



Evaluation of the effect of some chemicals used in the Nile tilapia (*Oreochromis niloticus*) culture

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ARTICLE INFO

Article History:

Received: May 3, 2021

Accepted: June 5, 2021

Online: June 9, 2021

Keywords:

Oreochromis niloticus,
oxytetracycline,
malathion,
qPCR,
DNA damage,
histopathological
alterations.

ABSTRACT

This study was conducted to assess genotoxic and histopathological changes induced by some chemicals applied in aquaculture as Oxytetracycline (OTC) from antibiotics group, Malathion (MAL) from pesticides group, or Formalin and malachite green FMG from disinfectants group in Nile tilapia (*Oreochromis niloticus*) culture as a model of toxicological studies in aquatic surroundings. Fish were exposed to OTC in a dose of 80 mg/kg/biomass/day, MAL in a dose of 0.1 ppm, or FMG in a dose of 100 ppm and 0.2 ppm respectively for 21 days. The genotoxic effect was determined through DNA damage detection by comet assay as revealed a significant increase in tail length, tail DNA%, and tail moment, all chemicals revealed DNA damage when compared to control. qPCR revealed significant ($p < 0.05$) down regulation of immunity-related genes, MHC IIA, Tlr7, and IgM heavy chain in head-kidney and significant ($p < 0.05$) upregulation in the expression level of the inflammatory gene, TNF- α , in liver tissue of all chemicals treated groups in comparison with a control group. Histopathological examination of liver and kidney revealed hepatocyte and renal damage.

INTRODUCTION

Fish has long been providing food or animal protein for millions of people to meet the increasing domestic and foreign demand for fish and fishery product, aquaculture has expanded and now become a booming industry around the world and it is expected to remain one of the most important rising animal food-producing sectors in terms of food nutrition, jobs, and foreign exchange earnings in any country economy (FAO, 2012). Global aquaculture production has doubled over the past decades, now accounting for 50% of fishery products for human consumption according to a report by the Food and Agriculture Organization (FAO, 2014). China produces 41.1 million tonnes of farmed aquaculture products in 2012, contributing to approximately 61.7% of total world output, as the main

supplier of aquaculture products (**Liu *et al.*, 2017**). Egypt aquaculture industry has grown at a faster rate than the world population growth over the last five decades (**Salih *et al.*, 2017**). Semi-intensive and extensive aquaculture are giving way to intensive aquaculture (**FAO, 2010**). Level of production reach to 200 tonnes/hectare/year in intensive aquaculture systems as it can produce very high densities of fish, up to 200 kg per m³ of water (**Shaalan *et al.*, 2018**). Feeding twice a day or constantly during daylight hours, resulting in a massive nutrient load (**Rosa *et al.*, 2020**).

Nile tilapia (*O. niloticus*), is an important freshwater fish species. It is one of the most cultivated species worldwide under a wide range of geographic, climatic, and technological conditions (**Van Doan *et al.*, 2019**), its tolerance to consuming a wide variety to natural food organisms to various environmental factors such as low water quality and diet types (**Shaheen *et al.*, 2013; Zulfiqar *et al.*, 2020**), It is considered the main cultivated and the most consumable fish in Egypt (**Ahmed *et al.*, 2021**), that contributes with about 65.15% of Egyptian fish production (**Gafrd, 2017**).

Fish commonly used as an animal model in toxicology studies, since fish occupy the top of the aquatic food chain, they are commonly used as a bio-indicator for assessing aquatic ecosystem health (**Burgos-Aceves *et al.*, 2018**), where the tilapia species is subjected to environmental stressors and rearing water toxicity (**Abdel-Daim *et al.*, 2019; Dawood *et al.*, 2020**).

Expansion usage of chemicals and drugs have been increased for many years as a part of management either to prevent or to treat diseases (**Dogan *et al.*, 2020**). It is reported that in commercial farming in the country the farmers exercise high stocking density, which subsequently necessitates larger use of fertilizers and supplementary feed references. Thus, the water quality and aquatic environment of the farms are deteriorated, which makes the fish/shrimp more susceptible to diseases. The disease outbreak in aquaculture occasionally leads to mass mortalities and economic losses references.

Therefore, the farmers/entrepreneurs indiscriminately use chemotherapeutics, feed additives, growth enhancers, etc. in aquaculture to increase fish/shrimp production and to combat diseases. For health control, the following are some of the commercial chemical products and medications currently used in aquatic life: Antibiotics (Tetracycline, Teramycene, Nitrofurantoin, Chloramphenicol, and others), methylene blue, sodium chloride, formalin, malachite green, potassium permanganate and glutaraldehyde (**DOF, 2002**).

Among pharmaceuticals, antibiotics (antibacterial or antimicrobials agents) are one of the most widely used groups, for human and veterinary uses having livestock and aquaculture growth promotion and prophylaxis (**Yi *et al.*, 2019**). They are extensively used in aquaculture where they may be applied directly into the

water or be incorporated in the food (Sharma, 2021). Owing to its higher effectiveness and lower cost as compared to other antibiotics, OTC is one of the most widely used antibiotics in fish farms (Ren *et al.*, 2017).

Pesticides are chemical compounds or a mixture of substances used in agricultural crops controlling a wide range of insectivorous and herbivorous pests used for repelling, preventing, destroying, mitigating or that would otherwise reduce food production quantity and quality (Abd El-satar *et al.*, 2019), MAL is a non-systemic, wide-spectrum organophosphorus insecticide. MAL is commonly used in agriculture. Aquatic organisms, particularly fish, are highly sensitive to MAL (Uikey, 2015).

Disinfectant are chemical mixtures that eliminate many or all undesirable microorganisms (Stephen & Hanne, 2013). Formaldehyde (formalin) is a chemical compound (CHO) that can be applied basically as an outside parasiticide on fish and fish eggs as indefinite treatment for parasite control (Leal *et al.*, 2018). Nowadays, the Food and Drug Administration (FDA) has approved and recommended three commercial products with formalin as an active principle for use in aquaculture, especially for the eradication of monogenean infestations (i.e. *Gyrodactylus* spp., *Dactylogyrus* spp., *Cleidodiscus* spp.) (Nasser *et al.*, 2017).

Malachite green seems to be an organic pigment that has long been used as a fish fungicide and parasiticide. Instead of building out structures, it is mainly used inside cultivation services. Because of the persistent residues, a long withdrawal period is required after submission (Sudova *et al.*, 2007). FMG can be used independently for treatment, but during treatment, they are usually used together because of the synergistic effect. They are more successful when they work together than when they work separately.

The present investigation has been carried out to determine the sub-chronic toxicity of OTC, MAL or FMG to assess the dangerous effect on the health of Nile tilapia, *O. niloticus*.

MATERIALS AND METHODS

2.1. Chemicals

2.1.1. Oxytetracycline

The commercial form of OTC (Terramycin[®]) used in the experiments was obtained from Pfizer (New York, New York, United States).

2.1.2. Malathion

The insecticide used in this study was 5% (Powder form) emulsifiable concentrate of MAL (O, O dimethyl S-1,2 dicarbo-ethyl-thiophosphate) with molecular weight 330.35, was purchased from a local retail pesticides shop.

2.1.3. Formalin

37% formaldehyde (El- Nasr Pharmaceutical Chemical Co., Egypt).

