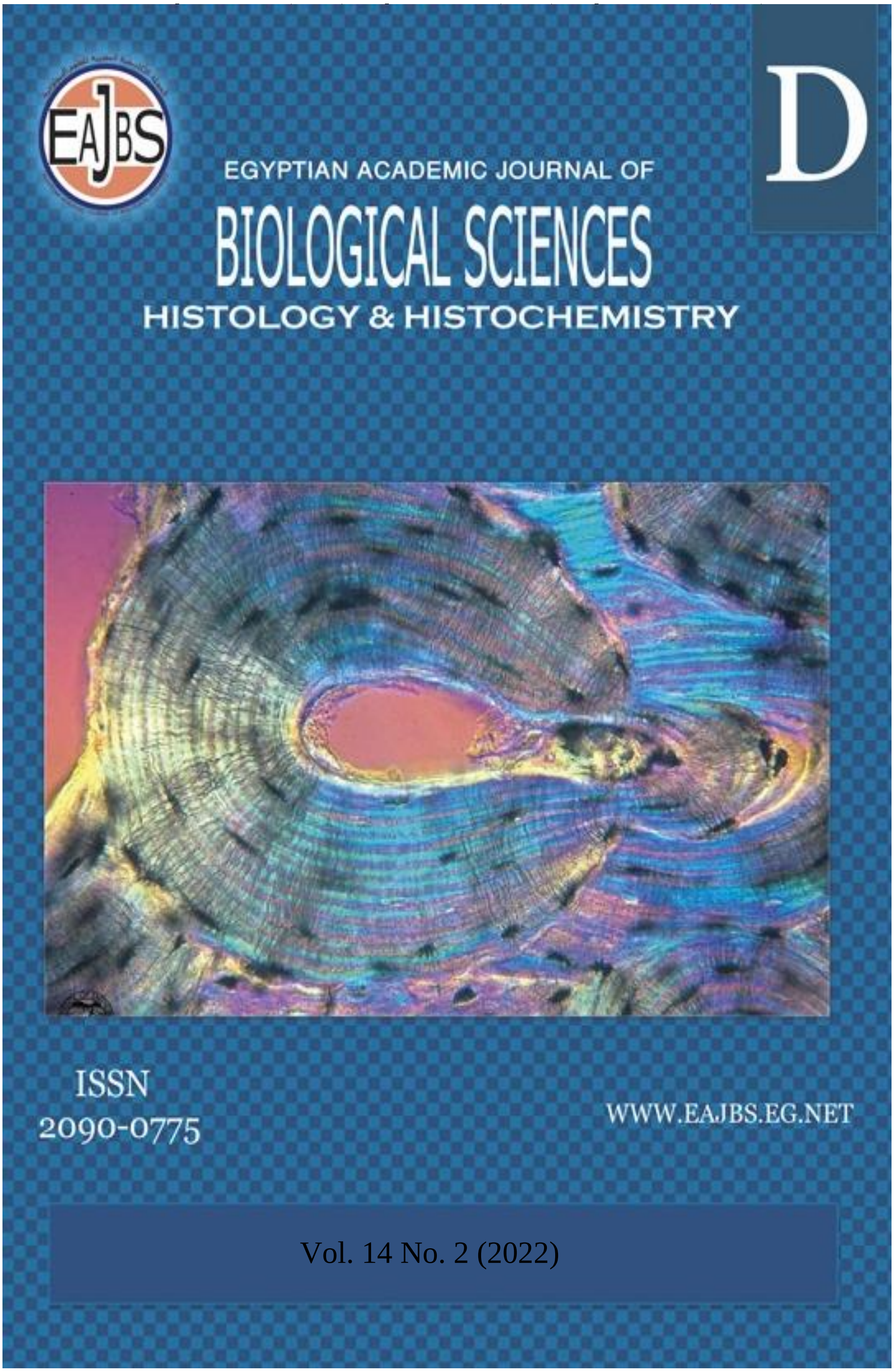
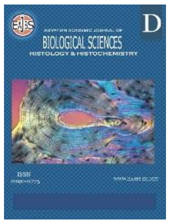


Citation: Egypt. *Acad. J. Biolog. Sci. (D-Histology and histochemistry) Vol.14(2) pp31-45(2022)*
DOI: 10.21608/EAJBSD.2022.259103

Egypt. Acad. J. Biolog. Sci., 14(2): 31-45 (2022)

Egyptian Academic Journal of Biological Sciences
D. Histology & Histochemistry
ISSN 2090 – 0775
http://eajbsd.journals.ekb.eg



Effect of Lead Toxicity on The Kidney of Nile Tilapia: Amelioration by Beta-MOS®

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ARTICLE INFO
Article History
Received:19/6/2022
Accepted:9/9/2022
Available:13/9/2022

