**Special Issue:**

Veterinary Medicine between Sustainable Development and Public Health to Confront Global Changes

# Ameliorative Effect of Dietary *Silybum marianum* on the Exposure of *Oreochromis niloticus* to Sublethal Doses of Lead Acetate

**Abd El-Alim F. Abd El-Alim[1], Abdelhakeem El-Murr[2*], Tahsein Hasan[1]**

*[1]Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt; [2]Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt.*

**Abstract** | This work assesses how well *Silybum marianum* extract (SME) protects against lead acetate poisoning in *Oreochromis niloticus* (*O. niloticus*). The work plan includes five experimental groups the negative control without any challenges in water and positive control water challenged with 1.45 mg lead acetate/liter for 20 days exposure both fed on the basal diet. The T1, T2, and T3: water challenged with 1.45 mg lead acetate/liter for 20 days exposure; T1: feed on basal diet supplemented with 50 mg/kg SME; T2: feed on basal diet supplied with 100 mg/kg SME; T3: feed on basal diet supplemented with 200 mg/kg SME. The lead acetate exposure decreases the weight gain in positive control (9.76 ± 0.37 g) in comparison with T3 (15.63 ± 0.68 g) after 20 days of exposure. The SME along with lead acetate exposure significantly improved feed conversion ratio (FCR) in T3 (1.26 ± 0.01g) in comparison to positive control (1.34 ± 0.02 g). One-way ANOVA revealed significant reduction ($P < 0.05$) in alanine aminotransferase (ALT) from 57.36 ± 1.02 to 36.27 ± 1.08 IU/ L, aspartate aminotransferase (AST) from 72.45 ± 1.86 to 72.45 ± 1.86 IU/ L, creatinine from 1.42 ± 0.14 to 0.75 ± 0.06 mg/dL and urea from 38.62 ± 1.12 to 23.69 ± 0.98 mg/dL in positive control and T3, respectively. Meanwhile, serum protein and hematological parameters enhanced in T1, T2 and T3 in comparing with positive control. The glucose and cortisol levels were 84.67 ± 2.97 mg/dL and 9.16 ± 0.89 ng/mL in positive control and significantly reduced in SME treated groups T1 (79.36 ± 2.65 mg/dL and 7.56 ± 0.85 ng/mL), T2 (78.89 ± 2.16 mg/dL and 5.79 ± 0.93 ng/mL) and T3(77.28 ± 3.11 mg/dL and 4.87 ± 0.81 ng/mL). The activities of catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), showed significant increase ($P<0.05$) in positive control and total antioxidant capacity (TAC) displayed a significant decrease ($P<0.05$) after lead acetate exposure. The supplementation of diets with SME is beneficial for *O. niloticus* reared in lead contaminated water as it enhances growth parameters, and antioxidant enzymes capacity.

**Keywords:** *Oreochromis niloticus*, lead, liver enzyme, oxidative stress, *Silybum marianum*, growth

**Received** | August 14, 2024; **Accepted** | September 25, 2024; **Published** | October 07, 2024
***Correspondence** | Abdelhakeem El-Murr, Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt; **Email:** Hakimelmor@hotmail.com
**Citation** | Abd El-Alim FA, El-Murr A, Hasan T (2024). Ameliorative effect of dietary *Silybum marianum* on the exposure of *Oreochromis niloticus* to sublethal doses of lead acetate. Adv. Anim. Vet. Sci. 12(s1): 161-172.
**DOI** | https://dx.doi.org/10.17582/journal.aavs/2024/12.s1.161.172
**ISSN (Online)** | 2307-8316; **ISSN (Print)** | 2309-3331



## INTRODUCTION

*Oreochromis niloticus* is one of the most important commercial species that has become a global staple in aquaculture in Egypt (Moyo and Rapatsa, 2021). Roughly 80% of all tilapia farmed worldwide is produced by the *Oreochromis niloticus* (Naylor *et al*., 2021).

Nile tilapia fish are considered a good model for study due to their widespread breeding in most countries due to their ability to withstand the climatic conditions of most African countries, in addition to providing abundant production to fill the deficit in animal protein (Chan *et al*., 2014). *Oreochromis niloticus* able to withstand a wide variety of temperatures and adverse environmental conditions implies that it could be a valuable bio-indicator of water contamination and toxicity studies (Henson *et al*., 2018; Yan *et al*., 2020).

The large-scale emissions of heavy metal into aquatic life results in interfering with several physiological, metabolic, and cellular processes in fish and upset the ecological balance (Arisekar *et al*., 2020). The higher concentrations pose a threat to aquatic environments, as the biological system is frequently unable to eliminate such toxins on its own rapidly (Abbas *et al*., 2021; Eroglu *et al*.,2015).

Lead (Pb), considered as a serious risk to aquatic life and is also the cause of the decline in ecological health in these habitats (Paul *et al*., 2017). It has been observed that prolonged exposure to the Pb toxicant increases the generation of reactive oxygen species (ROS), which results in oxidative damage and aberrant free radical growth (Patra *et al*., 2011; Kiran *et al*., 2021).

Fish physiology is disturbed, blood cells are damaged, and major changes to bodily tissues result from lead poisoning. Increased lead buildup in water sources may result in increased lead permeability in fish flesh, which may have harmful consequences on the health of consumers (Abbas *et al*., 2021). Thus, it would be crucial to identify effective strategies for lessening the detrimental impacts of heavy metals on aquatic life. A small number of research suggests that natural remedies such as herbal extracts and oils might lessen the harmful physiological effects of pollutants in fish (Dawood *et al*., 2020; Khafaga *et al*., 2020; Ahmadifar *et al*., 2021).

The therapeutic benefits of *Silybum marianum* have long been acknowledged as a medicinal herb (Křen and Walterová, 2005). Strong antioxidant activity is exhibited by silymarin, a polyphenolic molecule that was isolated from *S. marianum* (Kvasnička *et al*., 2003).

The mechanism of action is a reduction of the free radicles formed by toxins that damage the cell membranes (lipoperoxidation) and competitive inhibition through hepatocyte external cell membrane modification. Silymarin forms a complex that impedes the entrance of toxins into the interior of liver cells. Additionally, silymarin metabolically stimulates hepatic cells and activates the RNA synthesis of ribosomes to stimulate protein formation (Abou Zid, 2012). In addition to dietary *S. marianum*, a medicinal herb, boosted certain non-specific immune responses in fish (Alishahi *et al*., 2011).

Fish that are exposed to heavy metals showed a broad range of physiological and histological abnormalities due to oxidative stress (Chowdhury and Saikia, 2020). Biochemical alterations in the liver and blood are typically employed in toxicological investigations to assess the mode of action of toxicants (Chupani *et al*., 2017; Chupani *et al*., 2018). Hepatic and plasmatic enzymes offer a thorough picture of the state of immunity and were a good indicator of environmental stresses (Yousafzai *et al*., 2011).

Silymarin previously used in in aquaculture to mitigate the malathion toxicity in some fish species, Banaee *et al*. (2015), nickel oxide nanoparticles (Nazdar *et al*., 2018), *Streptococcus agalactiae* infection (Owatari *et al*., 2018). Lead is considered as water contaminant and up our knowledge there is no research article on the effect of SME on the *O. niloticus* exposed to lead toxicity. The current work conducted to study the effects of *S. marianum* on the growth, serum enzymes, cortisol, protein, stress markers, and hematological parameters of *O. niloticus* challenged with 1.45 mg of lead acetate $L^{-1}$.

## MATERIALS AND METHODS

### Detection Of Lead Acetate 96-H Lc50

From nursery ponds at the Aquaculture Research, *Oreochromis niloticus* fingerlings were obtained. In order to allow the fish to become used to the indoor wet laboratory, they were moved there and housed in fiberglass tanks for a period of 14 days. Fluorescent light were used to keep the photoperiod at 12–12 hours. As a source of lead acetate [Pb(C2H3O2)2], El-Gomhouria Company (Cairo, Egypt) was the supplier used. Dissolving lead acetate in deionized water produced newly generated Pb concentrations. Acclimatized fish (32.3–36.4 g) were divided into 10 fish per 100-L tank and subjected to 0.0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 mg Pb/L for 96 hours in duplicate in order to calculate the half-lethal concentration (LC50) of Pb (Table 1 and 2). Fish mortality was tracked every day for ninety-six hours at each Pb concentration. Utilizing the techniques outlined by Behreus and Karber (1953), the 96-hour limit of detection (96-h-LC50) for lead was found to be 14.5 mg/L. In the current investigation, a sub-lethal Pb concentration (1/10 of the LC50) of 1.45 mg/L was employed. The methodology and management of the experiment followed the directive and national legislation pertaining to animal care, as fish were reduced to a minimum without compromising the aims of the research, and procedures eliminated any needless pain, suffering, or discomfort to the fish. In addition to, the protocol of work approved by the institutional animal cate and use committee, Zagazig university (ZU- IACUC/2/F/150/2024).