2.2.4. Malachite Green

(Deisenhofer, Germany: Sigma). The dose was determined using the treatment concentration (0.20 mg L⁻¹) of fish exposed to malachite green for a long-term bath (1/4 or 1/5 of the LC₅₀ value).

2.2. Test organisms

A total number of one hundred and twenty (120) apparently healthy Nile tilapia, *O. niloticus* were collected from a private fish farm in Al-Manzala, Al-Dakahlia Governorate, Egypt. The fishes' weight and length ranged from 50± 5 g and 15±1 cm, respectively were used and transported in large plastic water containers to Animal Health Research Institute (AHRI), Fish disease and management Lab., Mansoura branch.

The fish were maintained in fully prepared glass aquaria (80 × 35 × 40 cm) filled with approximately 80 liters of dechlorinated tap water and left without any treatment for two weeks for acclimation before starting the experiment. Fish were kept in standard condition during the period of acclimation and the experiment in which provided with adequate aeration, 12:12hour light-to-dark photoperiod and underwater internal power filter keep at temperature 24±0.5 °C. Using digital pH meter where pH is 7.6 ± 0.4 and dissolved oxygen (5.4±0.4 mg/L). During the experiment, the fish were fed with commercial pellets purchased from same farm containing 30% crude protein at 3% of body weight twice a day, and during the acclimation period and experimental phase the aquaria were siphoned off daily for avoiding stress to the fish. Dead fish were removed as quickly as possible to maintain water quality.

2.3. Experimental design and treatment

One hundred and twenty (120) freshwater Nile tilapia were divided into four groups (30 fish each), fish were randomly assigned in two replicates into 4 groups of 15 fish / replicate. The experimental period lasted 21 days. One of the following treatments was offered to each group:

Group 1 (G1): Fish were reared in dechlorinated tap water free from any chemicals and fed a basal diet (30% crude protein) considered as a Control group.

Group 2 (G2): Fish were fed the basal diet supplemented with OTC at (80 mg/ kg/biomass/day) (**Julinta *et al.*, 2019**), antibiotics were coated with the fish diet by dissolving the antibiotics in liquid form on diet oil as a coating agent, mixing them and spray for fish diet. Next, let the fish diet containing antibiotics dry before administration.

Group 3 (G3): Fish were exposed to 1/ 4 LC₅₀ (0.1 ppm) of MAL 5% (powder form) in water and fed on a basal diet (30% crude protein). 1 mg of MAL was dissolved in 1 ml of distilled water to prepare a stock solution and then it

diluted to determine the test final concentration 0.1 mg / L (**Ibrahim, 2019**) of MAL in the tanks.

Group 4 (G4): Fish were exposed to Formalin 37% and Malachite green in the ratio (100 ppm (**Andem et al., 2015**), 0.2 ppm (**Sudova et al., 2007**) respectively in water and fed on the basal diet (30% crude protein).

The fish of all groups were fed 3% of their body weight two times daily for 21 days. The half amount of the water was changed daily to avoid the accumulation of fecal matter during acclimatization period. And after the start of the experiment, the water and chemicals were completely replenished day after day for 21 days of the experiment to maintain the toxicant concentration. Water quality parameters were monitored throughout the test.

2.4. Tissue sample collection and preparation

liver and kidney were collected. Liver was divided into three slices: the first one was kept in sterile Eppendorf tube, and then kept in -20 °C for determination of gene expression (Real-time PCR), the second part (0.5g) was homogenized in 4.5 ml ice-cold phosphate buffer saline (Oxoid) at PH 7.4 (Sigma, UK). Liver homogenate in sterile plastic tubes was stored in -20 °C was used for comet assay analysis and the third part of liver and kidney samples were stored in 10% formalin for histopathological analysis (mainly most affected whole liver and kidney tissue). Kidney sample was taken for gene expression and histopathological analysis.

Water samples were taken at the start and endpoint of the experiment for measuring of physical and chemical properties of it.

2.5. Genotoxicity (DNA damage detection)

The Comet assay (single cell gel electrophoresis, SCGE) was used to detect any potential DNA damage after various treatments. It investigates alkali labile sites and DNA strand breaks by measuring the migration of DNA from immobilized nuclear DNA. In the current study, the comet assay was conducted according to (**Singh et al., 1988**) and the calculations were done as previously described (**Badawy et al., 2018**).

2.6. Molecular investigations (RNA Extraction, Synthesis of cDNA, and Real-time quantitative PCR (RT-qPCR))

RT-qPCR was used to analyze gene expression, RNA was extracted from head kidney and liver tissues according to constructor's instructions, using the total RNA Purification Kit (Thermo Scientific, Fermentas, # K0731), quantification of the resulting RNA was done using Nanodrop, to ensure that the levels were pure enough just to perform real-time PCR. Use (Uv-Vis spectrophotometer Q5000/USA) to measure the sum of nucleic acids the absorption of Ultra-Violet light with OD at 260/280 nm by purines and pyrimidines ring structure can be used. To convert RNA into complementary DNA (cDNA), 5 µg of template RNA was

reverse transcribed using Revert Aid™ H Minus Reverse Transcriptase (Thermo Scientific, Fermentas, #EP0451), which is a genetically modified M-MuLV RT. The expression of target genes' mRNAs in the head kidney and hepatic tissue was examined using Real-time PCR with SYBR Green, with β -actin as an internal guide. Amplification of isolated cDNA was done using 2X Maxima SYBR Green/ROX qPCR Master Mix (# K0221, Thermo scientific, USA) and gene-specific primers according to manufacture's instructions. The primer pairs used in the amplification are listed in **Table 1**. The primer sets based on published Nile tilapia sequences and were developed using the web-based tool Primer 3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). To confirm that the primer sequence matches the template sequence exactly, we used BLAST (www.ncbi.nlm.nih.gov/blast/Blast.cgi) to compare it to other known sequences. RT-qPCR was carried out in a 25 μ l of 3 μ l cDNA template, 1 μ l of each Forward and reverse primer, 12.5 μ l of 2X Maxima SYBR Green and 7.5 μ l nuclease free water. Step One Plus real time thermal cycler used where PCR thermal condition as follow, cDNA initial denaturation at 95°C/ 10 min, then 40 cycles of denaturation at 95°C/15 sec, annealing 60°C/ 30 sec, and extension at 72°C/ 30 sec. The quantities critical threshold (Ct) of target gene were normalized with quantities (Ct) of housekeeping gene (β -actin). For the test groups, assessment of fold change of relative gene expression was calculated as follow: Fold change = $(2^{-\Delta\Delta Ct})$ (Livak & Schmittgen, 2001).

2.7. Physicochemical properties of waters

Water quality were monitored and sampled daily while remnants of the unconsumed feed and the excreta were also siphoned. Water quality parameters such as Temperature, pH, Ammonia, Nitrite, Total hardness, Total alkalinity and Dissolved oxygen were monitored and estimated, following the procedure of (APHA, 2005).

Table 1: Primers sequence for MHC IIA, Tlr7, IgM heavy chain, TNF- α , and B actin used for gene expression.