***Keywords*:**
Tilapia, Lead, Kidney, Histopathology, Creatinine.

ABSTRACT

The present study investigated the possible ameliorative effect of Beta-MOS® on lead-induced toxicity in Nile tilapia. A total of ninety *O. niloticus* (34.43 ± 0.31 g and 7 weeks age) were used in this study. Fish of mixed sex were divided into 4 groups. The first group which served as control received a basal diet (0% Beta-MOS®). The second group received a diet supplemented with 0.3% Beta-MOS®. The third group was exposed to 10 mg Pb acetate L^{-1} in water and received a basal diet. The fourth group was exposed to the same dose of Pb acetate (10 mg L^{-1}) in water and given 0.3% Beta-MOS®. Initial and final weight as well as weight gain were recorded. Red blood cell count and hemoglobin concentration were determined. Creatinine plasma level was estimated as well as histopathological changes in the kidney. The lead acetate induced a significant reduction in final body weight, weight gain, red blood cell count and hemoglobin concentration than control. Moreover, creatinine was significantly increased in the lead group than control, with retrogressive changes in histopathological sections. Dietary Beta-MOS® ameliorated all the lead-induced perturbations in the tested parameters. Dietary Beta-MOS® ameliorated lead acetate toxicity in Nile tilapia.

INTRODUCTION

In Egypt, Nile tilapia, *Oreochromis niloticus*, is an important species in commercial fisheries and it is a widely distributed freshwater fish that can persist in a highly polluted habitat (Carvalho *et al.* 2012; Al-Asgah *et al.* 2015). Its suitability for culture comes from its tolerance to a wide range of environmental conditions, such as high and low water temperature, low oxygen content, high salinities, somewhat acidic or alkaline water as well as its utilization as food from the lowest trophic levels (Abdel-Backy 1997).

In addition, tilapia is considered one of the most common freshwater fish used in toxicological studies (Garcia-Santos *et al.* 2006; Figueiredo-Fernandes *et al.* 2007). This species displays many characteristics making it an appropriate model to be used as an indicator species in bio-monitoring programs (Gadagbui *et al.* 1996) because it can persist in highly polluted habitats (Atli & Canli 2010), its high growth rate, strong immune system, significant tolerance to environmental stress, ease of reproduction, and high market demand (El-Sayed 2006; Eissa *et al.* 2013).

Water pollution with toxic substances such as heavy metals is one of the most dangerous hazards in Egypt due to agricultural, industrial and urban wastes generated by human activities (Abdel-Mohsien & Mahmoud 2015). Moreover, fish is the most affected species as they take these wastes through skin, gills and intestine (Drishya *et al.* 2016). Some metals such as iron and manganese are required for metabolic activities in organisms and other elements such as cadmium, lead, chromium, zinc, nickel, copper, and mercury showed toxic effects on the aquatic organisms.

Among the toxic heavy metals in the water bodies, Lead (Pb) is more abundant than cadmium, copper, chromium, manganese and mercury and is an emerging worldwide concern because it exerts damaging effects on human health. It acts as a cumulative poison and It is listed by Environmental Protection Agency as one of the 129 priority pollutants (Kumar *et al.* 2007; Rajeshkumar *et al.* 2017).

Lead is a natural component of the Earth's crust and the main natural source of Pb emission is windblown dust, forest fires, volcanic emission and sea salt sprays (Kim & Kang 2016). Generally, Pb is found in trace amounts in soils, plants and water (Cheng & Hu 2010). Additionally, Pb exposure causes a large range of toxic effects on physiological, behavioral and biochemical functions in animals (Hsu & Guo 2002); it also causes serious damage to different systems including the central nervous system, reproductive system, hematopoietic system, cardiovascular system and organs such as liver and kidney (Flora *et al.* 2012). Moreover, Pb has the capability to accumulate in different tissues of exposed fish causing hepatic and renal damage along with growth retardation and inducing stress which resulted in impaired cortisol levels and metabolic enzymes (Anbu 2014).

One of the most popular strategies to improve animal health, including fish, is the use of functional dietary supplements (Hoseinifar *et al.* 2018). Mannan oligosaccharides (MOS) and Betaglucans (βG) are prebiotics that is commonly used in fish food (Abu-Elala *et al.* 2018). Both of them are naturally found in the cell walls of the yeast *Saccharomyces cerevisiae*. Other sources, such as torula yeast *(Candida utilis*), fungi, and algae, are also currently used as sources of βG and MOS (Raa 1996). Dietary supplementation with prebiotics has proved to be effective in promoting growth performance and regulating the immune response (Selim & Reda 2015), modulating the intestinal beneficial flora (Andrews *et al.* 2009), improving nutrient availability and inhibiting the infection by pathogens in fish (Abu-Elala *et al.* 2018). However, the protective effects of MOS and βG against Pb toxicity have not been fully evaluated in Nile tilapia. This study aimed to investigate the possible protective effect of dietary MOS and βG supplementation against Pb toxicity in Nile tilapia through hematological parameters, histopathological and biochemical investigations.