**Table 1:** Zero and hundred % mortalities in O. niloticus exposed to different concentration of lead acetate for 96 hours.

| Conce (mg/L) | Time/day | | | | Dead fish No. |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 0 | 1 | 1 | 2 |
| 10 | 0 | 2 | 2 | 3 | 7 |
| 15 | 1 | 5 | 5 | 3 | 14 |
| 20 | 1 | 4 | 5 | 5 | 15 |
| 25 | 2 | 4 | 5 | 5 | 16 |
| 30 | 2 | 6 | 5 | 5 | 18 |
| 35 | 3 | 4 | 6 | 7 | 20 |
| 40 | 3 | 3 | 6 | 8 | 20 |
| 45 | 3 | 7 | 5 | 5 | 20 |
| 50 | 3 | 5 | 6 | 6 | 20 |

**Table 2:** Actual estimation of 96 hour LC50 of lead acetate in *O. niloticus*.

| Conce (mg/L) | Dead fish Np. for 96h | a | b | axb | £ axb |
|---|---|---|---|---|---|
| 0 | 0 | | | | |
| 5 | 2 | 5 | 1 | 5 | |
| 10 | 7 | 5 | 4.5 | 22.5 | |
| 15 | 14 | 5 | 10.5 | 52.5 | |
| 20 | 15 | 5 | 14.5 | 72.5 | |
| 25 | 16 | 5 | 15.5 | 77.5 | |
| 30 | 18 | 5 | 17 | 85 | |
| 35 | 20 | 5 | 19 | 95 | 410 |

96hrs LC50=highest dose -∑axb/n; 96 h LC50 of lead acetate =35-410/20=14.5 mg/L; a=constant factor between 2 successive doses; b=mean of dead fish between two groups; ∑axb=sum of axb

## Feed Preparation

Fish meal (72% crude protein) (85), soybean meal (45% crude protein) (465), wheat bran (183), ground corn (100), corn oil (20), cod liver oil (20), mineral mixture (30), vitamin mixture (30), and starch (67) make up the basic diets in grammes per kilogram. To form a dough, the feed ingredients including *S. marianum* extract were combined with an appropriate quantity of water. The dough was ground into 1.5-mm pellets by first running it through a meat mincer. After that, the diets were produced and stored at 4 ◦C until they were needed.

## Experimental Design

Fish weighing between 32.3 and 36.4g were placed into 100 L glass aquariums (10 fish/aquarium) and allowed to acclimate to the laboratory environment for 14 days Using an aquarium air pump and air-stones, compressed air was added to each tank. During the acclimatization period, fish were given the control meal three times a day until they were satisfied. For 20 days, acclimated fish were split into five groups of five duplicates. five experimental groups the negative control without any challenges in water and positive control water challenged with 1.45 mg lead acetate/liter for 20 days exposure both fed on the basal diet. The T1, T2, and T3: water challenged with 1.45 mg lead acetate/liter for 20 days exposure; T1: feed on basal diet supplemented with 50 mg/kg SME; T2: feed on basal diet supplied with 100 mg/kg SME; T3: feed on basal diet supplemented with 200 mg/kg SME, all of positive control. Every two days, the water in each tank was completely emptied out and refilled with fresh water that had the same Pb acetate. All water quality parameters are maintained according to Boyd (1984). The Lead concentrations were 0.17–0.19 and 8.01–8.07 µg/L in aquariums that were Pb-free and Pb-exposed, respectively. The following growth and feed consumption data were ascertained after the experimental trial by gathering, tallying, and group-weighing all the fish in each tank:

$$W2 - W1$$

Where;
W1 and W2 are the starting and ending weights, respectively, equals weight gain (g). Fish number in aquarium divided by total feed consumed equals feed consumption (g feed per fish).

Feed input / fish weight increase equals the feed conversion ratio (FCR). (Final fish number/Initial fish number) = 100 is the fish survival percentage.

## Sampling Methods

Fish are fasted for twenty-four hours before their blood are sampled. To reduce handling stress on the fish tested, a 0.02 percent benzocaine solution was used to anesthetize the fish. Using 2-mL sterile hypodermic needles, blood samples were extracted from the caudal vein of three fish, per tank (15 fish/treatment). Two sections of the drawn blood samples were separated. For hematological analysis, a fraction was put into Eppendorf tubes filled with heparin. After being placed into Eppendorf tubes, the second part was centrifuged for 15 minutes at 1500 × g after clotting for 30 minutes at room temperature. Following collection, the serum was stored at 20 ◦C to facilitate further biochemical analyses. Fish were dissected after blood collection, liver tissues were collected, and the liver tissues were homogenised in cold physiological buffer saline (PBS) using a Potter-Elvehjem glass/Teflon homogenizer. After filtering and centrifuging for 10 minutes at 4 °C at 1600 × g, the resultant supernatant was stored at 20 °C for further analysis.

## Haemato-Biochemical Assay

The counts of white blood cells (WBCs) and red blood cells (RBCs) were calculated using Brown's (1980) techniques.

**Table 3:** Effect of *Silybum marianum* on growth performance, feed conversion ratio and feed intake of *O. niloticus* challenged with 1.45 mg lead acetate $L^{-1}$.

| Parameters | Time | -ve control | +ve control | T1 (50 mg) | T2 (100 mg) | T3 (200 mg) |
|---|---|---|---|---|---|---|
| Weight | Initial | 35.21 ± 0.32 | 35.16 ± 0.30 | 35.33 ± 0.31 | 35.06 ± 0.31 | 35.40 ± 0.31 |
| | 20 days | 56.44 ± 0.53$^{a}$ | 52.40 ± 0.78$^{b}$ | 56.73 ± 0.80$^{a}$ | 56.20 ± 0.51$^{a}$ | 56.65 ± 0.50$^{a}$ |
| | 20 days | 71.44 ± 1.32$^{a}$ | 62.16 ± 1.04$^{b}$ | 71.06 ± 0.90$^{a}$ | 71.60 ± 1.24$^{a}$ | 72.20 ± 0.51$^{a}$ |
| Weight gain | 10 days | 21.24 ± 0.78$^{a}$ | 17.23 ± 1.06$^{b}$ | 21.40 ± 1.11$^{a}$ | 21.13 ± 0.80$^{a}$ | 21.16 ± 0.90$^{a}$ |
| | 20 days | 15.54 ± 0.91$^{a}$ | 9.76 ± 0.37$^{b}$ | 14.33 ± 0.85$^{a}$ | 15.40 ± 0.92$^{a}$ | 15.63 ± 0.68$^{a}$ |
| FCR | 10 days | 1.22 ± 0.02$^{b}$ | 1.34 ± 0.02$^{a}$ | 1.23 ± 0.01$^{b}$ | 1.23 ± 0.01$^{b}$ | 1.22 ± 0.02$^{b}$ |
| | 20 days | 1.25 ± 0.06$^{b}$ | 1.34 ± 0.02$^{a}$ | 1.24 ± 0.02$^{b}$ | 1.24 ± 0.03$^{b}$ | 1.23 ± 0.01$^{b}$ |
| Feed intake | 10 days | 25.87 ± 1.11 | 23.11 ± 1.86 | 26.18 ± 1.53 | 26.06 ± 1.09 | 25.95 ± 0.96 |
| | 20 days | 19.72 ± 1.16$^{a}$ | 13.14 ± 0.27$^{b}$ | 17.85 ± 0.78$^{a}$ | 19.20 ± 1.12$^{a}$ | 19.79 ± 0.66$^{a}$ |

$^{(a,b,c)}$ different superscript letters in the same row indicate significant differences ($p < 0.05$).

Van Kampen and Zijlstra's (1961) cyanmethemoglobin technique was used to measure hemoglobin (Hb). Fresh blood was placed in capillary glass tubes, spun for 10 minutes in a microhematocrit centrifuge, and the packed cell volume was measured to calculate the hematocrit (Ht) values right away after the sample (Brown, 1980). In accordance with Dacie and Lewis (1984), The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) of the generated blood indices were calculated.

As directed by the manufacturer, the biochemical, oxidative stress, and immunological biomarkers were identified using the diagnostic reagent kits (Biodiagnostic Co., Cairo, Egypt). Cortisol and blood glucose levels were measured using the procedures outlined in Foster and Dunn (1974) and Trindern (1969), respectively. The colorimetric techniques outlined by Wotton and Freeman (1982) and Henry (1964) were used to assess serum total protein (TP) and albumin (ALB), respectively. By deducting albumin from the total protein in the sample, serum globulin (GLO) was computed. Reitman and Frankel (1957) reported that the activity of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured colorimetrically.