Gene	Forward primer (5' ----- 3')	Reverse primer (5' ----- 3')
<i>MHC IIA</i>	CATCAAAGGAAACGAGTGCA	CTACTTTCAGCCGCTCTCCC
<i>Tlr7</i>	TCAGCAGGGTGAGAGCATAC	ACATATCCCAGCCGTAGAGG
<i>IgM heavy chain</i>	AGGAGACAGGACTGGAATGCACAA	GGAGGCAGTATAGGTATCATCCTC
<i>TNF-α</i>	GGTTAGTTGAGAAGAAATCACCTGCA	GTCGTCGCTATTCCCGCAGATCA
<i>B-actin</i>	CAGCAAGCAGGAGTACGATGAG	TGTGTGGTGTGTGGTTGTTTTG

2.8. Study of Histopathological Alterations

Tissue specimens from liver and kidney were collected by sacrificing the fish. For a period of 24 hours, the tissues were held in 10% formaldehyde solution. The fixed tissues were exposed to increasing concentrations of ethanol (30, 50, 70, 90 and 100 percent) (Al-Gomhoureia Co., Egypt) to dehydrate them. The dehydrated liver samples were cleared in xylene until they became translucent. Then transferred to molten paraffin wax for 1h to remove xylene completely and then embedded in wax. The embedded liver samples were sectioned at 4 to 6 μm thickness. Tissue slices were adhered to clean and dry slides. Staining with haematoxylin and eosin after drying. Lastly, the liver as well as kidney slices were investigated under light optical microscope for any structural alterations and compared to the control group (Bancroft & Gamble, 2008).

2.9. Statistical analysis

Means \pm S.E. were used to explain the experimental results. One-way analysis of variance (ANOVA) was used to determine the factual significance using SPSS ver. 18.0 software, 2011. and Duncan's multiple range test was used to assess the individual associations, when $p \leq 0.05$ was achieved, the values were deemed statistically significant. The data were presented as mean \pm standard error of the mean (SEM), with a significance level at $p \leq 0.05$.

RESULTS

DNA damage detection

DNA damage in liver of fish following exposure to OTC (G2), MAL (G3) or FMG (G4) determined by comet assay were shown in (Table 2 and Fig. 1). Exposure of fish to OTC (G2), MAL (G3) or FMG (G4) result in significant increases in DNA damage ($P < 0.05$), with highest damage in G3 followed by G4 then G2, as compared to control group (G1) (Table2, Fig.1). Increases in tail length, tail DNA percent, and tail moment suggested this damage.

Table 2: Parameters of comet assay obtained by image analysis in liver cells of all groups after 21 days' treatment experiment.

<i>Group</i>	<i>Tailed %</i>	<i>Untailed %</i>	<i>Tails length μm</i>	<i>Tail DNA%</i>	<i>Tail moment</i>
<i>G1</i>	2.5 ^d	97.5 ^a	1.80 \pm 0.15 ^d	1.52 ^d	2.74 ^d
<i>G2</i>	10 ^c	90 ^b	3.79 \pm 0.19 ^c	2.75 ^c	10.42 ^c
<i>G3</i>	31 ^a	69 ^d	10.48 \pm 0.71 ^a	8.83 ^a	92.54 ^a
<i>G4</i>	18 ^b	82 ^c	6.27 \pm 0.29 ^b	5.01 ^b	31.41 ^b

Different superscript letters in the same column of tail length showed significance difference at $P < 0.05$

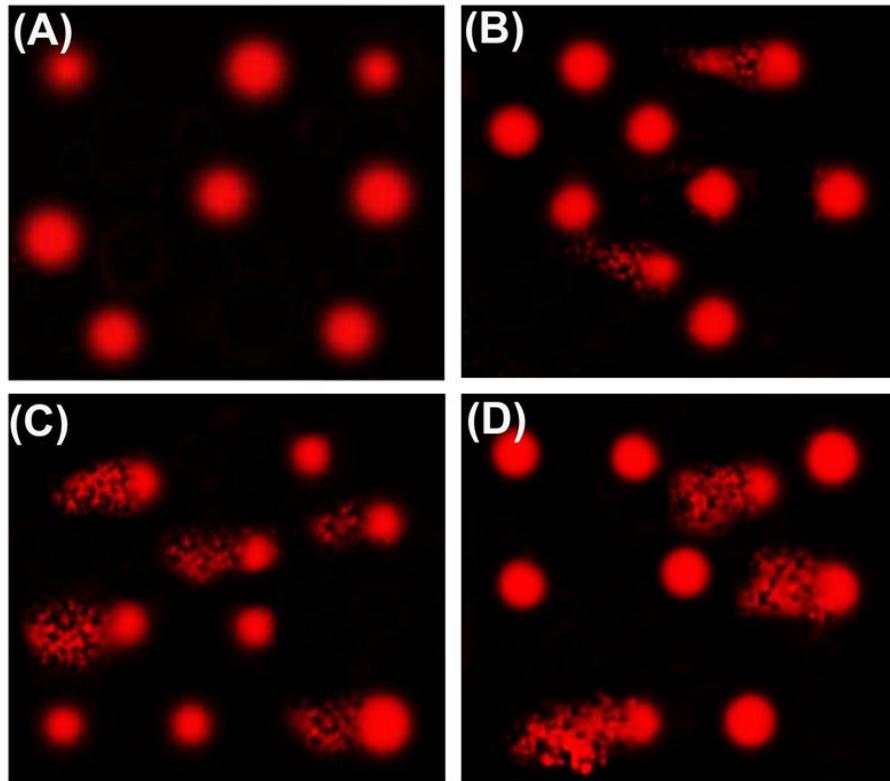


Fig.1. DNA damage photomicrographs representation in hepatic sample tissues, in control group (A), OTC(B), MAL (C), or FMG (D) following 21 days' exposure.

Immune gene expression

The obtained qPCR results showed a significant downregulation in the expression levels of the MHC IIA, IgM heavy chain, Tlr7 genes (**Table 3, Figs 2, 3, 4**) respectively in head kidney of fish treated with OTC (G2), MAL (G3) or FMG (G4) with lowest expression in G3 followed by G4 then G2 compared to control, but significant upregulation appeared in the expression levels of the TNF- α gene (**Table 3, Fig. 5**) in the liver of fish treated with OTC (G2), MAL (G3) or FMG (G4) with highest expression in G3 followed by G4 then G2 compared to control.

Table 3: Relative expression of *MHCIIA*, *IgM heavy chain*, *TLR7* in *O. niloticus* head-kidney and *TNF- α* in liver following treatments with OTC(G2), MAL (G3) and FMG (G4) for 21 days.

Group	<i>MHC IIA</i>	<i>IgM heavy chain</i>	<i>TLR7</i>	<i>TNF-α</i>
<i>G1</i>	1.00 ^a \pm 0.05	1.00 ^a \pm 0.07	1.00 ^a \pm 0.05	1.00 ^d \pm 0.08
<i>G2</i>	0.46 ^b \pm 0.02	0.53 ^b \pm 0.04	0.67 ^b \pm 0.03	2.55 ^c \pm 0.13
<i>G3</i>	0.24 ^d \pm 0.01	0.07 ^d \pm 0.003	0.19 ^d \pm 0.01	5.74 ^a \pm 0.27
<i>G4</i>	0.32 ^c \pm 0.02	0.31 ^c \pm 0.02	0.42 ^c \pm 0.03	3.89 ^b \pm 0.21

Means within the same column carrying different superscript letters are significantly different ($P \leq 0.05$).