MATERIALS AND METHODS

Fish and Experimental Conditions:

Ninety freshwater mixed sex of Nile tilapia *O. niloticus* (34.43 ± 0.31 g and 7 weeks age) were used in this study. They were purchased from a private fish farm, Kafr EL-Sheikh governorate, Egypt and transported to the laboratory of Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt. They were treated with a 4% NaCl solution for 10 min for dermal infection removal (Suomalainen *et al.* 2005). Fish were sexed and distributed at the rate of 15 fish per 60 L aerated glass aquaria (60 × 30 × 40 cm^3) prewashed with NaCl solution (Suomalainen *et al.* 2005).

The water in aquaria was supplied from a reservoir constantly containing aerated water to remove any residual chlorine, water parameters (temperature, pH, salinity and dissolved oxygen) were controlled daily before and during the experimental period. Water temperature was kept between 26 and 28 ˚C using submerged heaters and pH of 7.56. The dissolved oxygen in each aquarium was maintained at close to saturation by using air pumps. The photoperiod was the natural daylight rhythm. A static/renewal bioassay was done; the aquaria were cleaned up (3 times/week) by siphoning half of the water from the aquaria and replacing it with an equal volume of water and adjusting the concentration of the tested chemical.

Experimental Design and Diets:

Fish of mixed sex were divided into 4 equal groups (3 replicates/ group); (Table 1). The first group which served as control received a basal diet (0% Beta-MOS®). The second group received a diet supplemented with 0.3% Beta-MOS® (βG & MOS) (EURO MARK Company, Italy). The third group was exposed to 10 mg Pb acetate 99.9% L^{-1} (6080-56-4, Sigma-Aldrich Company, Germany) in water and received a basal diet. The fourth group was exposed to the same dose of Pb acetate (10 mg L^{-1}) in water and nourished on 0.3% Beta-MOS®. The concentration of Pb was determined to be equal to the average Pb concentration in Suez Canal area. The experimental design was shown in Table 1.

Fish were acclimated in laboratory conditions for 2 weeks prior to the experimental work and were fed at a rate of 3% of their body weight with commercial submersible formulated dry pellets at least twice daily. Only disease-free and healthy fish were selected for experimentation. Initial body weight (W_I). The treatments were continued for 60 days. The mortality rate of each group was recorded daily from day one of the experiment and the dead fish immediately were removed to prevent contamination.

The experimental and control diets were formulated according to Jobling (2012) to fulfill the nutritional requirements of growing fish. The first diet was the control diet and the second one was incorporated with 0.3% Beta-MOS®. The two diets were formulated to supply 29% crude protein (CP) and 4100 kcal kg^{-1} gross energy as described in Table 2**.** Ingredients were ground into fine powder through a 175-µm mesh before pelleting and an appropriate amount of water was added to produce pellets. The pellets were produced by using California pelleting machine with a 2mm diameter at Fish Research Center, Faculty of Agriculture, Suez Canal University. All diets were packed in clean dry plastic jars and stored at 4 ºC in the refrigerator until use.

Table 1: Experimental design.

Fish group (45 fish/group)	Basal diet	0.3% Beta-MOS®	10 mg Pb L^{-1}
Group 1 Control	√		
Group 2		√	
Group 3	√		√
Group 4		√	√

Table 2: Composition of experimental and control Nile tilapia diets.

Ingredient	Unit	Basal diet	0.3% βG & MOS diet
Fish meal (67%)	%	12	12
Soybean meal (48%)	%	25	25
Yellow corn (9%)	%	31	31
Corn gluten meal (60%)	%	10	10
Wheat bran (8%)	%	18.6	18.3
Corn oil	%	1.85	1.85
Mono calcium phosphate (23.7%)	%	0.15	0.15
Vitamins & mineral premix*	%	0.3	0.3
Salt	%	0.5	0.5
Methionine	%	0.2	0.2
Lysine	%	0.3	0.3
Vitamin C	%	0.1	0.1
Beta-MOS	%	0	0.3
Total	%	100	100

Sampling and Body Weight:

Blood samples were obtained from the caudal vein, at the end of the experiment in EDTA tubes. The plasma was separated for creatinine estimation. The growth performance in terms of final body weight (W_F), and weight gain (W_G) were determined.

Hematology and Plasma Creatinine Level:

Blood collected in EDTA tubes was used to count RBCs and Hemoglobin (Hb) concentrations were determined spectrophotometrically via cyanomethaemoglobin procedure using Drabkin's solution and compared with the standard cyanmethaemoglobin (Qualigens) (Drabkin 1946).