### Statistical Analysis

The normality and homogeneity of variances were assessed using Barlett's test and significant differences between the various experimental groups, one-way ANOVA was employed. At $P < 0.05$, significant differences across groups were evaluated using Duncan's test. The statistical software version 20 (SPSS, Richmond, VA, USA) was used for all statistical analyses (Dytham, 2011).

## RESULTS AND DISCUSSION

One-way ANOVA analysis demonstrated that exposure to lead acetate significantly decrease the weight (62.16 ± 1.04 g) and weight gain (9.76 ± 0.37 g) in positive control group on comparing with negative control and *S. marianum* treated groups ($p< 0.05$) after 20 days exposure. In addition, supplementing diets with *S. marianum* induced significant ($P < 0.05$) decreases in feed conversion ratio on treated groups T1 (1.24 ± 0.02), T2 (1.24 ± 0.03) and T3 (1.23 ± 0.01). The addition of *S. marianum* to the diet, induce a noticeable increase in the amount of feed that the fish consumed; however, after 20 days, exposure to lead acetate significantly ($P < 0.05$) decreased the amount of feed that the fish consumed (Table 3). When lead acetate exposure (T1, T2, and T3) was combined with feeding *S. marianum* to *O. niloticus*, the feed consumption was considerably higher than in the positive control. On the tenth day of exposure to lead acetate, there were not statistically significant ($P > 0.05$) changes in feed consumption among the various treatments (Table 3). One-way ANOVA analysis revealed that AST and ALT were notably ($P < 0.05$) higher due to Pb toxicity in control positive group. Meanwhile, the inclusion of 50 mg (T1), 100 mg (T2) and 200 mg (T3) of *S. marianum* in *O. niloticus* diets for Pb-exposed fish significantly restored their values in comparison to control (Table 4). The creatinine level in positive control group was 1.42 ± 0.14 mg/dL that significantly higher than ($P< 0.05$) negative control 0.72 ± 0.06 mg/dL and *S. marianum* treated groups T1 (0.92 ± 0.08 mg/dL), T2 (0.81 ± 0.07 mg/dL) and T3 (0.75 ± 0.06 mg/dL). The urea level in *O. niloticus* serum was significantly lower in all *S. marianum* treated groups T1 (31.65 ± 1.06 mg/dL), T2 (28.12 ± 0.89 mg/dL) and T3 (23.69 ± 0.98 mg/dL) in comparing to positive control group (38.62 ± 1.12 mg/dL). The finding in Table 4 demonstrated that the positive control group's cortisol and glucose levels were 9.16 ± 0.89 ng/mL and 84.67 ± 2.97 mg/dL considerably ($P < 0.05$) higher than those of the negative control 4.49 ± 0.62 ng/mL and 76.16 ± 3.36 mg/dL, respectively. Giving *O. niloticus* a meal containing 100 mg or 200 mg *S. marianum* increases protein synthesis, whereas giving it a negative control group resulted in a substantial decrease in total protein, albumin, globulin,

**Table 4:** Effect of *Silybum marianum* on aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, glucose and cortisol of O. *niloticus* challenged with 1.45 mg lead acetate $L^{-1}$.

| Parameters | -ve control | +ve control | T1 (50 mg) | T2 (100 mg) | T3 (200 mg) |
|---|---|---|---|---|---|
| AST(IU/ L) | 49.66 ± 1.78$^{c}$ | 72.45 ± 1.86$^{a}$ | 52.17 ± 1.46$^{b}$ | 48.45 ± 1.35b$^{c}$ | 44.35 ± 1.54$^{c}$ |
| ALT(IU/ L) | 35.21 ± 0.92$^{c}$ | 57.36 ± 1.02$^{a}$ | 42.61 ± 1.22$^{b}$ | 38.14 ± 1.35$^{b}$ | 36.27 ± 1.08$^{c}$ |
| Creatinine (mg/dL) | 0.72 ± 0.06$^{c}$ | 1.42 ± 0.14$^{a}$ | 0.92 ± 0.08$^{b}$ | 0.81 ± 0.07$^{bc}$ | 0.75 ± 0.06$^{c}$ |
| Urea (mg/dL) | 22.36 ± .82$^{b}$ | 38.62 ± 1.12$^{a}$ | 31.65 ± 1.06$^{ab}$ | 28.12 ± 0.89$^{ab}$ | 23.69 ± 0.98$^{b}$ |
| Glucose (mg/dL) | 76.16 ± 3.36$^{b}$ | 84.67 ± 2.97$^{a}$ | 79.36 ± 2.65$^{a}$ | 78.89 ± 2.16$^{ab}$ | 77.28 ± 3.11$^{b}$ |
| Cortisol (ng/mL) | 4.49 ± 0.62$^{c}$ | 9.16 ± 0.89$^{a}$ | 7.56 ± 0.85$^{ab}$ | 5.79 ± 0.93$^{b}$ | 4.87 ± 0.81$^{c}$ |

$^{(a,b,c)}$ different superscript letters in the same row indicate significant differences ($p < 0.05$).

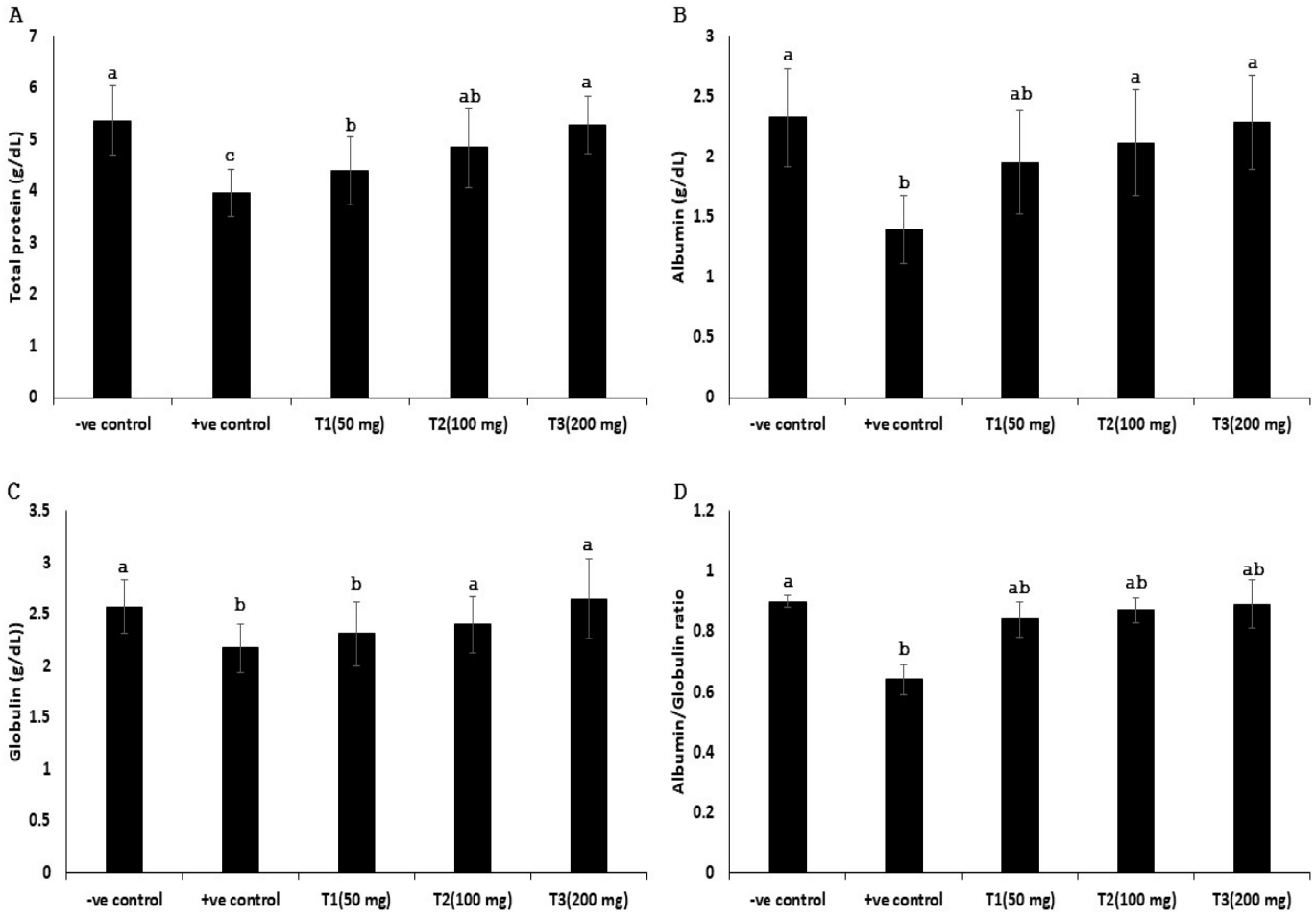


**Figure 1:** Effect of *Silybum marianum* on (A) serum total protein (B) albumin (C) globulin, (D) albumin/globulin ratio of O. *niloticus* challenged with 1.45 mg lead acetate $L^{-1}$.
$^{(a,b,c)}$ different letters indicate significant differences ($p < 0.05$).