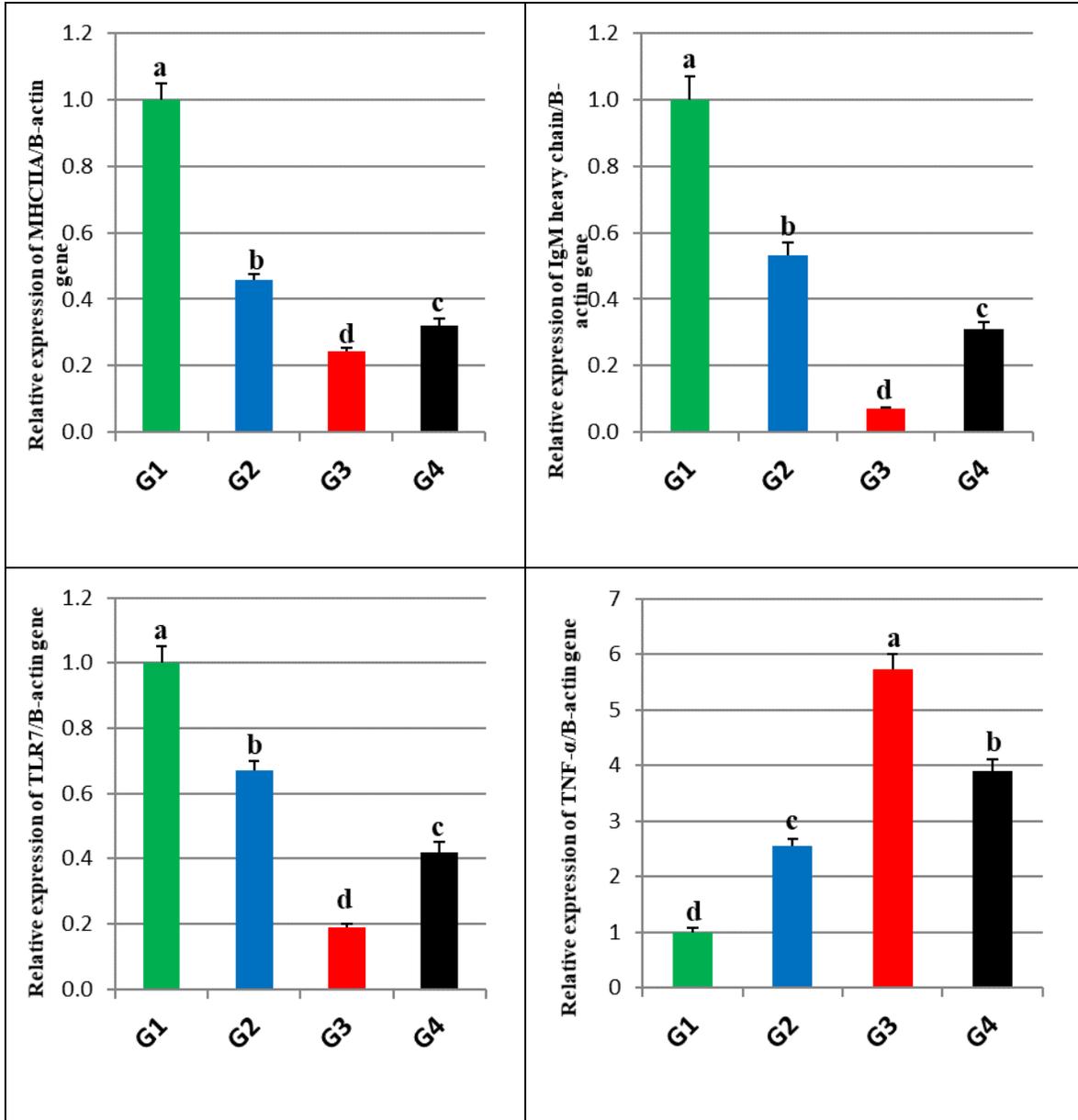


Fig. 2. Effect of OTC, MAL, or FMG on expression of *MHCIIA*, *IgM heavy chain*, *TLR7* genes in head-kidney and *TNF- α* gene in liver after 21 days of treatment. Data presented as mean \pm SEM (n = 7/experimental group). Columns with dissimilar letters are significant.

Physicochemical Properties of Test Water

There was no mortality in any experimental group during experimental period. The physico-chemical characteristics of the water for all the experimental periods remained constant and found to be within the limits of EOS- (1589/2005) as presented in (Table 4).

Table 4: Physico-chemical water parameters used in the experiments. Values are shown as mean \pm SD (n = 5).

Analysis	Result	Permissible limits by EOS- (1589/2005)
Appearance / Color	Clear	Clear
Odor	Odourless	Odourless
pH	7.4	6-7.5
Temperature	25	23.6-25
Conductivity (ms/m)	1520	2000 (Microms/m)
Calcium(mg/l)	80	Up to -150
Calcium Carbonate(mg/l)	200	Up to -400
Magnesium(mg/l)	24	Up to -50
Magnesium Carbonate(mg/l)	84	Up to -175
Total hardness (mg/l)	284	Up to -500
TSS(mg/l)	150	Up to -250
TDS(mg/l)	1110	Up to -750
TS(mg/l)	1260	Up to -1000
Chloride(mg/l)	350	Up to -250-H Up to -500-A
Ammonia(mg/l)	-Ve	Up to -0.5
Nitrite(mg/l)	-Ve	Up to -0.02
Sulphate(mg/l)	54	Up to -25
Phosphours(mg/l)	-Ve	No limit
Phosphate(mg/l)	-Ve	Up to -5
Free Chlorine(mg/l)	0.32	0.4-5
Total alkalinity (mg/l)	160	Up to -100
Bicarbonate alkalinity (mg/l)	160	No limit
Carbonate alkalinity (mg/l)	0	No limit
Hydroxide alkalinity (mg/l)	0	No limit
Lead(ppb)	-Ve	0.01
Copper(ppm)	-Ve	2
Iron(ppm)	0.006	
Manganise(ppm)	-Ve	0.1
Zinc (ppm)	-Ve	3
Cynide test	-Ve	

The results of histopathological examination

1- Liver histopathology

Histopathologically, liver section of G1 (non- treated) control group showed intact normal pancreatic acini and blood sinusoids surrounded with intact hepatocytes (**Fig.3 A**). G2 (OTC treated) revealed pancreatic acini, small areas of hemorrhage, congested blood sinusoids, degeneration of pancreatic and hepatic cells in addition to severe vacuolation in hepatocytes (**Fig.3 B**). G3 (MAL treated) showed shrinkage, congested blood sinusoids, vacuolation and degeneration hepatocytes (**Fig.3 C**). On other hands, G4 (FMG treated) showed sever effect after 21 days of exposure where, necrosis and degeneration occurred in pancreatic acini and hepatic parenchyma, lymphocytes aggregation in addition to hemorrhage and hemosiderosis (**Fig.3 D**).

2- Kidney histopathology

Histopathologically, kidney section of G1 (non- treated) control group showed normal-sized blood vessel, melanomacrophage center, hemopoietic tissue and lymphopoietic tissue (**Fig.4 A**). G2 (OTC treated) revealed blood vessel, increase in melanomacrophage center, mild increase in lymphopoietic tissue over hemopoietic tissue with presence of hemosiderosis (**Fig.4 B**). G3 (MAL treated) showed severe congestion of the blood vessel, degeneration in the kidney parenchyma and increase in melanomacrophage center (**Fig.4 C**). On other hands, G4 (FMG treated) showed degenerative changes in the parenchymatous tissue with increase in melanomacrophage center as shown in (**Fig.4 D**).

