

Effect of the Leaf Extract of *Nerium Nerium indicum* on Health Status of the Common Carp *Cyprinus carpio*

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Abstract: This experiment was conducted for 14 days on 80 common carp, *Cyprinus carpio* fingerlings to study the effect of *Nerium indicum* leaf extract on blood serum enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and creatine kinase (CK)), number of red blood cells (RBC), number of white blood cells (WBC), haemoglobin (Hb) concentration and histological aspects of liver. Fishes were distributed among experimental tanks with mean weight of 7.72±1.19 g. They were pre-acclimated to laboratory conditions for 10 days prior to the trials. The experiment was done by using eight glass tanks (100 L) represented four treatments with two replicates; each tank was stocked with 10 fish. Fishes were treated with 0.0, 0.2, 0.4 and 0.6% of *N. indicum* leaf extract for 14 days. No significant differences occurred in blood serum enzymes ($P>0.05$) between fish control and fish treatments except fishes treated with 0.6% of *N. indicum* extract, showed significantly decreased values ($P<0.05$). Highest concentrations were achieved by fishes treated with 0.4% in number of blood cells and haemoglobin concentration significantly ($P<0.05$) increased compared with all treatments. Levels of number of white blood cells that significantly ($P<0.05$) increased in fishes with 0.6% treatment compared with other treatments. Cloudy tumor and minifying in liver tissue pockets and bleeding in center of liver vein for common carp after 14 days of treating with 0.6% of *N. indicum* extracts which noticed. So, based on the current findings, together with the low cost and the health effect of *N. indicum* leaf extract, it can be recommended to be used (at 0.4%) in aquaculture.

Keywords: *Nerium indicum*, *Cyprinus carpio*, Health status, Haematology, Histopathology

Introduction

Fish culturists are continuously seeking for inexpensive and safe ways used to manage their fish farms. Recently on application of medicinal plants related to different families are used in diets formation of fish cultured in earthen ponds because these plants are safe, effective, widely available and inexpensive. Using these plants enable culturist to produce fishes that are free from any chemicals of public health hazards. Some species of plants and herbs also have antimicrobial

and antioxidant activities (Aly et al., 2010; Sivagurunathan et al., 2011; Yılmaz et al., 2012). It is estimated and stated that products obtained from plants are used to improve and enhance different activities e.g. growth promotion, anti-stress, appetite stimulation and immune system in aquaculture (Aly et al., 2010; Manal et al., 2014).

Natural plant products have antimicrobial characteristics on some aquatic animals due to their bio active compounds such as polypeptide, terpenoids, quinones, lectins, alkaloids, polyphenols and phenolics and other synthetic compounds and effective alternatives of antibiotics (Yılmaz et al., 2012; Olusola et al., 2013). Oral intake of these compounds increases growth and stimulation of immune (Olusola et al., 2013). To improve immunity and increase growth rate, different medicinal plants are used in birds, fishes, and animals diet. *Nerium* (*N. oleander*) is one of these medicinal plants that contain diverse chemical active substances affecting on several biological properties of animals.

In Iraq, different medicinal plants are used in fishes to improve immunity and increase growth rate (Al-Atabi, 2012; Abdulrahman, 2014; Albadran et al., 2018; Taher et al., 2018; Al-Niaeem, 2019). This study was carried out to investigate the effect of different concentrations of *N. indicum* leaf extract on health status in fingerlings of the common carp, *Cyprinus carpio*.

Materials and Methods

Current experiment was conducted on young common carp with average weight of 7.72 ± 1.19 g, which were brought from fish farm of Marine Sciences Center, University of Basrah. These fishes were acclimated to laboratory conditions (10 days) and fed on commercial pellets diets prior to the feeding trials. A total of 84 specimens were randomly distributed among eight aquaria (ten fishes per tank and 10 fishes per tank for control, two replicates per treatment). Water temperature was measured by using simple thermometer, while dissolved oxygen, pH and salinity were measured by digital meter (YSI).

The current experiment was conducted to investigate the effect of nerium extract (after 14 days exposure) on tissues of liver, and on the concentrations of serum enzymes. These parameters were alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and creatine kinase (CK). The blood samples were taken from caudal peduncle vein and transferred to heparinized sterile tubes for the proposed serum biochemical tests. Serum was isolate by centrifugation at 1500 rpm for three minutes (Yang & Chen, 2003). The levels of serum enzymes were assayed according to the instructions provided with the corresponding enzymatic kits. Three concentrations (0.2, 0.4 and 0.6%) of nerium extract were used after preparing according to Al-Niaeem (2006) and Al-Niaeem et al. (2017).