and albumin globulin ratio (Figure 1). When the positive group was exposed to lead acetate in water for 20 days, their levels of catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) significantly increased ($P < 0.05$) in comparison to the negative control group (Figure 2). Conversely, following exposure to 1.45 mg lead acetate/L, the activity of total antioxidant capacity (TAC) showed a significant reduction ($P < 0.05$). Feeding on diet containing 50, 100 and 200 mg *S. marianum* tolerates the effect of lead acetate decreasing oxidative stress and enhanced total antioxidant capacity (Figure 2). After 20 days of trial, the positive control showed substantially lower levels of RBCs, Hb, PCV, platelets, and MCV compared to the negative control ($p<0.05$). Meanwhile, there were no significant changes in MCH or MCHC across all groups. The *S. marianum*-treated groups are comparable to the negative control groups, particularly the group treated with 200 mg of *S. marianum* (Table 5). The count of WBCs significantly reduced ($P < 0.05$) in positive control group in comparison to negative control and all *S. marianum* treated groups as well as the differential leukocyte count (Table 6).

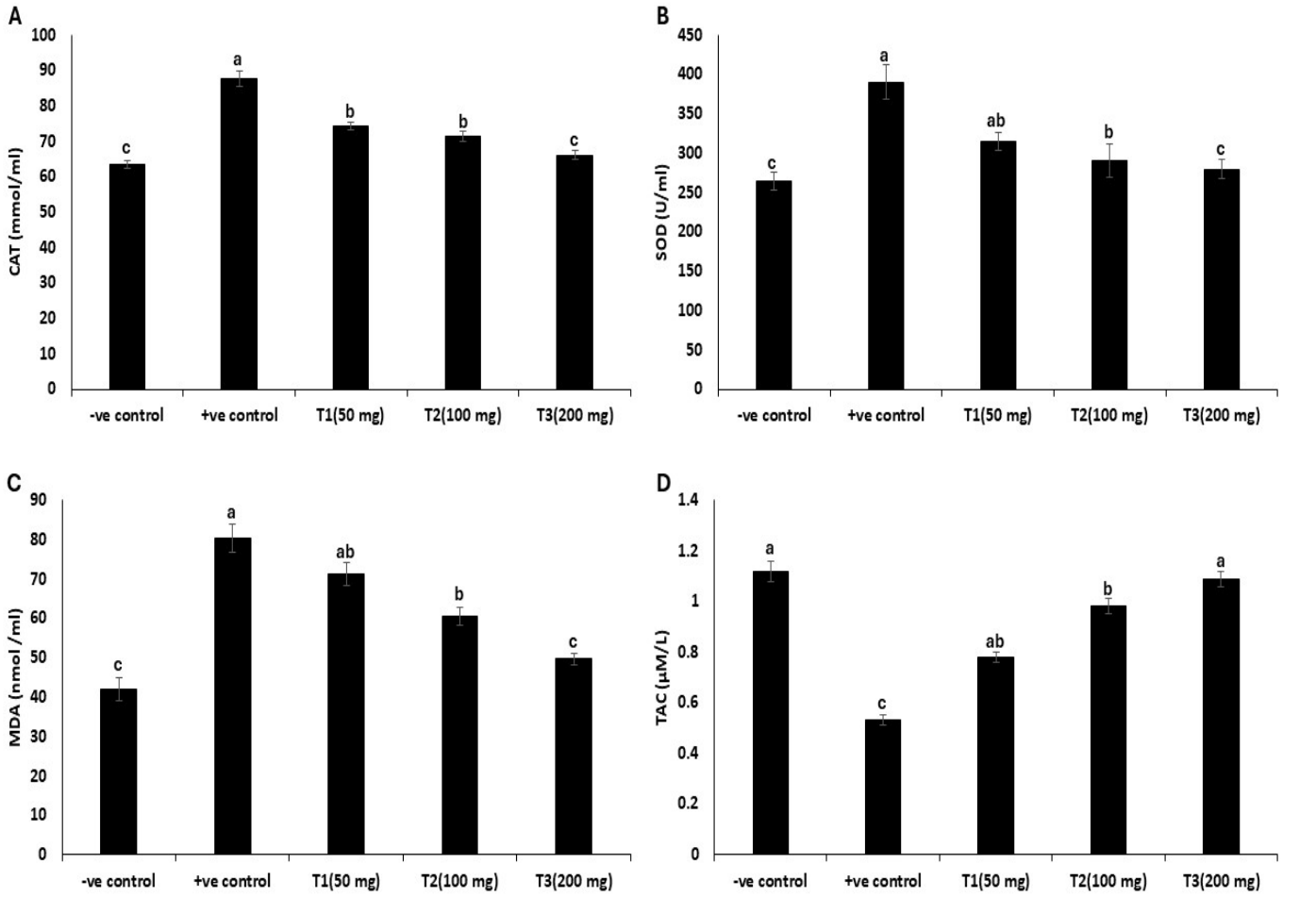


**Figure 2:** Effect of *Silybum marianum* on (A) catalase (CAT), (B) superoxide dismutase (SOD), (C) lipid peroxidase (MDA), (D) total antioxidant capacity (TAC) of O. *niloticus* challenged with 1.45 mg lead acetate $L^{-1}$.
$^{(a,b,c)}$ different letters indicate significant differences ($p < 0.05$).

**Table 5:** Effect of *Silybum marianum* on heamatological parameters of O. *niloticus* challenged with 1.45mg lead acetate $L^{-1}$.

| Parameters | -ve control | +ve control | T1(50 mg) | T2(100 mg) | T3(200 mg) |
|---|---|---|---|---|---|
| RBCs count (×$10^6$/cm) | 2.84 ± 0.26$^a$ | 1.82 ± 0.19$^c$ | 2.14 ± 0.21$^b$ | 2.56 ± 0.23$^{ab}$ | 2.75 ± 0.28$^a$ |
| Hb g/dl | 8.52 ± 0.67$^a$ | 5.89 ± 0.74$^c$ | 6.75 ± 0.65$^b$ | 7.36 ± 0.85$^{ab}$ | 8.24 ± 0.59$^a$ |
| PCV% | 25.65 ± 2.13$^a$ | 17.23 ± 1.52$^c$ | 20.21 ± 2.33$^c$ | 22.78 ± 2.49$^c$ | 24.28 ± 1.98$^c$ |
| platelets count (×$10^3$/cm) | 71.45 ± 4.24$^a$ | 37.36 ± 3.16$^c$ | 42.38 ± 1.45$^{bc}$ | 55.78 ± 2.93$^b$ | 68.27 ± 2.75$^a$ |
| MCV (fl) | 101.23 ±6.35$^a$ | 87.29 ± 5.24$^b$ | 88.49 ± 6.27$^{ab}$ | 96.24 ± 5.22$^a$ | 98.87 ± 5.47$^a$ |
| MCH (pg) | 29.96 ± 3.14$^a$ | 30.26 ± 2.34$^a$ | 30.41 ± 3.24$^a$ | 30.45 ± 2.39$^a$ | 30.12 ± 2.94$^a$ |
| MCHC (g/dl) | 32.24 ± 3.75$^a$ | 32.25 ± 3.45$^a$ | 31.42 ± 2.65$^a$ | 30.58 ± 3.21$^a$ | 31.42 ± 3.47$^a$ |

$^{(a,b,c)}$ different superscript letters in the same row indicate significant differences ($p < 0.05$).