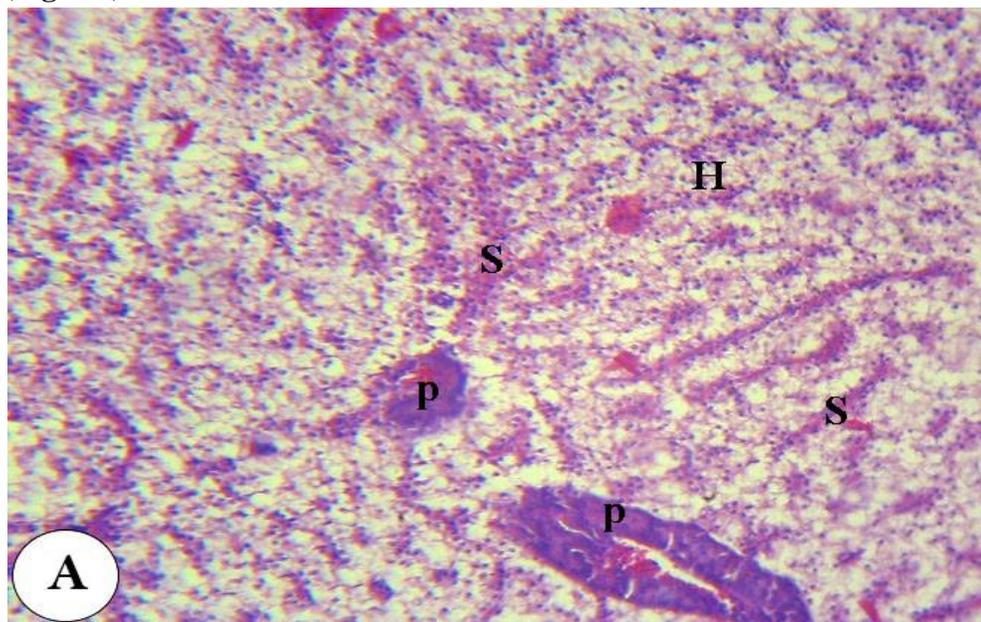


Fig. 3. A: hepatopancreas of control group showing intact normal pancreatic acini (P) and blood sinusoids (S) surrounded with intact hepatocytes (H). H&E, X

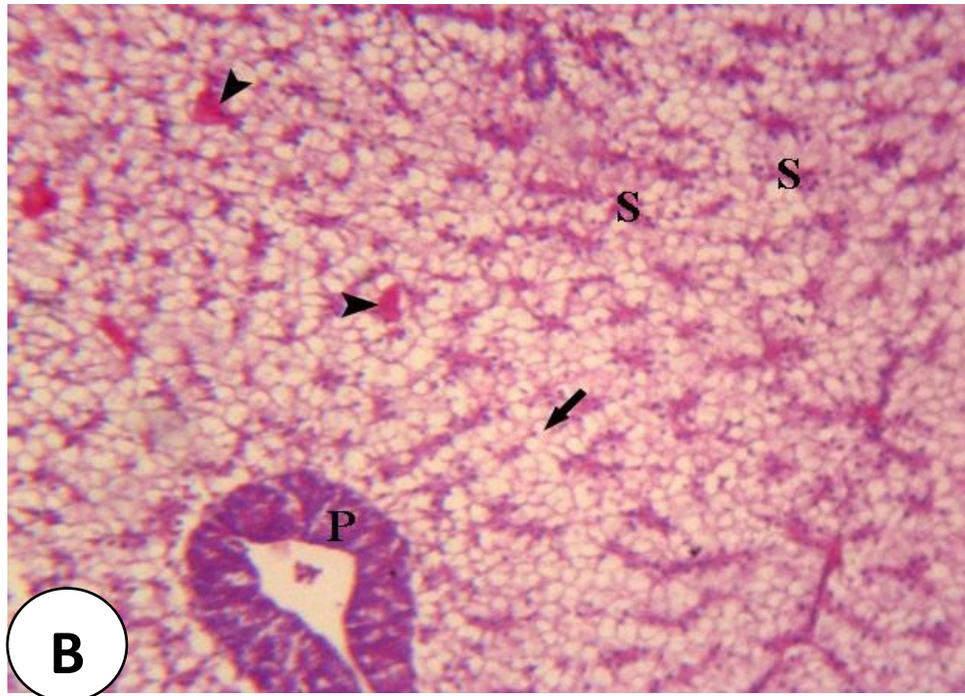


Fig. 3 (B): Hepatopancreas of OTC group after 21 days showing pancreatic acini (P), small areas of hemorrhage (arrow head), congested blood sinusoids (s) and vacuolation of hepatocytes (arrow). H&E, X 100.

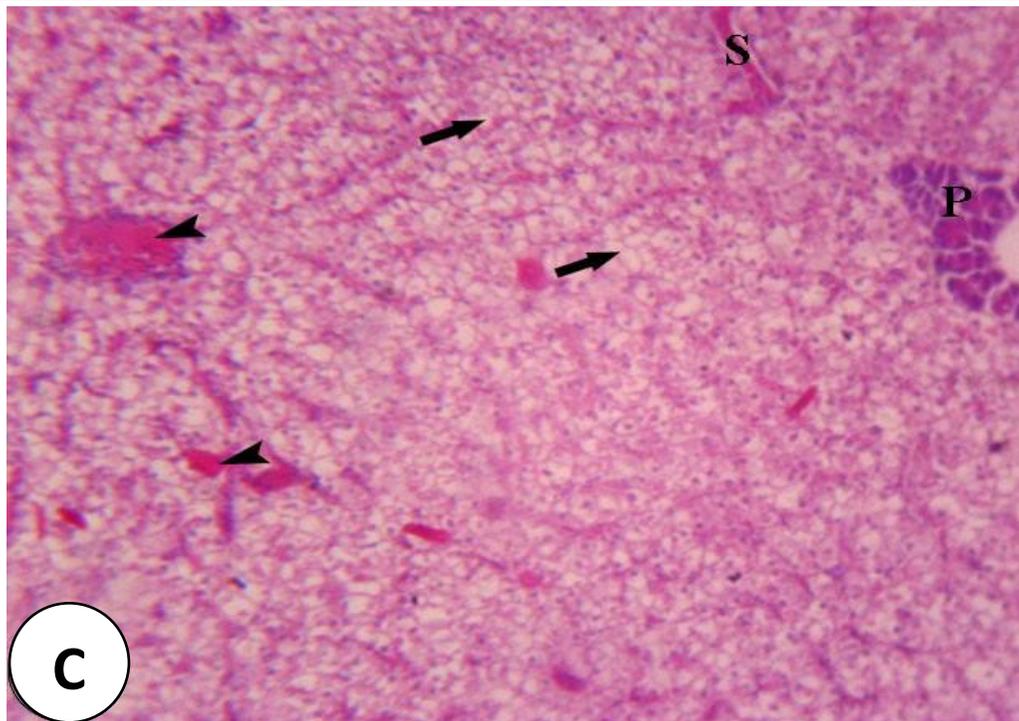


Fig. 3 (C): Hepatopancreas of MAL group after 21 days showing shrinkage of pancreatic cells (P), congested blood sinusoids (S), focal areas of hemorrhage (arrow head) and vacuolar degeneration of hepatocytes (arrow). H&E, X 100.