The number of blood cells (RBC) and white blood cells (WBC) were calculated by using a haemocytometer. Haemoglobin was measured by mixing 20 μ L whole blood with 5 ml Drabkin's reagent and by reading the absorbance at 540 nm on spectrophotometer.

Liver tissues of the fish were fixed on 10% neutral buffered formalin for 24 hrs, then dehydrated in ascending alcohol and cleared in xylene before embedding in paraffin, sections (4-5 μ) were prepared. According to application of SPSS (version 22), the data were subjected to One-way analysis of variance (ANOVA) by using SPSS software (version 18) and the differences between means verified by using RLSD test.

Results and Discussion

Biochemical parameters are considered as useful measuring instruments in order to check the quality of fish health, physiological responses, nutrients absorption and animal surroundings that affect fishes (Albadran et al., 2018). Values of water quality parameters are shown in Figure (1). The general serum enzymes of the common carp after exposure by *N. indicum* leaf extract for 14 days are presented in Table (1), and number of RBC, WBC and Hb of the common carp after exposure to *N. indicum* leaf extract for 14 days are presented in Figures (1-3).

Table 1: Water quality parameters during the experiment.

Parameter	Treatment (Mean \pm S.D)			
	Control	0.2%	0.4%	0.6%
Temperature (C ⁰)	25.5 \pm 0.22	25.2 \pm 0.22	25.4 \pm 0.80	25.2 \pm 0.20
pH	8.65 \pm 0.02	8.65 \pm 0.02	8.65 \pm 0.02	8.65 \pm 0.02
Dissolved oxygen (mg/l)	8.8 \pm 0.4	8.8 \pm 0.4	8.8 \pm 0.2	8.8 \pm 0.4
Salinity (‰)	2.3 \pm 0.20	2.1 \pm 0.03	2.2 \pm 0.20	2.3 \pm 0.10

Statistical analyses of current experiment results revealed that there were significant differences ($P < 0.05$) in concentrations of all examined enzymes between control fish and fish treated with 0.6% of nerium extracts (After 14 days), while there weren't any significant differences ($P > 0.05$) between control fish and fish treated with other two concentration of nerium extracts, and the best results were with fish treated with 0.4% of nerium extracts after 14 days ($P < 0.05$) compare with the control fish and other fish treated (Table 2 and Figures 1-3). No significant differences ($P < 0.05$) were observed in WBC for common carp treated with 0.4% of nerium extracts after 14 days. ($P < 0.05$)

The blood parameters in fishes can be changed with factors such as nutrition, environmental conditions, diseases, stocking density and environmental pollutants (Adewolu & Aro, 2009). The haematological parameters in the assessment of fish health has been confirmed by Abasali & Mohamad (2010). Therefore, the blood parameters in the assessment of the health status of fishes is one of the most important indicators (Alizadeh & Shaabani, 2012). Immunostimulants such as herbs, probiotics and vitamins can elevate the non-specific defense mechanisms, the specific immune response and disease resistance of fishes (Tanekhy et al., 2016).

Other than that, in some cases the additives' properties or their high concentrations can inhibit the haematological parameters of fishes (Abasali & Mohamad, 2010).

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In the current experiment, the decrease in concentrations of plasma enzymes (ALP, AST, ALT, LDH and CK) for common carp treated with 0.6% nerium extract may be attributed to the hepatocellular damage as a result of toxic effect of high concentrations of nerium leaf water extract comparing with low concentrations (0.2 and 0.4 %) and control. Toxic effects of these high concentrations were due to the inhibition of synthesis for investigated enzymes (ManiRam et al., 2011). The biochemical results confirm the demonstration to the histopathological finding of liver (ManiRam et al., 2015). The significant changes in the activities of two enzymes (AST and ALT) in fish blood indicate the tissue impairment caused by stress and liver damage produced by toxicity of high concentrations of nerium extract. Alternatively, the cell membranes were disrupted, allowing the enzymes to leak out of the cells (ManiRam et al., 2011, 2015). Toxicity greatly impairs the liver regeneration and also decrease the synthesis of mitochondrial protein after causing a deficiency in cytochrome oxidase C and ATP synthetase enzymes necrosis degeneration in the liver. As shown by the liver tissue in the current experiment for common carp after 14 days of treating with 0.6% of nerium extracts, cloudy tumor and minifying in liver pockets and the bleeding in center of liver vein were noticed (Figures 4-6).