Bioaccumulation is a characteristic aspect of toxicity caused by Pb exposure. Toxic effects are induced in fish due to Pb exposure affecting its biochemical and physiological functions. Exposure pathway (dietary and waterborne), environmental factors (salt-water or fresh water) and Pb binding capacity with protein, SH and sulfur group decide accumulation pattern of Pb exposure. The accumulated lead in fish flesh is considered a serious issue to human health (Hussein *et al*., 2023). The 96 h-LC50 of lead acetate in the present study was 1.45 mg/L. In previous studies LC50 value of lead acetate for *O. mossambicus* is 20.03 mg/l (Meena *et al*., 2017), 18.7mg/l (Latif *et al*., 2013). For the same species of fish, different concentrations of the same heavy metal were obtained. This might be because to variations in sex, age, and eating habits, in addition to the experimental setup (Witeska *et al*., 1993; Elora *et al*., 2011). The exposure of *O. niloticus* to sub lethal dose of lead acetate for 20 days retarded its growth performance, as shown

**Table 6:** Effect of *Silybum marianum* on total and differential leukocyte count of O. *niloticus* challenged with 1.45 mg lead acetate $L^{-1}$.

| Parameters | -ve control | +ve control | T1(50 mg) | T2(100 mg) | T3(200 mg) |
|---|---|---|---|---|---|
| WBCs (×$10^3$/cm) | 15.8 ± 0.41[a] | 5.78 ± 0.57[c] | 8.81 ± 0.62[b] | 13.52 ± 0.67[ab] | 14.26 ± 0.48[a] |
| Lymphocytes | 10.80 ± 0.13[a] | 3.09 ± 0.32[b] | 4.84 ± 0.37[b] | 9.02 ± 0.42[ab] | 10.06 ± 0.41[a] |
| Heterophils | 3.35 ± 0.11[a] | 2.28 ± 0.21[b] | 2.74 ± 0.25[b] | 3.41 ± 0.26[ab] | 2.96 ± 0.31[a] |
| Monocytes | 0.86 ± 0.09[a] | 0.22 ± 0.11[b] | 0.33 ± 0.14[b] | 0.67 ± 0.14[b] | 0.80 ± 0.13[a] |
| Eosinophils | 0.33 ± 0.03[a] | 0.13 ± 0.03[b] | 0.18 ± 0.11[b] | 0.29 ± 0.09[a] | 0.30 ± 0.07[a] |
| Basophils | 0.16 ± 0.02[a] | 0.06 ± 0.01[b] | 0.09 ± 0.01[b] | 0.13 ± 0.03[a] | 0.14 ± 0.06[a] |

[(a,b,c)] different superscript letters in the same row indicate significant differences ($p < 0.05$).

by decreased weight and weight gain and increased feed conversion ratio. These declines in fish performance might be linked to the harmful effects of lead poisoning on many metabolic processes in fish exposed to lead. According to Bengani *et al.* (2015), lead (Pb) is a very hazardous element that negatively impacts the biological functions of inebriated organisms. Regarding this, Ikeogu *et al.* (2016) recorded that fish exposed to lead poisoning had significant mortality rates and reduce development in some fish species. In addition, Kumar *et al.* (2021) recorded that Pb-exposed *O. niloticus* showed a significant reduction in growth when compared to the control group. Contrariwise, dietary *S. marianum* increased the growth rate as recorded in pervious works (Hassaan *et al.*, 2019; Abdel-Latif *et al.*, 2023). The digestion-related enzyme activities may be enhanced by the increased growth and feed intake following dietary supplementation with *S. marianum* levels (Abdel-Latif *et al.*, 2023). When *S. marianum* is fed to fish together with Pb exposure, the harmful effects of Pb acetate on *O. niloticus* growth are retrieved. Dietary *S. marianum* may have a mitigating effect on lead acetate toxicity because of its high antioxidant content 92.25 and 123 mg/ kg dry weight, of flavonolignans, which activated the enzymatic system to accelerate protein synthesis (Banaee *et al.*, 2011). Hassan *et al.* (2019) reported comparable results when *S. marianum* was used to treat Pb-induced toxicity in *O. niloticus*. In addition, fish fed a diet enriched with *S. marianum* seeds showed the lowest FCR in comparison to positive control. The FCR decreased in poultry and quail feed silymarin for 4 weeks (Khaleghipour *et al.*, 2019). The obtained results might be explained by the presence of bioactive components in *S. marianum*, which affect protein retention and increase feed efficiency. The active components of plant extract might enhance the availability and digestibility of nutrients, leading to increased levels of protein synthesis and feed utilization (Citarasu, 2010). In line with this, silymarin extract was reported to have a positive impact on the growth performance and feed consumption efficiency of fish species (Jia *et al.*, 2013; Xiao *et al.*, 2017). Meanwhile, *Carassius auratus* growth performance and feed consumption were unaffected by the addition of silymarin extract (Yi *et al.*, 2012). These disparities in the outcomes might be caused by various fish species, culture circumstances, or dietary components. ALT is dispersed throughout the hepatic cells and bile duct, whereas AST is often found in the mitochondria of hepatocytes. Fish with elevated blood AST and ALT levels may be exhibiting increased hepatic tissue production of enzymes or enzyme leakage through damaged plasma membranes (Yang and Chen, 2003). Accordingly, serum AST and ALT activity are employed as critical markers to represent the liver's health and the fish's activities (Zhai *et al.*, 2014). They may also be used to evaluate the fish's health and serve as stress markers (Satheeshkumar *et al.*, 2010). In the present investigation, a better trend was seen in the liver cells of *O. niloticus* as serum ALT and AST activity reduced with increasing dietary level of S. marianum extract. Additionally, as previously stated by Kvasnicka *et al.* (2003) and Akrami *et al.* (2015), the lowest level of AST and ALT may be related to the strong antioxidant activity of *S. marianum* and their hepatoprotective properties, which led to prevent lipid peroxidation of cell membranes and ultimately suppress the release of liver damage enzymes like AST and ALT into plasma. According to Davila *et al.* (1989), naturally occurring antioxidant flavonoids such silybin and catechin increased liver function and strengthened its resistance to oxidative stress and tissue damage. Moreover, Banaee *et al.* (2011) found that include *S. marianum* extract in meals controlled the activities of ALT and AST in rainbow trout plasma. The action of SME attributed to increasing the regenerative ability of the liver cells by enhancing the synthesis of DNA and RNA, as silymarin has a steroid structure; Altering the structure of the hepatocyte external membrane, that prevents entrance of the xenobiotics into the cell (Karimi *et al.*, 2011).

Lead exposure in current study increased the blood cortisol and blood glucose levels in *O. niloticus*, suggesting that the stress response to Pb poisoning is intensifying. Sayed *et al.* (2017) and Kumar *et al.* (2021) who reported that Pb exposure significantly raised the levels of cortisol and hyperglycemia in African catfish and *O. niloticus*, respectively made similar findings. Furthermore, *S. marianum* has hepatoprotective effects, maintaining hepatocytes from

toxin-induced damage. Considering the significance of the liver in glucose metabolism, maintaining liver function in fish exposed to lead acetate by supplementing the diet with *S. marianum* may help maintain glucose homeostasis.

The waste product creatinine is created during muscle metabolism and is removed from the blood by the kidneys. As a result, increased plasma creatinine levels may be a sign of compromised renal function. In current study, dietary supplementation with *S. marianum* reduced serum urea and creatinine levels in serum. Fish exposed to lead acetate may experience oxidative stress, inflammation, and damage to their kidney tissues. These side effects may disrupt regular kidney function, which might have an impact on urea and uric acid metabolism and excretion. Purine metabolism produces uric acid, whereas urea is a consequence of protein metabolism (Banaee *et al*., 2011; Abdelkhalek *et al*., 2017).

Elevations in fish serum total protein, albumin, and globulin levels are often associated with a more robust innate immune response (Bernet *et al*., 2001). According to the data presented here, fish given all diets including *S. marianum* extract showed significant (P<0.05) enhancement of "total protein, albumin, and globulin." This suggests that the rise in serum total protein level is likely closely related to the increase in protein synthesis in liver tissue. Fish have a robust immune system, as evidenced by the increased serum albumin content, which may be linked to the enhancing effect of silymarin flavonolignans on ribosome formation and stimulation of DNA and protein synthesis in liver tissues (El-Kamary *et al*., 2009; Akrami *et al*., 2015). According to Ahmadi *et al*. (2012), young *Oncorhynchus mykiss* with serum albumin levels greater than those of the control group were fed a diet supplemented with 0.1 g/ kg of *S. marianum* extract. The presence of antioxidant and radical-scavenging properties in silymarin promote its cytoprotective properties (Karimi *et al*. 2011). The inhibition and modification of cell transporters, pglycoprotein, estrogenic and nuclear receptors are among the putatively understood mechanisms of action of silymarin protection (Ardo *et al*., 2008).