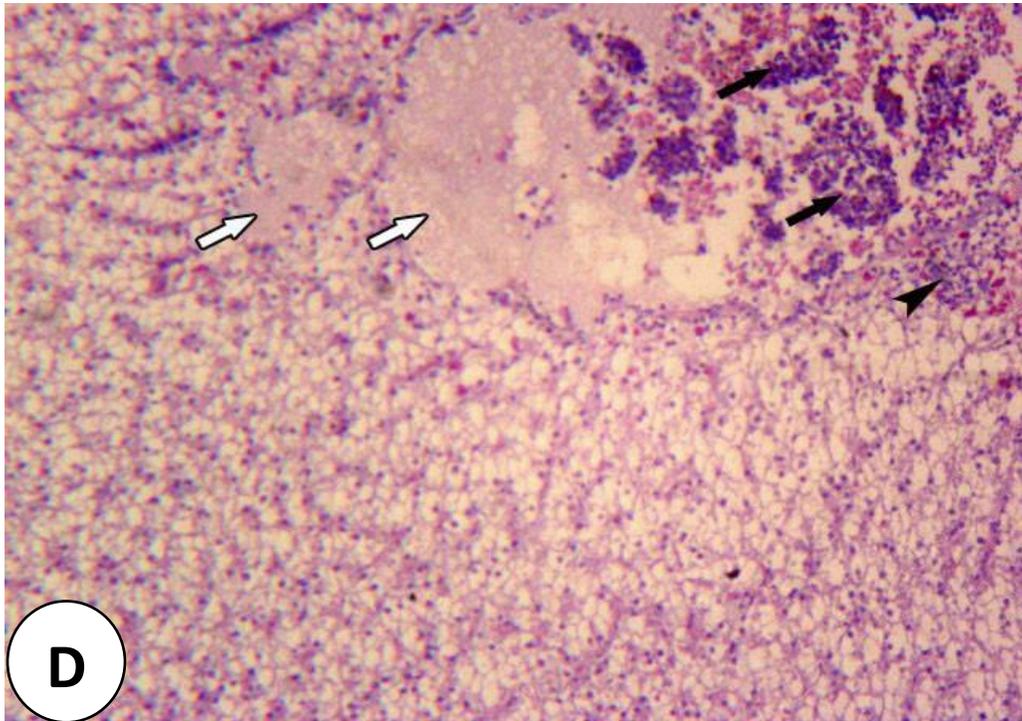


Fig. 3 (D): Hepatopancreas of FMG group after 21 days showing necrosis of pancreatic acini (black arrow) and hepatic parenchyma (white arrow) in addition to lymphocytes aggregation (arrow head). H&E, X 100.

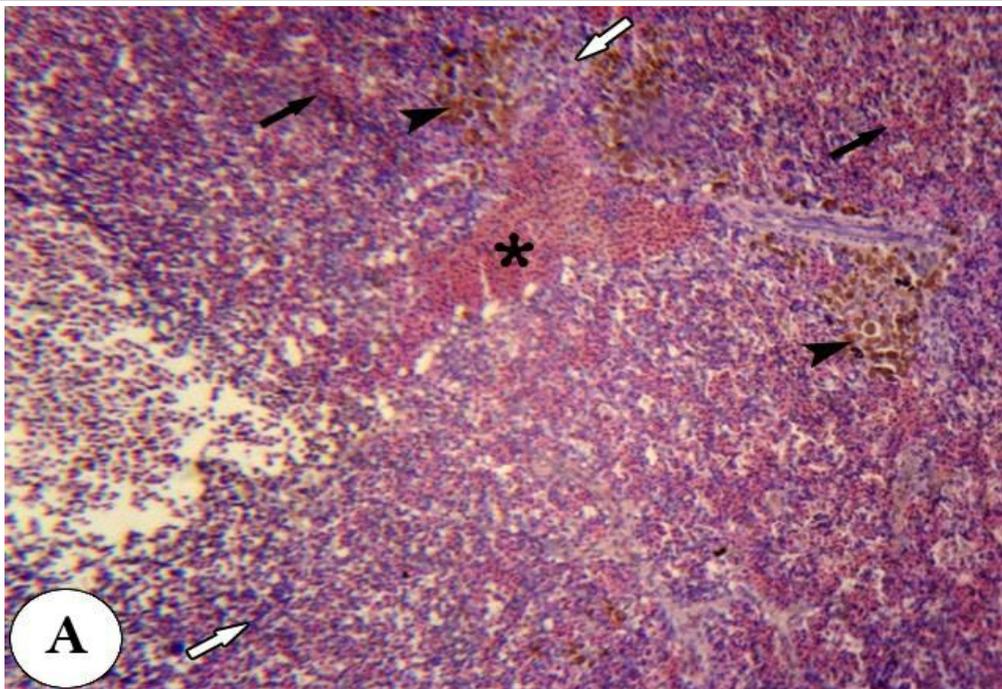


Fig. 4 (A): Anterior kidney of control group showing normal-sized blood vessel (asterisk), melanomacrophage center (arrow head), hemopoietic tissue (black arrow) and lymphopoietic tissue (white arrow). H&E, X 100.

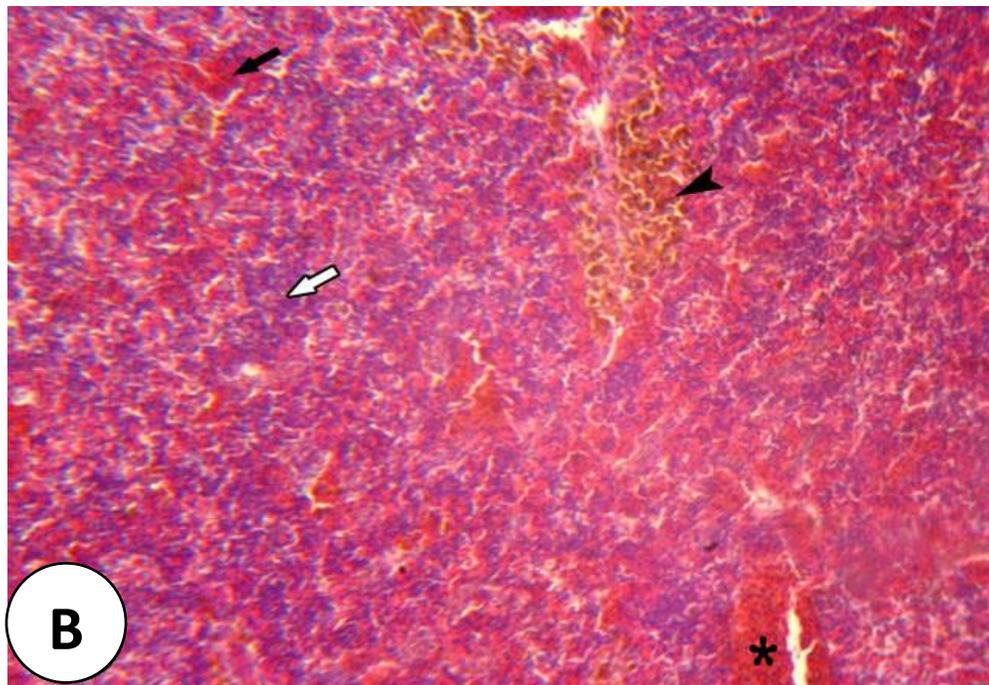


Fig. 4 (B): Anterior kidney of OTC group after 21 days showing blood vessel (asterisk), increase in melanomacrophage center (arrow head), mild increase in lymphopoietic tissue (white arrow) over hemopoietic tissue (black arrow). H&E, X 100.