Plasma creatinine was determined calorimetrically according to Tietz *et al.* (1995) method by using Diamond Diagnostics, Co, kit (Catalog No. CR 12 50, detection limit of 0.09 mg/dL to linearity limit of 15 mg/dL, sensitivity: 1 mg/dL), Egypt.

Histopathological Examination:

For histopathological investigation, treated as well as control fish were dissected at the end of Pb-exposure (60 days). kidneys were dissected out and washed in in physiological saline solution (NaCl; 0.60%). Small pieces from each organ were cut out and immediately fixed in Boun's fluid according to Wolf *et al.* (2004) for 48 h. The fixed tissues were dehydrated in ascending grades of ethyl alcohol, 1 h for each, then they were transferred in two changes of absolute alcohol for 1 h each. Tissues were cleared in two changes of xylene for 30 min and impregnated in soft paraffin wax at 60^o C for 2 h followed by embedding in hard paraffin wax. Sections of 5µm thickness were made using a rotary microtome, the sections were mounted on clean glass slides and stained with Mayer's Haematoxylin and counter-stained with aqueous Eosin solutions (Avwioro 2010). Six specimens of each tissue were sectioned per treatment and examined by light microscopy.

RESULTS

Body weight

No significant differences were observed in body weight values between all groups at day zero of the experimental period. The results revealed that Beta-MOS® supplemented group showed a significant increase ($p < 0.05$) in final body weight and weight gain than control. Meanwhile, the Pb-exposed group showed lower values in the final body weight and weight gain than the control. Administration of dietary Beta-MOS® to Pb-exposed fish significantly ($p < 0.05$) increased final body weight and weight gain in Pb-exposed group (Table 3).

Table 3: Effect of dietary Beta-MOS® on Weight final and gain in Pb-intoxicated Nile tilapia.

	Control	0.3 % Beta-MOS®	10 mg Pb L^{-1}	10 mg Pb L^{-1} + 0.3 % Beta-MOS®
$W_{initial}$ (g)	34.43 ± 0.31	34.11 ± 0.52	34.24 ± 0.61	34.12 ± 0.52
W_{final} (g)	55.65 ± 0.37 ^{b}	66.09 ± 0.59 ^{a}	46.45± 0.98 ^{c}	59.09 ± 0.86 ^{b}
W_{gain} (g)	20.25 ± 0.52 ^{b}	30.85 ± 0.63 ^{a}	11.21 ± 0.28 ^{c}	± 0.28 ^{b} 23.86

Different superscript letters (a, b and c) within the same raw indicate a significant difference at $p < 0.05$.

Hematology and Plasma Creatinine Level:

The effect of Beta-MOS® supplemented diet on the hematological parameters of Pb-intoxicated Nile tilapia after 60 days of the experiment were summarized in Figures 1 and 2. Beta-MOS® supplemented Nile tilapia showed a significant ($p < 0.05$) increase in RBCs count and Hb concentrations in control and other groups. While the Pb-exposed group showed significant ($p < 0.05$) lower values of RBCs counts and Hb concentrations than the control group. Incorporation of dietary Beta-MOS® to Pb-intoxicated fish enhanced RBCs count and Hb concentrations caused by Pb and increased these values.

Higher levels of serum creatinine were detected in Pb-exposed Nile tilapia than in control fish ($p < 0.05$). 0.3% Beta-MOS® incorporated diet caused a significant ($p < 0.05$) decrease in creatinine level compared to control and other groups. Moreover, the addition of 0.3% dietary Beta-MOS® to the Pb-exposed group lowered the creatinine level of than Pb-intoxicated group (Fig. 3).