It is well known that heavy metals cause oxidative stress in aquatic organisms by inhibiting the activity of antioxidant enzymes. This increased cellular generation of reactive oxygen species (ROS) causes the oxidation of biomolecules. Lead is a redox inactive heavy metal that causes oxidative damage by changing the functions of enzymes that contain thiols (Pinto *et al*., 2003). All aquatic creatures have antioxidant defense mechanisms to counteract the harmful effects of ROS (Lushchak, 2011). Antioxidant defense systems, such as antioxidant enzymes, avert tissue oxidative damage (Hoseinifar *et al*., 2020). Fish are commonly utilized as non-specific immunological indices due to the synthesis of superoxide dismutase (SOD) and catalase (CAT), both of which are important markers of a cell's capacity to withstand oxidative damage (Shiau *et al*., 2015). In current study CAT, SOD and MDA significantly increased (P< 0.05) in control positive group on contrary TAC decreased in the same group responding to the oxidative stress induced by the lead The *S. marianum* treated groups T1, T2 and T3 were tolerated the oxidative stress due to lead exposure, which may be attributed to the active silymarin molecule in *S. marianum* seeds contains flavonoids and vitamin E, which have a highly effective scavenging effect on free radicals inside tissues (Doehmer *et al*., 2008). Banaee *et al*. (2011) state that silymarin has healing capabilities that include "antioxidant, anti-inflammatory, anti-carcinogenic, and anti-fibrotic properties." It also affects the efficacy of cellular protein manufacturing, the liver's decreased glutathione (GSH) concentration, and the cell membrane's enhanced stability. Moreover, Chand *et al*. (2011) found that the active silymarin in *S. marianum* seeds significantly raises the oxidative status of the liver and directly impacts immune cells. Furthermore, Astuya *et al*. (2017) observed that the pro-inflammatory enzymes' relative expression in salmonid cell lines was dramatically decreased by the phenolic compounds that were isolated from Pinus radiata bark. Other phenolic substances have been shown in comparable experiments to have favorable effects on SOD activity under situations of oxidative stress (Subash and Jayanthi, 2010). According to the current study, *O. niloticus* fed diets supplemented with *S. marianum* showed improvements in its erythrocyte and leucocyte counts, especially in the T2 and T3 groups, suggesting that *S. marianum* has immunostimulant qualities. Nearly similar results obtained in fish fed dietary silymarin showed improvements in blood parameters erythrocyte count, white blood cell count, hemoglobin, and packed cell volume (Sherif *et al*., 2023). Deficit in the antioxidant system is directly linked to low erythrocyte and haemoglobin levels. The alterations in the erythrocyte profile point to the body compensating for an oxygen shortage brought on by lead exposure-related gill damage. Haematological traits are commonly used to identify fish contaminated with metals. (Shah and associates, 1995; Drastichova and associates, 2004). Metals typically have an impact on fish hematological parameters through osmotic changes that cause hemoconcentrations, or an increase in blood cell concentration brought on by the loss of plasma or water from the bloodstream, or hem dilution, which is an increase in plasma volume and a decrease in red blood cell concentration in blood (Tort and Torres, 1988). The current investigation found that lead exposure lowered hematological parameters. A prior study found that exposure to lead acetate significantly reduced hemoglobin, packed cell volume, RBC count, WBC count, and lymphocytes, especially after the tenth week (Ilesanmi *et al*., 2022). The spleen is typically to blame for this alteration. Because the

spleen functions as a powerful blood storage organ, storing blood cells during rest and releasing them into the bloodstream in response to different forms of stress (Yamamoto, 1988). According to Bano and Hasan (1990), exposure to lead causes spleen damage, which lowers fish hematological and immunological indices.

## CONCLUSIONS AND RECOMMENDATIONS

Lead acetate decreases the growth performance parameters, antioxidants capacity and immunological indicators. Meanwhile, increase liver enzymes (AST and ALT), cortisol, glucose, CAT, SOD, and MDA. The addition of dietary *S. marianum* extract plays in the protection of *O. niloticus* from the harmful effects of Pb poisoning in water and enhance immunological biomarkers, antioxidant capacity, and performance of fish. Future studies are needed for evaluation of of other potential physiological parameters and lead residue in fish flesh.

## ACKNOWLEDGMENTS

The authors thank faculty of veterinary medicine, Zagazig University for their support of this research. This work has not been published or submitted to other journals previously is included.

## AUTHOR'S CONTRIBUTION

AFA designed the experiment, AE, TH carry out experiment, wrote the manuscript, analyzed the data and reviewed the manuscript.

### Conflict Of Interest

The authors have declared no conflict of interest.

## REFERENCES

Abbas, W.T., Ali, S.E., Melegy, A.A., Gamil, A. Fish Diet Supplemented with Yemeni Zeolite Improves Growth Performance and Reduces Lead Toxicity in Nile Tilapia (*Oreochromis Niloticus*). Aquatic Res., 2021; 52: 6678–6688. https://doi.org/10.1111/are.15537

Abdelkhalek, N.K., Eissa, I.A., Ahmed, E., Kilany, O.E., El-Adl, M., Dawood, M.A., Hassan, A.M., Abdel-Daim, M.M. Protective role of dietary Spirulina platensis against diazinon-induced Oxidative damage in Nile tilapia; *Oreochromis niloticus*. Environ. Toxicol. Pharmacol., 2017; 2(54): 99–104. https://doi.org/10.1016/j.etap.2017.07.002

Abdel-Latif, H.M., Shukry, M., Noreldin, A.E., Ahmed, H.A., El-Bahrawy, A., Ghetas, H.A., Khalifa, E. Milk thistle (Silybum marianum) extract improves growth, immunity, serum biochemical indices, antioxidant state, hepatic histoarchitecture, and intestinal histomorphometry of striped catfish, Pangasianodon hypophthalmus. Aquaculture, 2023; 562: 738761. https://doi.org/10.1016/j.aquaculture.2022.738761

Abou Zid S. Silymarin, Natural Flavonolignans from Milk Thistle. In: Venketeshwer R, editor. *Phytochemicals-A Global Perspective of Their Role in Nutrition and Health*. Rijeka: Croatia InTech; 2012; 255–272. https://doi.org/10.5772/26027

Ahmadi, K., Banaee, M., Vosoghei, A.R., Mirvaghefei, A.R., Ataeimehr, B. Evaluation of the immunomodulatory effects of silymarin extract (Silybum marianum) on some immune parameters of rainbow trout, Oncorhynchus mykiss (Actinopterygii: Salmoniformes: Salmonidae). Acta Ichthiol., Et Piscato, 2012; 42(2): 113–120. https://doi.org/10.3750/AIP2011.42.2.04

Ahmadifar, E., Kalhor, N.., Dawood, M.A., Ahmadifar, M., Shahriari, M.M., Yousefi, M. Effects of dietary p-coumaric acid on the growth performance, digestive enzyme activity, humoral immunity and immune-related gene expression in common carp, Cyprinus carpio. Aquacult. Nutr., 2021; 27: 747–756. https://doi.org/10.1111/anu.13220

Akrami, R., Gharaei, A., Mansour, M.R., Galeshi, A. Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato-biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. Fish Shellfish Immunol., 2015; 45:828–834 https://doi.org/10.1016/j.fsi.2015.06.005

Alishahi, M., Soltani, M., Mesbah, M., Rad, A.E. Effects of dietary Silybum marianum extract on immune parameters of the common carp (Cyprinus carpio). J. Vet. Res., 2011; 66: 255–286.

Ardo, L., Yin, G., Xu, P., Varadi, L., Szigeti, G., Jeney, Z. Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. Aquaculture, 2008; 275(1): 26–33. https://doi.org/10.1016/j.aquaculture.2007.12.022

Arisekar, U., Jeya, R., Shalini, R., Jeyasekaran, G. Human Health Risk Assessment of Heavy Metals in Aquatic Sediments and Freshwater Fish Caught from Thamirabarani River, the Western Ghats of South Tamil Nadu. Marine Pollut. Bullet., 2020; 159: 111496. https://doi.org/10.1016/j.marpolbul.2020.111496

Astuya, A., Ziehe, J., Rivera, A., Ortiz, S., Ulloa, V., Roeckel, M., Aspe, E., Fernandez, K. Antioxidant and anti-inflammatory activities of *Pinus radiata* bark extract in salmonid cell lines. Aquacult. Res., 2017; 48: 3568–3578. https://doi.org/10.1111/are.13182

Banaee, M., Sureda, A., Shahaf, S., Fazilat, N. Protective effects of silymarin extract on malthion-induced zebra cichlid (Cichlasoma nigrofasciatum) hepatotoxicity. Iranian J. Toxicol., 2015; 9: 1239–1246.

Banaee, M., Sureda, A., Mirvaghefi, A.R., Rafei, G.R. Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (Oncorhynchus mykiss). Fish Physiol. Biochem., 2011; 37:885-896. https://doi.org/10.1007/s10695-011-9486-z

Bano, Y., Hasan, M. Histopathological Lesions in the Body organs of cat-fish (*Heteropneustes fossilis*) following mercury intoxication. Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes, 1990; 25(1): 67-85. https://doi.org/10.1080/03601239009372677

Behreus, A.S., Karber, L. Determination of LC50. Archives of Experimental Pathology and Pharmacology, 1953; 28: 177.