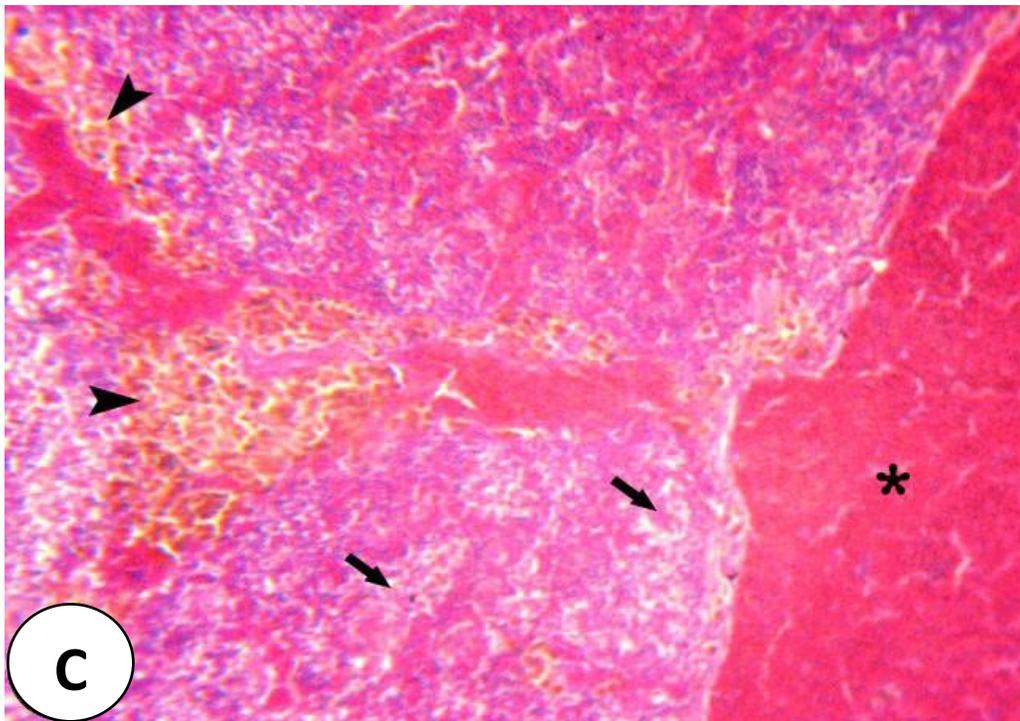


Fig. 4 (C): Anterior kidney of MAL group after 21 days showing severe congestion of the blood vessel (asterisk), degeneration in the kidney parenchyma (arrow) and increase in melanomacrophage center (arrow head). H&E, X 100.

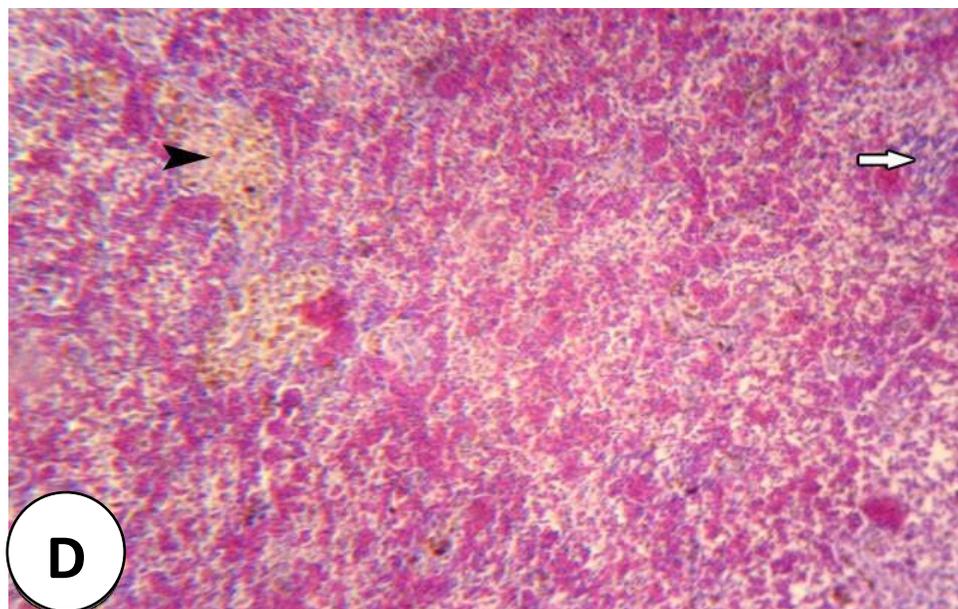


Fig. 4 (D): Anterior kidney of FMG group after 21 days showing increase in melanomacrophage center (arrow head) in addition to depletion of lymphopoietic tissue (white arrow). H&E, X 100.

DISCUSSION

In many developing countries, aquaculture is crucial for providing food security for millions of People. It is therefore important to assess the full impact of the environmental changes on the Aquaculture sustainability. Because of over population and unintended civilization, many aquatic ecosystems are facing complicate problems of contamination (El-Adawy *et al.*, 2018).

Pressure outbreaks of disease caused by viral, bacterial, fungal and parasitic infections or Direct emissions of micro-contaminants from fish farming facilities in seawater may result in contamination of the marine environment have led to the use of chemicals (Lulijwa, 2020). Nile tilapia (*Oreochromis niloticus*) is an excellent model for aquaculture, fundamental research, and environmental contamination studies so present work was planned to illustrate, the genotoxic and histopathological effect of most common applied chemicals in aquaculture as OTC from antibiotics group, MAL from pesticides group or FMG from disinfectant group.

In the present study we found chemicals treated groups can significantly induce DNA damage of liver tissue cells indicating the genotoxic potential of this chemicals in Nile tilapia with high reference to MAL treated group. In support, similar results were reported by other studies such as (**Kushwaha *et al.*, 2000; Kumar *et al.*, 2010**), who concluded that, when teleost fish *Channa punctatus* (Bloch) was exposed to MAL levels, DNA damage levels increased in gill, kidney, and lymphocyte. The organ produces a number of metabolites during the detoxification process that challenge the integrity of prominent biomolecules such as proteins and DNA of liver cells and cause serious damage to DNA strands (**Cajaraville *et al.*, 2003**). Similarly, **Yin *et al.* (2008)** reported that genotoxicity in RBCs and liver cells of Chinese toad (*Bufo gargarizans*) when exposed to the sub lethal dose of chlorpyrifos. Also triclosan elicited genotoxicity in hemocytes of zebra mussel (**Hemalatha *et al.*, 2019**). Also, **Gyimah *et al.* (2020)** reported DNA damage in liver tissue of adult zebrafish after exposure to antimicrobial agent triclosan.

Other studies have also reported lead exposure causing genotoxic effects, such as chromosome aberration, mutation, inhibition of DNA synthesis and breakage of DNA (**Celik *et al.*, 2005**). **Abd El-satar *et al.* (2019)** reported similar result when exposed *Claries gariepinus* to lead acetat and chlorpyrifose.