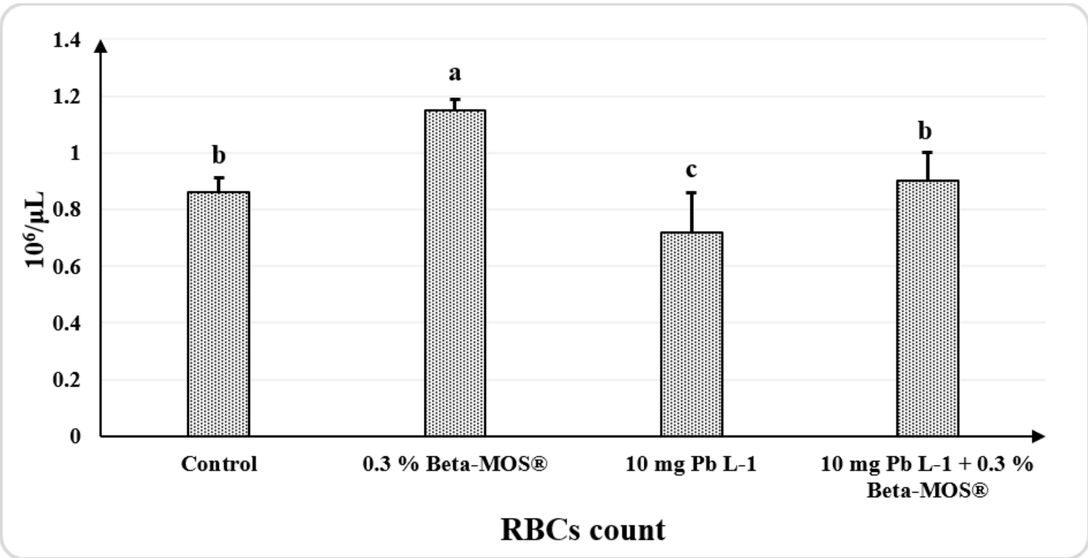

Fig 1: Effect of dietary Beta-MOS® on RBCs count in lead (Pb) intoxicated Nile tilapia, after 60 days. Data (n=9/group) were expressed as mean ± SE. Different letters (a,b&c) on the same bar were significantly different at $p < 0.05$.

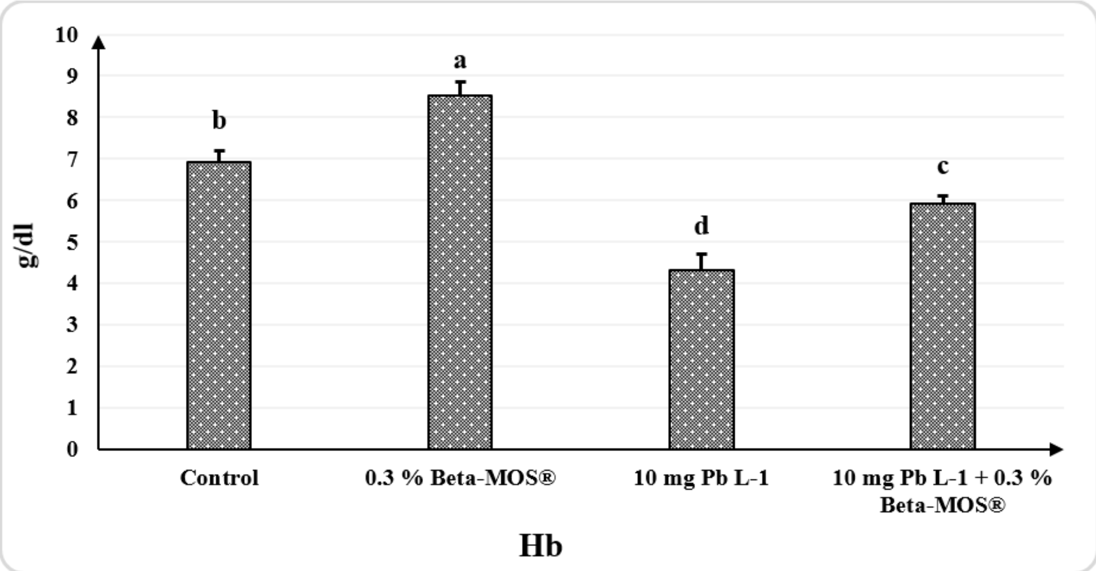

Fig. 2: Effect of dietary Beta-MOS® on hemoglobin (Hb) concentrations in lead (Pb) intoxicated Nile tilapia, after 60 days. Data (n=9/group) were expressed as mean ± SE. Different letters (a,b,c&d) on the same bar were significantly different at $p < 0.05$.