Table 2: Concentrations of blood serum enzymes for common carp during the experiment.

Parameters (U/l)	Treatments			
	Control	0.2%	0.4%	0.6%
ALP	50.52±1.31 ^a	52.04±2.01 ^a	53.13±1.15 ^a	40.2±2.34 ^b
ALT	5.13±0.20 ^a	4.52±0.31 ^a	5.10±0.20 ^a	2.53±2.23 ^b
AST	30.24±1.02 ^a	32.21±2.20 ^a	35.02±0.13 ^a	24.13±3.22 ^b
LDH	52.41±1.50 ^a	50.32±3.12 ^a	49.10±2.25 ^a	24.21±2.54 ^b
CK	32.51±1.21 ^a	30.02±1.40 ^a	29.50±2.22 ^a	20.67±3.60 ^b

Values in the same raw with different letters are significantly different (P<0.05).

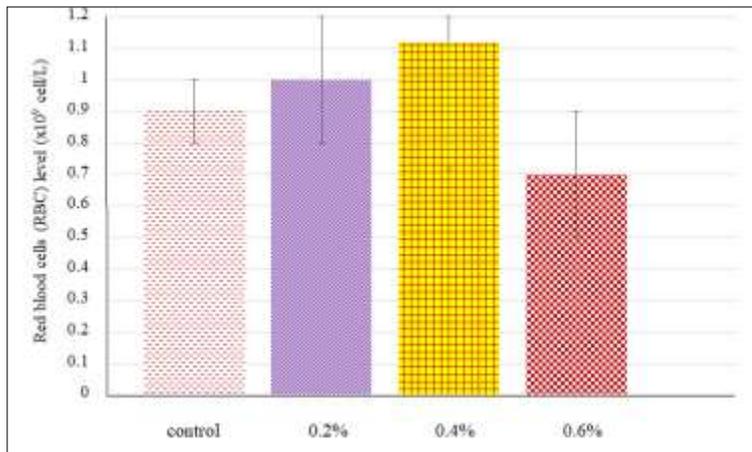


Figure 1: Red blood cells for common carp during the experiment.

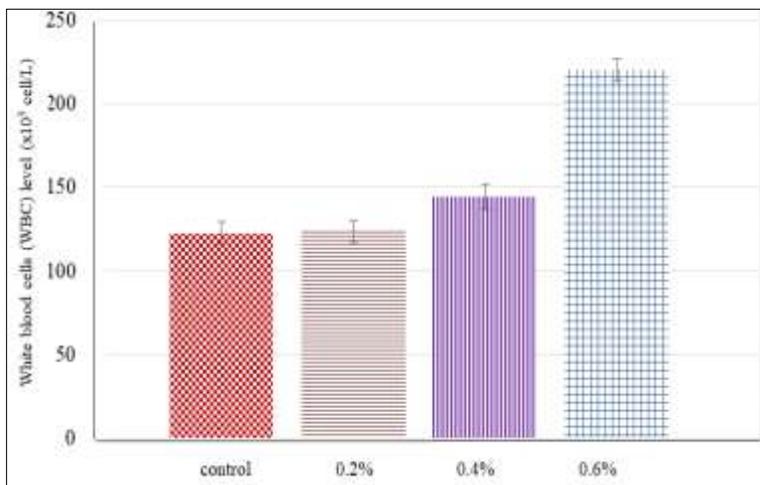


Figure 2: White blood cells for common carp during the experiment.

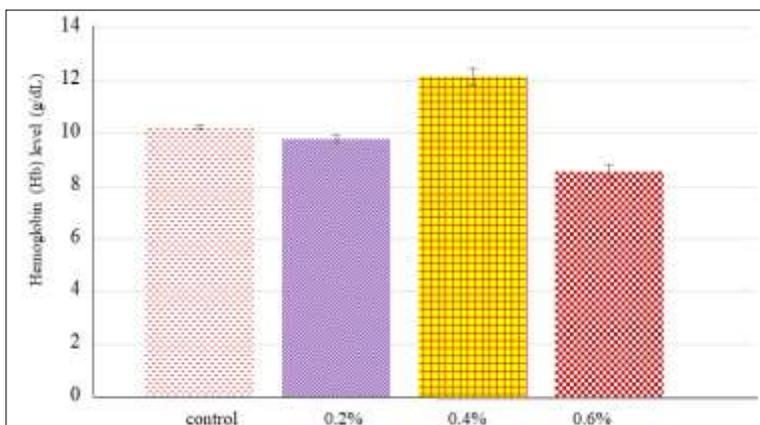


Figure 3: Haemoglobin for common carp during the experiment.