Relative Changes in mRNA expression levels of MHC IIA, Tlr7, IgM heavy chain, TNF- α were revealed in response to OTC, MAL or FMG exposure in comparison with those in the non-treated group. The obtained result indicated that MAL exposure could significantly inhibit the MHC IIA, Tlr7 and IgM heavy chain gene expression followed by FMG then OTC, reducing the immune response of *O. niloticus* resulting in increased susceptibility to infection (**Blakley *et al.*, 1999**). In line with our results **Tang *et al.* (2021)** who found exposure of crucian carp to diazinon revealed that, the expression levels of TLR4, MyD88, NF-kB p100 and IL-8 genes were significantly reduced compared to those of the controls. In opposite the gene of mediators involved in the inflammation response as TNF- α gene, TNF- α is a proinflammatory cytokine involved in inflammation, apoptosis, cell proliferation, and the stimulation of various aspects of the immune system (**Ma *et al.*, 2014**). qPCR results showed a significant upregulation in the expression levels of the TNF- α gene in the treated chemicals liver group with highest expression in MAL exposure followed by FMG then OTC. In agree with our results **Liu *et al.* (2018)**, in the inflammatory response in zebrafish after paraquat (PQ) exposure, upregulation of IL-8 and NF-kB gene expression was found. TNF- α and TNF- β are important mediators of inflammatory and immune response mechanisms. TNF- α and TNF- β expression were also significantly up-regulated in

zebrafish exposed to fluoroquinolone and tetracycline (**Zhang et al., 2016**); TNF- α activation could trigger leukocytes to express adhesion molecules on their cell surface, resulting in diapedesis through individual endothelial vascular cells (**Wu & Chen, 2014**). In addition, **Ma et al. (2014)** reported that PQ significantly increased the expression levels of TNF- α gene expression in the spleen (1 d) and liver (3-7 d) of crucian carp. **Ryu et al. (2014)** showed that exposure to Perfluorooctanoic acid (PFOA) causes a marked increase in the mRNA expression level of IFN. **Wang et al. (2011)** reported that following exposure to atrazine, chlorpyrifos, or a mixture of both, IL-1 β expression was upregulated in the kidney and spleen of common carp. **Zhong et al. (2020)** found that after exposure to an organic pollutant (PFOA), the expression levels of both TLR-2 and MyD88 genes in zebra fish increased, implying that the immune response was influenced through the TLR/MyD88/NF- κ B pathway.

Physicochemical parameters such as temperature, pH, total dissolved solids, dissolved oxygen and electric conductivity are essential aquatic indices which determine growth, reproduction and fish health (**Audu et al., 2014**). All the water quality parameters measured in this study were within the Permissible limits by EOS- (1589/2005), for general fish survival and growth. Since MAL has a half-life of 0.2 weeks at pH 8.0 and 21 weeks at pH 6.0 (**Hazardous Substances Databank, 1988**), it was necessary to adjust the test water every other day with freshly prepared solution to keep the pesticide test concentration during the experiment.

In this study, MAL caused several kidney and liver lesions. While the liver is responsible for removing poisonous compounds and detoxify it, high levels of these toxic compounds cause tissue harm. Histopathological changes in the liver were similar to the organophosphate pesticide's typical histological reactions (**Subburaj, 2018**). **Dawood et al. (2020)** found the hepatotoxicity due to deltamethrine caused most hepatocytes to vacuolate, revealing pyknotic nuclei and fatty degeneration in the vacuolated hepatocytes. Our results are also in the same manner of the study carried out by (**Muttappa et al., 2015**) who revealed histopathological changes in the liver of Nile tilapia such as necrosis, vacuolization, increase in number of kupffer cells, and aggregation of melanomacrophge after exposure to ciprofloxacin, Histopathological alterations in the experimental organs of *Channa punctatus* fresh water fish were observed after exposure to sublethal concentrations of MAL for 7 days, according to (**Magar & Shaikh, 2013**), also reported Highly degenerative renal and collecting tubules with necrosis, shrinkage of glomeruli and renal tubules and spaces in Bowman's capsule. According to the finding of this study, *Channa punctatus* hepatic tissues exposed to MAL for 4 days showed degeneration of cytoplasm and vacuolization of hepatocyte.

The current findings are analogous with previous studies showing numerous histopathological changes caused by pesticides in the livers of various fish species as, zebra fish *Danio Rerio* (**Bhuvaneshwari *et al.*, 2015**) *Channa punctata* (**Tabassum *et al.*, 2016**), *Tilapia mossambica* (**Reddy, 2017**) *Labeo rohita* (**Ullah *et al.*, 2018**).

Histological examination is also applied to determine the risk effects of xenobiotics on fish (**Bojarski *et al.*, 2020**). In the present study areas of hemorrhage, congested blood sinusoids, degeneration of pancreatic and hepatic cells in addition to severe vacuolation in hepatocytes have been observed in the liver, increase in melanomacrophage center and mild increase in lymphopoietic tissue over hemopoietic tissue with presence of hemosiderosis observed in kidney following OTC exposure. In the same manner **Gyimah *et al.* (2020)**, histopathological alteration in zebra fish liver after chronic exposure (6 hpf to 90 dpf) to triclosan. **Islam *et al.* (2015)** found addition of 4 g/kg of OTC to *Barbonymus gonionotus* diet for 45 days displayed histopathological lesions in liver and kidney: fatty liver degeneration, hepatocytes vacuolation, and kidney lymphocyte aggregations. Therefore, because the liver is a vital organ in the metabolism and detoxification of xenobiotics, the damaging effects of OTC on the liver warrant scientific attention. Contrary to our results (**Dobšíková *et al.*, 2013**), histopathological examination of liver, gills, skin, spleen, and head kidneys of the common carp fed on OTC showed no pathological lesions.

In the current research, histological results of Nile tilapia treated with the FMG represented by detrimental effects in hepatic focal areas of hemorrhage, degenerative changes in hepatocytes and mono nuclear cells infiltration. These findings were in accordance with the experimental studies conducted by **Mert *et al.* (2015)** who postulated that Nile tilapia liver exhibited hyperaemia, hydropic degeneration and cloudy swelling, after exposure to formalin. (**El-Neweshy & Abou Srag, 2011**) found hydropic degeneration of the hepatic cells, cellular swelling of renal tubular and hemopoietic tissue necrosis after chronic malachite green toxicity in Nile tilapia, (**Adeyemo & Akano, 2012**) observed multifocal necrosis of hepatocytes in the liver after 3 days of exposure to 37 mg L⁻¹ formalin for 30 minutes per treatment. Malachite green affects mitochondria and causes nuclear defects in the liver, causing sinusoidal congestion and focal necrosis (**Gerundo *et al.*, 1991**). Following malachite green therapy, hepatocytes of *Heteropneustes fossilis* showed vacuolisation and hypertrophy, followed by necrosis and cirrhosis (**Srivastava *et al.*, 1998**). **Nouh & Selim (2013)**, intertubular capillary congestion, perivascular oedema, and tubular nephrosis were discovered when *O. niloticus* was exposed to 25.0 mg/L formalin, it developed a focal loss of haematopoietic tissue and vacuolization in the renal epithelium.

From our result, liver and kidney tissue damages were clearer in the all chemicals treated groups. In response to different chemicals, a number of histological changes in the liver have been observed in some studies (**Rafieepour et al., 2020; Tang et al., 2021**).

CONCLUSION

This study showed that even in small doses, MAL has a stronger toxic effect than OTC or FMG so we recommend that major principles underlying strategies to improve the efficiency and effectiveness of fish culture control should be followed, with efforts focused on issues that pose the greatest risk. The government must lay down requirements for fish culture safety and ensure that the requirements are through a range of regulatory measures.

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