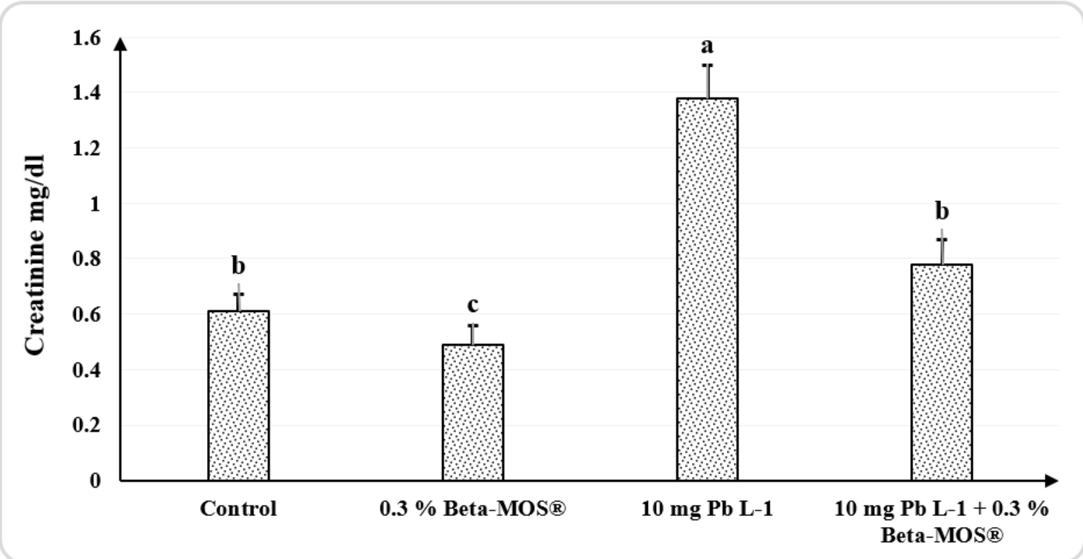

Fig. 3: Effect of dietary Beta-MOS® on serum creatinine in lead (Pb) intoxicated Nile tilapia, after 60 days. Data (n=9/group) were expressed as mean ± SE. Different letters (a b&c) on the same bar were significantly different at $p<0.05$.

Histopathological Examination:

No signs of pathological alterations were detected in the renal tissues of all examined sections from both control and 0.3% Beta-MOS® supplemented groups (Figs. 4 A & B). Pb-exposed kidney showed severe pathological alterations; including a glomerular expansion that resulted in the reduction of Bowman's space and few appeared atrophied. In renal tubules, the most frequent necrosis of renal tubules is associated with lymphocytic infiltration and hemorrhage. Additionally, there was hyperplasia of hematopoietic tissue, dilation and congestion with partial hemolysis in the epithelial lining of the blood vessel and hyperplasia of MMCs (Fig. 4 C). Compared to the previous, Pb-exposed group supplemented with Beta-MOS® revealed marked improvement with slight pathological changes in renal tissues of almost examined sections such as hyperplasia f hematopoietic tissue with slight activation of MMCs and hemorrhage and congestion of blood vessel. In the Bowman's capsule, a glomerular expansion that resulted in the reduction of Bowman s space (Fig. 4 D) was the less frequent alteration detected. All supplemented kidney sections exhibited, without necrosis when compared to Pb group.