Bengani, R., Gadhia, M., Ansari, E. Accumulation of lead in the muscle of brackish water fish (*Boleopthalmus dussumieri*). J. Appl. Nat. Sci., 2015; 7: 662–665. https://doi.org/10.31018/jans.v7i2.663

Bernet, D., Schmidt, H., Wahli, T., Burkhardt-Holm, P. Effluent from a sewage treatment works causes changes in serum chemistry of brown trout (Salmo trutta). Ecotoxicol. Environ. Safety, 2001; 48: 140–147. https://doi.org/10.1006/eesa.2000.2012

Boyd, C.E. 1984: Water Quality in Warm Water Fishponds. Auburn University Agriculture Experimental Station, Auburn, Alabama, USA.

Brown, B.A. 1980: Hematology: Principles and Procedures. Lea and Febiger, Philadelphia, PA, USA.

Chan, C.Y., Tran, N., Pethiyagoda, S., Crissman, C.C., Sulser, T.B., Phillips, M.J. Prospects and challenges of fish for food security in Africa. Glob. Food Sec., 2019; 1(20):17-25. https://doi.org/10.1016/j.gfs.2018.12.002

Chand, N., Muhammad, D., Durrani, F.R., Qureshi, M.S., Sahibzada, S., Ullah, S.S. Protective effects of milk thistle (*Silybum marianum*) against aflatoxin B1 in broiler chicks. Asian-Aust. J. Anim. Sci., 2011; 24 (7): 1011–1018. https://doi.org/10.5713/ajas.2011.10418

Chowdhury, S., Saikia, S.K. Oxidative stress in fish: a review. Journal of Scientific Research, 2020; 12: 145–160. https://doi.org/10.3329/jsr.v12i1.41716

Chupani, L., Niksirat, H., Velíšek, J., Stará, A., Hradilová, Š., Kolařík, J., Panáček, A., Zusková, E. Chronic dietary toxicity of zinc oxide nanoparticles in common carp (*Cyprinus carpio* L.): tissue accumulation and physiological responses. Ecotoxicol. Environ. Safety, 2018; 147: 110–116. https://doi.org/10.1016/j.ecoenv.2017.08.024

Chupani, L., Zusková, E., Niksirat, H., Panáček, A., Lünsmann, V., Haange, S.B., von Bergen, M., Jehmlich, N. Effects of chronic dietary exposure of zinc oxide nanoparticles on the serum protein profile of juvenile common carp (*Cyprinus carpio L.*). Sci. Total Environ., 2017; 579: 1504–1511. https://doi.org/10.1016/j.scitotenv.2016.11.154

Citarasu, T. Herbal biomedicines: a new opportunity for aquaculture industry. Aquacult. Int., 2010; 18: 403–414. https://doi.org/10.1007/s10499-009-9253-7

Dacie, J.V., Lewis, S.M. 1984: Practical Haematology, 6th ed. Churchill Livingstone, London, UK.

Davila, C., Lenherr, A., Acosta, D. Protective effect of flavonoids on drug induced hepatotoxicity *in vitro*. Toxicology, 1989; 57: 267–286. https://doi.org/10.1016/0300-483X(89)90116-9

Dawood, M.A., Abdel-Tawwab, M., Abdel-Latif, H.M. Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. Rev. Aquacult., 2020; 12: 2511–2526. https://doi.org/10.1111/raq.12455

Doehmer, J., Tewes, B., Klein, K.U., Gritzko, K., Muschick, H., Mengs, U. Assessment of drug–drug interaction for silymarin. Toxicol. Vitro, 2008; 22 (3): 610–617. https://doi.org/10.1016/j.tiv.2007.11.020

Drastichova, J., Svobodova, Z., Luskova, V., Machova, J. Effect of Cadmium on Hematological Indices of Common Carp (*Cyprinus carpio L.*). Bullet. Environ. Contamination Toxicol., 2004; 72: 725-732. https://doi.org/10.1007/s00128-004-0305-4

Dytham, C. 2011; Choosing and Using Statistics: A biologist's Guide. Blackwell Science Ltd, London, UK.

El-Kamary, S.S., Shardell, M.D., Abdel-Hamid, M., Ismail, S., El-Ateek, M., Metwally, M., Mikhail, N., Hashem, M., Mousa, A., Aboul-Fotouh, A., A randomized controlled trial to assess the safety and efficacy of silymarin on symptoms, signs and biomarkers of acute hepatitis. Phytomedicine, 2009; 16, 391–400. https://doi.org/10.1016/j.phymed.2009.02.002

Eroglu, A., Dogan, Z., Kanak, E.G., Atli, G., Canli, M. Effects of Heavy Metals (Cd, Cu, Cr, Pb, Zn) on Fish Glutathione Metabolism. Environ. Sci. Pollut. Res., 2015; 22: 3229–3237. https://doi.org/10.1007/s11356-014-2972-y

Foster, L., Dunn, R. Single antibody technique for radioimmunoassay of cortisol in extracted serum or plasma. Clin. Chem., 1974; 20: 365. https://doi.org/10.1093/clinchem/20.3.365

Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., El-Garhy, H.A., Moustafa, M.M., Mohamed, S.A., El-Haroun, E.R. Effect of Silybum marianum seeds as a feed additive on growth performance, serum biochemical indices, antioxidant status, and gene expression of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. Aquaculture, 2019; 509: 178-187. https://doi.org/10.1016/j.aquaculture.2019.05.006

Henry, R. 1964: Colorimetric determination of total protein. Clinical Chemistry. Harper and Row Publ, New York, USA.

Henson, M.N., Aday, D.D., Rice, J.A. Thermal Tolerance and survival of nile tilapia and blue tilapia under rapid and natural temperature declination rates. Transactions of the American Fisheries Societies, 2018; 147(2):278-86. https://doi.org/10.1002/tafs.10023

Hoseinifar, S.H., Yousefi, S., Van Doan, H., Ashouri, G., Gioacchini, G., Maradonna, F., Carnevali, O. Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev. Fish. Sci. Aquacult., 2020; 29 (2):198-217. https://doi.org/10.1080/23308249.2020.1795616

Hussein, M. A., Morsy, N. S., Mahmoud, A. F., Darwish, W. S., Elabbasy, M. T., Zigo, F., Farkašová, Z., & Rehan, I. F. Risk assessment of toxic residues among some freshwater and marine water fish species. Front. Vet. Sci., 2023; 10, 1185395 https://doi.org/10.3389/fvets.2023.1185395.

Ikeogu, C.F., Nsofor, C.I., Igwilo, I.O., Ngene, A.A. Haematological and serological responses of *Clarias gariepinus* to sublethal concentrations of lead nitrate. J. Pharmacol. Sci. Biosci. Res., 2016; 6 (3): 442–446.

Ilesanmi, O.B., Adeogun, E.F., Odewale, T.T., Chikere, B. Lead exposure-induced changes in hematology and biomarkers of hepatic injury: protective role of TrévoTM supplement. Environ. Analitic. Health Toxicol., 2022; 37(2):e2022007. https://doi.org/10.5620/eaht.2022007

Jia, R., Cao, L., Du, J., Xu, P., Jeney, G., Yin, G. The protective effect of silymarin on the carbon tetrachloride ($CCl_4$)-induced liver injury in common carp (*Cyprinus carpio*). In Vitro Cell. Dev. Biol. Anim., 2013; 49:155–161. https://doi.org/10.1007/s11626-013-9587-3

Karimi, G., Vahabzadeh, M., Lari, P., Rashedinia, M., Moshiri, M. "Silymarin", a promising pharmacological agent for treatment of diseases. Iran. J. Basic Med. Sci., 2011; 14(4): 308–317.

Khafaga, A.F., Naiel, M.A., Dawood, M.A., Abdel-Latif, H.M. Dietary Origanum vulgare essential oil attenuates cypermethrin-induced biochemical changes, oxidative stress, histopathological alterations, apoptosis, and reduces DNA damage in Common carp (*Cyprinus carpio*). Aquatic Toxicol., 2020; 228: 105624. https://doi.org/10.1016/j.aquatox.2020.105624

Kiran, K., Midhun, S.J., Vysakh, A., James, T.J. Antagonistic Effects

of Dietary Moringa Oleifera on Hemato-biochemical and Oxidative Stress of Lead Nitrate Intoxicated Nile Tilapia, Oreochromis Niloticus. Aquacult. Res., 2021; 52: 6164–6178. https://doi.org/10.1111/are.15478

Khaleghipour, B., Khosravinia, H., Toghiyani, M., Azarfar, A. Effects of silymarin on productive performance, liver function and serum biochemical profile in broiler Japanese quail challenged with dietary aflatoxins. Italian J. Anim. Sci., 2019; 18(1): 564-573. https://doi.org/10.1080/1828051X.2018.1548310

Křen, V., Walterová, D. Silybin and silymarin-new effects and applications. Biomedical Papers, 2005; 149: 29–41. https://doi.org/10.5507/bp.2005.002

Kvasnička, F., Bıba, B., Ševčık, R., Voldřich, M., Kratka, J. Analysis of the active components of silymarin. J. Chromatograph., A, 2003; 990: 239–245. https://doi.org/10.1016/S0021-9673(02)01971-4

Kvasnicka, F., Byba, B., Sevcyk, R., Voldrich, M., Kratka, J. Analysis of the active components of silymarin. J. Chromatograph., A, 2003; 990: 239–245. https://doi.org/10.1016/S0021-9673(02)01971-4

Latif, A., Ali, M., Sayyed, A.H., Iqbal, F., Usman, K., Rauf, M., Kaoser, R. Effect of copper sulphate and lead nitrate, administered alone or in combination, on the histology of liver and kidney of Labeo rohita, Pak. J. Zool., 2013; 45(4): 913-920.

