التغيير في بعض علامات الدم المصلية علي أن هناك استجابة حيوية فيسيولوجية للإجهاد الناجم عن التعرض للمبيدات الحيوية.

الكلمات المفتاحية: كلوريد البنزالكونيوم ، المؤشرات الحيوية ،المطهر، Oreochromis niloticus ، السمية.