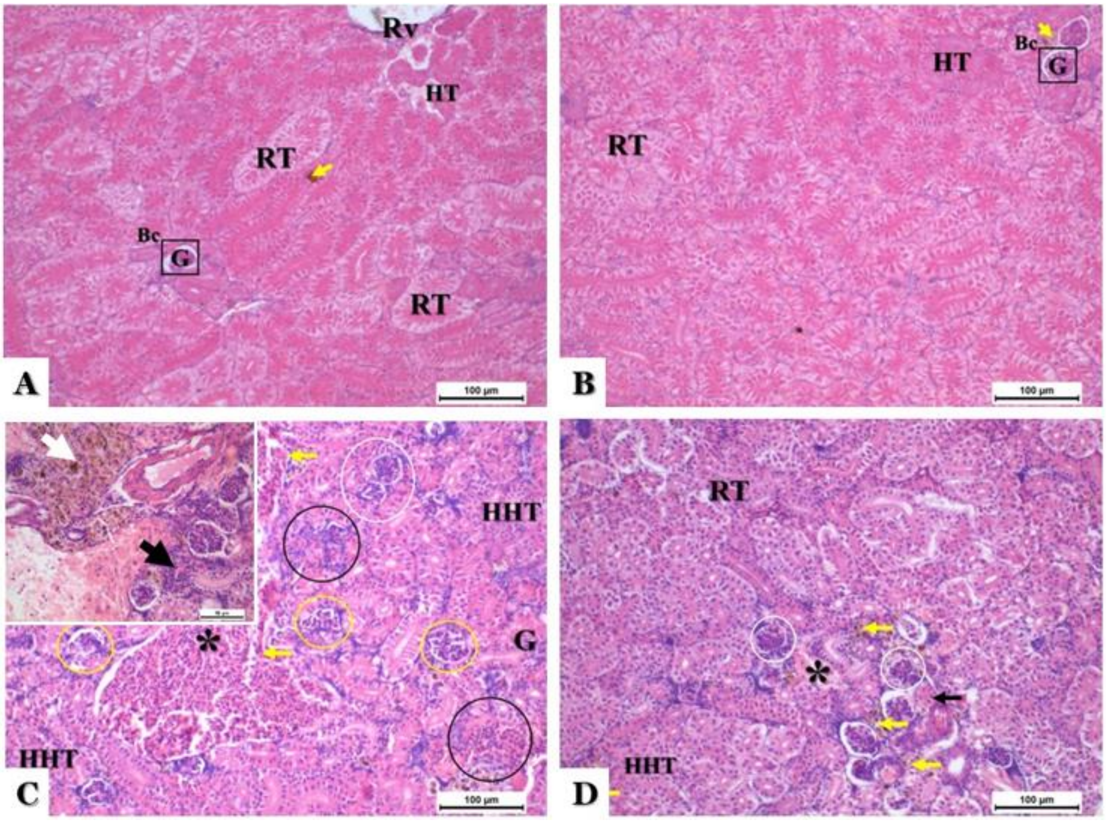

Fig. 4: Kidney longitudinal sections of normal control and 3% Beta-MOS® supplemented diet **(A)** and **(B)** respectively, showed normal renal tubules (RT), Bowman's capsule (Bc) included glomerulus (G), hematopoietic tissue (HT). **(C)** Kidney longitudinal section of Pb-exposed Nile tilapia fed basal diet, showed two adjacent Bowman's capsules with glomerular expansion that resulted in the reduction of Bowman's space (white circle) and some appeared atrophied (yellow circles), hyperplasia of hematopoietic tissue (HHT) and focal necrosis associated with lymphocytic infiltration (black circles) were observed. Besides, congestion (asterisk) and partial hemolysis in the epithelial lining of the blood vessel and slight hemorrhage (yellow arrows). **(C) Window** showed hyperplasia of MMCs (white arrow) and focal necrosis of renal tubules (black arrow) associated with lymphocytic infiltration. **(D)** Section of Pb-exposed kidney supplemented with 3% Beta-MOS®, showed marked improvement of renal tissues with slight pathological changes included; glomerular expansion (white circle), hyperplasia of hematopoietic tissue (yellow circles) and slight activation of MMCs (yellow arrows), mild hemorrhage (black arrows). Besides, congestion (asterisk) of the blood vessel and slight hemorrhage. H&E stain (X 200).

DISCUSSION

Lead in the water system, as one of the environmental pollutants, causes a serious problem around the world. These problems result in toxic damage to fish and threaten human health through the food chain (Yesilbudak and Erdem, 2014; Zhang *et al*., 2017). Pb can easily pass into the environment, then to human, and animal blood streams and enter various organs such as gills, intestine, brain, kidneys, liver, gonads and bones (Ali *et al.* 2019; Giri *et al.* 2021). Nowadays, there is an increasing demand for using eco-friendly feed supplements instead of antibiotics or chemicals in order to diminish the toxic effects of pollutants, including heavy metals on farmed fish. To date, no effective treatment method has been developed for Pb poisoning in aquaculture (Giri *et al.* 2021). The use of prebiotics as immunostimulants in aquaculture is expanding worldwide due to their lower price, easy availability, ease of preparation, and low toxicity to humans and aquatic life (Davani-Davari *et al.* 2019). The present study investigated the possible protective effects of prebiotics βG and MOS (Beta-MOS®) against Pb-induced toxicity in Nile tilapia.

The concentration of Pb in the present study was equivalent to the average concentrations of Pb in fish farms in the Suez Canal area as estimated in the current study. Exposure to Pb (10 mg L^{-1}) significantly decreased the final body weight reflected on weight gain than control. These results might be due to both reduction in feed intake and the energy lost in repairing Pb-induced tissue damage rather than growth processes (Ding *et al.* 2019). In addition, Pb might cause nutrient malabsorption and/or impaired protein synthesis (Minnema & Hammond 1994). Present results were in agreement with Giri *et al.* (2021) who confirmed that waterborne Pb exposure caused retardation in *Cyprinus carpio* growth reflected as a reduction in Fish final body weight.

Administration of dietary 0.3% Beta-MOS®, alone or in combination with Pb improved weight final and weight gain of Nile tilapia. Usually, dietary yeast product supplementation enhances protein deposition and growth performance of Nile tilapia (Abdel-Tawwab *et al.* 2008). Yeast supplements improve nutrient digestibility (Waché *et al.* 2006) by increasing intestinal enzyme activities, intestinal villus height, epithelial thickness and enterocytes' brush border (lara-flores *et al.* 2010). Ayyat *et al.* (2020) showed similar results, they reported the ameliorative effect of dietary 4 g/ kg MOS against weight loss caused by 50 ppm Pb in Nile tilapia. Improved growth performance and feed utilization were previously reported with the use of βG and MOS mixture in the fish diet of different species such as; common carp *Cyprinus carpio* (Ebrahimi *et al.* 2012), Beluga *Huso huso* (Ta'ati *et al.* 2011), Nile tilapia *Oreochromis niloticus* (Selim & Reda 2015) and sea cucumbers *Apostichopus japonicus* (Gu *et al.* 2011). On contrary, previous researchers found that administration of MOS and βG to Nile tilapia diet had no positive effect on growth performance and feed utilization (Whittington *et al.* 2005; Sado *et al.* 2008; Shelby *et al.* 2009). Lin *et al.* (2011) detected that β-glucan induced inflammation in the gut of Carp *Cyprinus carpio koi* that led to growth retardation. The variation in results might be associated with; the difference in the chemical structure of the food additives and their solubility, the period of feeding trial and the experimental conditions, as well as fish age, sex and species (Dalmo & Bøgwald 2008; Akrami *et al.* 2015). Moreover, the actions which produced by the yeast cell wall derivatives changes according to the chemical composition and their fermentation processes (Sado *et al.* 2008; Akrami *et al.* 2015; Selim & Reda 2015). The dietary MOS can bind mannose receptors on microbes. Binding and blocking of microbes via mannose receptors leading them eliminated out the intestine instead of colonizing and invading the host, thus considered suitable status for growing of the beneficial bacteria (Staykov *et al.* 2007; Refstie *et al.* 2010; Zorriehzahra *et al.* 2016).