It is evident from this study that aqueous extract of nerium leaf powder could enhance fish hematological parameters after 14 days exposure (0.4%). It suggests that health status would improve the non-specific immune infections in aquaculture. This study is a preliminary investigation conducted to provide an insight for the use of *N. indicum* leaf extract improving the health of the fishes. Future research should focus on the purification of the active compounds and their evaluation in order to improve quality and their usage in culture system.

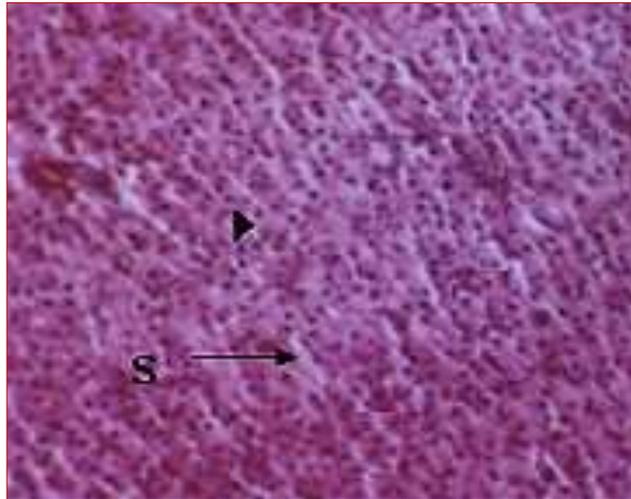


Figure 4: Section of liver tissues for common carp before treatment (200X, H&E). S: Hepatic cords or platescolumns (hepatocytes extending from the portal region to central vein); ◄: liver sinusoid (type of capillary known as a sinusoidal capillary).

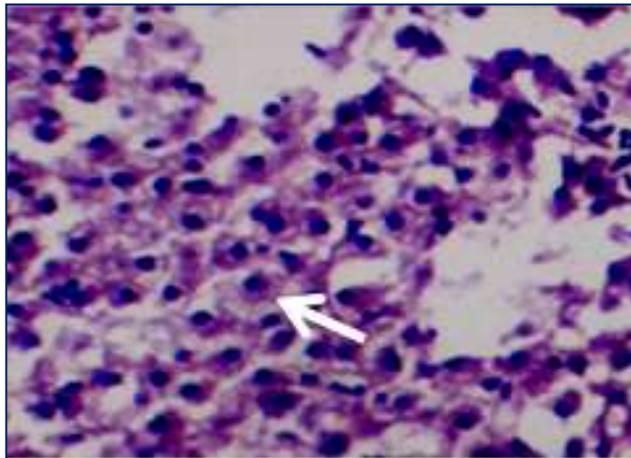


Figure 5: Section of liver tissues of common carp after 14 days of treatment with 0.6% of nerium extracts. Notice cloudy tumor and minifying in liver pockets (400X, H&E).

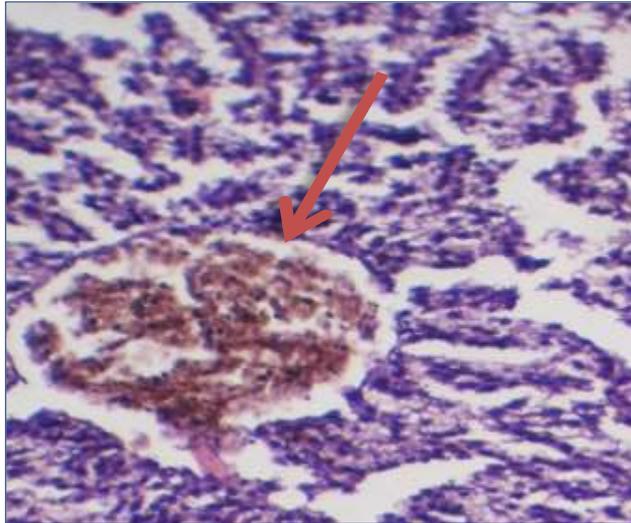


Figure 6: Section of liver tissues for common carp after 14 days of treating with 0.6% of nerium extracts. Notice the bleeding in center of liver vein (400X, H&E).

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