Livingstone, D.R. Contaminantstimulated reactive oxygen species production and oxidative damage in aquatic organisms. Marine Pollut. Bullet., 2001; 42: 656-666. https://doi.org/10.1016/S0025-326X(01)00060-1

Lushchak, V.I. Environmentally induced oxidative stress in aquatic animals. Aquatic Toxicology, 2011; 101(1):13-30. https://doi.org/10.1016/j.aquatox.2010.10.006

Meena, R., Chandrakala, N., Indra, N. Studies on the bioaccumulation of lead in Oreochromis mossambicus during short term toxicity. Int. J. Appl. Res., 2017; 3(1):468-470.

Moyo, N.G., Rapatsa, M.M. A Review of the Factors Affecting Tilapia Aquaculture Production in Southern Africa. Aquaculture, 2021; 535: 736386. https://doi.org/10.1016/j.aquaculture.2021.736386

Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao, L., Klinger, D.H., Little, D.C., Lubchenco, J., Shumway, S.E., Troell, M. A 20-year retrospective review of global aquaculture. Nature, 2021; 591(7851): 551-563. https://doi.org/10.1038/s41586-021-03308-6

Nazdar, N., Imani, A., Noori, F., Moghanlou, K.S. Effect of silymarin supplementation on nickel oxide nanoparticle toxicity to rainbow trout (*Oncorhynchus mykiss*) fingerlings: pancreas tissue histopathology and alkaline protease activity. Iran. J. Sci. Technol., 2018; 42: 353–361. https://doi.org/10.1007/s40995-016-0052-5

Owatari, M. S., Alves Jesus, G. F., Brum, A., Pereira, S. A., Lehmann, N. B., de Pádua Pereira, U., Martins, M. L., & Pedreira Mouriño, J. L. (2018). Sylimarin as hepatic protector and immunomodulator in Nile tilapia during *Streptococcus agalactiae* infection. Fish Shellfish Immunol., 82, 565–572. https://doi.org/10.1016/j.fsi.2018.08.061

Patra, R.C., Rautray, A.K., Swarup, D. Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. Vet. Med. Int., 2011; 2011: 457327. https://doi.org/10.4061/2011/457327

Paul, S., Mandal, A., Bhattacharjee, P., Chakraborty, S., Paul, R., Kumar, M., Ukhopadhyay, B. Evaluation of Water Quality and Toxicity after Exposure of Lead Nitrate in Fresh Water Fish, Major Source of Water Pollution. Egypt. J. Aquat. Res., 2019; 45: 345–351. https://doi.org/10.1016/j.ejar.2019.09.001

Pinto, E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.K., Morse, D., Colepicola, P. Heavy metal induced oxidative stress in algae. J. Physiol., 2003; 39: 1008-1018. https://doi.org/10.1111/j.0022-3646.2003.02-193.x

Reitman, S., Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 1957; 28(1): 56–63. https://doi.org/10.1093/ajcp/28.1.56

Satheeshkumar, P., Ananthan, G., Senthilkumar, D., Khan, A.B., Jeevanantham, K. Comparative investigation on haematological and biochemical studies on wild marine teleost fishes from Vellar estuary, southeast coast of India. Comp. Clin. Pathol., 2010; 21: 275–281. https://doi.org/10.1007/s00580-010-1091-5

Sayed, A.H., El-Sayed, Y.S., El-Far, A.H. Hepatoprotective efficacy of *Spirulina platensis* against lead-induced oxidative stress and genotoxicity in catfish; *Clarias gariepinus*. Ecotoxicol. Environ. Safety, 2017; 143: 344–350. https://doi.org/10.1016/j.ecoenv.2017.05.013

Shah, S.L., Hafeez, M.A., Shaikh, S.A. Changes in Hematological parameters and Plasma Glucose in the fish *Cyprinus watsoni*, in the Exposure to Zinc and Copper Treatment. Pak. J. Zool., 1995; 27: 50-54.

Sherif, A.H., Toulan, A.E., El-Kalamwi, N., Farag, E.A.H., Mahmoud, A.E. Silymarin enhances the response to oxytetracycline treatment in Oreochromis niloticus experimentally infected with *Aeromonas hydrophila*. Sci. Rep., 2023; 13(1):16235. https://doi.org/10.1038/s41598-023-43270-z

Shiau, S.Y., Gabaudan, J., Lin, Y.H. Dietary nucleotide supplementation enhances immune responses and survival to *Streptococcus iniae* in hybrid tilapia fed diet containing low fish meal. Aquacult. Reprod., 2015; 2: 77–81. https://doi.org/10.1016/j.aqrep.2015.08.002

Subash, P., Jayanthi, R. Antioxidant effect of Caffeic acid on oxytetracycline induced lipid peroxidation in albino rats. Indian J. Clin. Biochem., 2010; 25: 371–375. https://doi.org/10.1007/s12291-010-0052-8

Tort, T., Torres, P. The Effects of sublethel concentrations of cadmium on hematological parameters in the Dogfish, *Scyliorhinus canicula*. J. Fish Biol., 1988; 32: 277-282. https://doi.org/10.1111/j.1095-8649.1988.tb05361.x

Trinder, P. Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J. Clin. Pathol., 1969; 22: 246-253. https://doi.org/10.1136/jcp.22.2.246-b

Van Kampen, E.J., Zijlstra, W.G. Recommendations for haemoglobinometry in human blood. Brazilian J. Hematol., 1961; 13:71-78.

Vinodhini, R., Narayanan, M. Biochemical changes of antioxidant enzymes in common carp (Cyprinuscarpio) after heavy metal exposure. Turkish J. Vet. Anim. Sci., 2009; 33: 273-278. https://doi.org/10.3906/vet-0711-18

Witeska, M., Jezierska, B., Chaber, J. The influence of cadmium on common carp embryos and larvae. Aquaculture, 1993; 129:129-132 https://doi.org/10.1016/0044-8486(94)00235-G

Wotton, I.D., Freeman, H. 1982: Microanalysis in Medical Biochemistry. Churchill, New York, USA.

Xiao, P., Ji, H., Ye, Y. Dietary silymarin supplementation promotes

growth performance and improves lipid metabolism and health status in grass carp (*Ctenopharyngodon idellus*) fed diets with elevated lipid levels. Fish Physiol. Biochem., 2017; 43: 245–263. https://doi.org/10.1007/s10695-016-0283-6

Yamamoto, K.I. Contraction of Spleen in Exercised Fresh water Teleost. Comp. Biochem. Physiol., 1988; 89A: 65-66. https://doi.org/10.1016/0300-9629(88)91139-5

Yan, W., Hamid, N., Deng, S., Jia, P.P., Pei, D.S. Individual and Combined Toxicogenetic Effects of Microplastics and Heavy Metals (Cd, Pb, and Zn) Perturb Gut Microbiota Homeostasis and Gonadal Development in Marine Medaka (Oryzias melastigma). J. Hazardous Mater., 2020; 397: 122795. https://doi.org/10.1016/j.jhazmat.2020.122795

Yang, J.L., Chen, H.C. Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. Zool. Stud., 2003; 42: 455–461.

Yi Dan, Y., Gu, L., Ding, B., Li, M.L., Hou, Y., Wang, L., Joshua, G. Effects of dietary silymarin supplementation on growth performance and oxidative status in Carassius auratus. Gibelio, 2012; 11(18): 3399-3404. https://doi.org/10.3923/javaa.2012.3399.3404

Yousafzai, A.M., Shakoori, A.R. Hepatic responses of a freshwater fish against aquatic pollution. Pak. J. Zool., 2011; 43: 209–221.

Zhai, S.W., Lu, J.J., Chen, X.H. Effects of dietary grape seed proanthocyanidins on growth performance, some serum biochemical parameters and body composition of tilapia (*Oreochromis niloticus*) fingerlings. Italian J. Anim. Sci., 2014; 13: 3357. https://doi.org/10.4081/ijas.2014.3357