Hematological and blood biochemical parameters are reliable tools for evaluating the metal toxicity in an aquatic environment, the status of the oxygen-carrying ability, as well as the fish's physiological responses (Kim & Kang 2014; Shah *et al.* 2020). In the current study, the negative impacts of Pb on hematological parameters were evidenced by decreasing in RBCs count, and Hb concentration. Decreased RBCs count and Hb were detected in common carp exposed to Cd and Pb (Khalesi *et al.* 2017), and *Ctenopharyngodon idella* exposed to Pb, Cr and Cu (Shah *et al.*

2020). African catfish (*Clarias gariepinus*) exposed to 24.4 mg L^{-1} Pb revealed similar results which were represented by a reduction of RBCs count and Hb concentration (Abd El-satar *et al.* 2019). Hb is an oxygen carrier and reflects the fish's anemic conditions (Parekh & Tank 2015). Therefore, the significant decline in Hb concentration in Pb intoxicated group revealed that tilapia suffered from severe anemia. The reduction of RBCs and Hb might be due to the Pb deleterious effects on the mitochondrial enzymes incorporated in heme and Hb synthesis, as well as iron metabolism leading to anemia and RBCs depletion (Gürer *et al.* 1998). ROS production also might deteriorate the membrane of RBCs integrity resulting in a shortening of RBCs lifespan and RBCs count (Jacob *et al.* 2000). Moreover, Pb could cause a reduction in erythropoietin release leading to anemia (Shah *et al.* 2020).

Serum creatinine is filtered out by the kidneys (glomerular filtration) and blood levels rise in severe kidney dysfunction (Kulkarni & Pruthviraj 2016). The present study detected an increase in serum creatinine level in Pb intoxicated group. This elevation might be related to impaired kidney function whereas, several studies detected higher levels of creatinine in the plasma of intoxicated fish, which is considered a good indicator of alteration in glomerular filtration and kidney dysfunction (Ayyat *et al.* 2003; Zaki *et al.* 2010; Parekh & Tank 2015; Osman *et al.* 2018). High creatinine in fish bloodstream might be linked to numerous factors acting simultaneously like disorders in cell membrane integrity with further cell damage that had resulted from increased ROS production and lipid peroxidation (Jurczuk *et al.* 2007) due to Pb toxicity. This was confirmed by the existence of histopathological lesions in Pb intoxicated kidneys. Nourian *et al.* (2019) reported high creatinine levels in Pb-exposed *Cyprinous carpio* with kidney disorders that led to many waste products in the fish's bloodstream.

The fish kidney is one of the most vital organs affected by different contaminants in the water (Al-Balawi *et al.* 2013) and received the largest proportion of post-branchial blood (Thophon *et al.* 2003). Therefore, renal lesions are expected to be good indicators of heavy metal pollution (Alm-Eldeen *et al.* 2018). Under the present investigation, the kidney of Nile tilapia exposed to Pb revealed several histological lesions, after 60 days. Glomerulus showed expansion resulting in a reduction of Bowman's space, necrosis, or atrophied, architecture degeneration of renal tubules were the most frequent and observed alterations in the current study. These lesions were also observed previously in Nile tilapia (Al-Faragi *et al.* 2017) and common carp (Mustafa *et al.* 2017). In the present study, the kidney of Pb-exposed fish showed severe lesions such as congestion, edematous fibrosis, necrosis associated with lymphocytic infiltrations and hemorrhage within hematopoietic tissues. These toxic effects of Pb on the kidney were in accordance with previous studies in fish exposed to different contaminants (Mohamed 2009; Al-Balawi *et al.* 2013; Alibraheemi 2019). Also, Pb-exposed kidney showed marked aggregation of MMCs, this result might related to renal retrogressive changes and necrosis (Steinel & Bolnick 2017). The kidney lesions in the present study supported by elevation of creatinine levels in sera of Nile tilapia which reflected kidney failure.

In conclusion, it was clear that dietary MOS and βG exerted an ameliorative effect against Pb toxicity through improvement in weight gain and hematological parameters. Also, renal function and histopathology were improved.

Ethical Statements:

This study was carried out in strict accordance with the guidelines of the National Health and Medical Research

Council for the Care and Use of Animals.

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