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HEMATOLOGICAL EFFECTS OF GRAPHENE NANOPARTICLES EXPOSED TO COMMON CARP *CYPRINUS CARPIO* L.

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ABSTRACT

The current was research conducted to study the hematological effects of graphene nanoparticles exposed to common carp *Cyprinus carpio* L. A total of 48 specimens of *Cyprinus carpio* with average of 68.44 g in weight were examined to detect the expose graphene nanoparticles on experimental fish. Experimental fish were acclimated for two weeks prior to initiation of the experiment to adapt to laboratory conditions. Fish were exposed to graphene nanoparticles as followed T1, 10; T2, 20 mg / l and control group (without graphene) for 10 days. The study included measuring the hematological parameters of the experimental fish including RBC, WBC, PCV and Hb. Results of statistical analysis showed significant decrement at $P \leq 0.05$ for both RBC (0.770 Cells / μlx10^6) in T1 and 0.850 Cells / μlx10^6 in T2 compared with negative control (1.410 Cells / μlx10^6). No significant decrease $P > 0.05$ of each PCV in T1 and T2 (31 and 27%) respectively compared with control negative (35%) and hemoglobin (10.30 and 9 mg/dl) in T1 and T2 respectively compared with negative control (11.65 mg /dl), while the addition of graphene nanoparticles did not effect on the number of WBC in T1 and T2 (15.1 and 19.1 Cells / μlx10^{3})) respectively compared with negative control (15.6 Cells / μl x10^3). The present study was suggested to investigate of the graphene nanoparticles effects to clarify a clear representation about on fish and its danger of its accumulation in aquatic environment.

Keywords: *Cyprinus carpio*, Nanoparticles, Graphene, Hematology

1. INTRODUCTION

THe term "Nano" used to describe the size measurement; a nanometer (nm) is a millionth of a millimeter. Any material size is between 0.1 - 100 nm describe as nanoparticle (NP) (Demirkalp, 1992)**.** The development of nanotechnology has encouraged the requirement to study the environmental effects of nanomaterials (San-chez et al., 2011; Bianco, 2013). Graphene-family nanomaterials (GFNs) including reduced graphene oxide (rGO) pristine graphene, and graphene oxide (GO) present wide application prospective, leading to the probability of their accumulate in aquatic environments, the hazard of graphene family nanomaterials to aquatic environments has acquired rising attention, with these materials nowadays considered to be possible environmental contaminants of emerging concern (Zhao et al., 2014). Graphene could take place from consumption of profitable products containing the material, from its degradation during consumption and from the remaining disposal of such products (Muzi et al., 2015), and can present into the aquatic environment. The material would begin interacting with abiotic compounds such as natural organic material and other molecules naturally present in waters (inorganic and organic macro-molecules, colloidal particles, etc.), but also with alive organisms (Muzi et al., 2015). However, the existing information of the potential harmfulness of graphene family nanomaterials towards aquatic organisms is still poor (Muzi et al., 2015). Hematological parameters are one of the important examinations of toxic material in fish is essentially used to evaluation of toxicity and physiological status of the animals' health (Hedayati and Ghaffari, 2013). Several studies showed hematological disturbance in fish that exposed to different nano materials (Jahanbakhshi, 2015; Suganthi et al., 2015; Kanwal et al., 2016). The mechanism of graphene nanoparticles toxicity is not clear to researchers, few studies showed the impacts of these materials on fish. Therefore, the purpose of current study was to explain the poisonous effects of graphene on *Cyprinus carpio*.

2. MATERIALS AND METHODS

2.1 Experimental fish: The current research was conducted at the Fish Laboratory of Veterinary Medi-cine College, University of Baghdad. A total of 48 specimens of *Cyprinus carpio* with average of 68.44 g in weight were examined to detect the expose graphene nanoparticles on experimental fish. Experimental fish were acclimated for two weeks prior to initiation of the experiment to adapted to laboratory conditions, fed with commercial diets.

2.2 Experimental designs

2.2.1 Preparation of graphene suspension: Graphene was purchased from Areej Alfurat Bureau added to deionized water and submitted to bath sonication for 10 minutes, this suspension regarded as stock solution, prepared 10 and 20 mg/l of graphene.

2.2.2 Limit test for graphene: Using the techniques described in (OECD, 1992), a limit test should be accomplished at 100 mg graphene/l for seven fish under optimum environmental conditions for 96 hr. to determine the LC50.

2.2.3 Fish exposure: Fish exposure to graphene was done in glass containers filled with 28 L water with constant oxygen provides, without feeding. The graphene concentrations were designated based on former acute toxicity tests in which no death was detected for concentrations up to 100 mg/l. Sublethal concentrations of 0 (control), 10 T1 and 20 mg/l T2 were evaluated in following assays (duplicate in each exposure, 16 fish per treatment). Fish were exposed to graphene for 10 day.

2.3 Hematological examination: Blood was collected in the end of the experiment from the caudal vessels using a sterile disposable plastic syringe 3 ml, transferred immediately to a test tube containing (EDTA) (Ethylene di amine tetra-acetic acid) for studying hematological parameters, including the Red Blood Cell (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV) and Haemoglobin concentration (Hb), these parameters were determined as described by (Blaxhall and Daisley, 1973).

2.4 Statistical analysis: One-way analysis of variance (ANOVA) was used to determine the significant differences between the variables. The differences of means were analyses at probability value 0.05. Probability levels equal or less than $P < 0.05$ were considered significantly different.

3. RESULTS

3.1 Hematological parameters: The present study showed that the mean of RBCs count ranged between 0.770 to 1.410 Cells/µl 10^6 in T1 and CN respectively (Tab. 1). Results of statistical analysis showed significantly increase ($P \leq 0.05$) in CN compared with T1 and T2, and no significant difference ($P > 0.05$) between T1 and T2. Results of WBCs values ranged between 15.1 in T2 to 19.1Cells/µl 10^3 in T1 (Tab. 1). The results of statistical analysis showed no significant differences ($P > 0.05$) among studied treatments. Table 1 showed that the highest values of PCV was recorded in negative control reached 35%, while the lowest values was recorded in T2 approached 27 %. Mean values of Hb concentration reached 11.65 in control negative and when use 10 mg/l of graphene the values drop to 10.30gm/dl, when increase concentration of graphene to 20mg/l, the value of Hb decrease to 9.00 gm/dl. Result of statistical analysis showed no significant difference ($P>0.05$) among treatments of PCV as well as Hb.

Table 1: Hematological parameters levels (Mean ± SE) of *Cyprinus carpio* exposed to graphene

parameter / Treatment	RBCs count Cells/µl 10^6	WBC count Cells/µl 10^3	PCV %	Hb concentration gm/dl
Control Negative CN	**1.410 ± 3.25 $^{E+04}$ A**	**15.6 ± 2.810^{E+03} A**	**35 ± 5 A**	**11.65 ± 1.65 A**
T1	**0.770 ± 5.00 $^{E+04}$ B**	**19.1 ± 0.937^{E+03} A**	**31 ± 3 A**	**10.30 ± 1.00 A**
T2	**0.850 ± 0.10 $^{E+04}$ B**	**15.1± 0.187^{E+03} A**	**27 ± 3 A**	**9.00 ± 1.00 A**
LSD values	**0.274^{E+06}**	**6.146^{E+03}**	**16.59**	**5.10**

4. DISCUSSION

Turbulences in blood biomarkers, such as RBC, WBC, PCV and Hb reveal the physiological injury of the fish under stress conditions (Shaw et al., 2012). The blood parameters revealed a significant decrement in the RBCs count in T1 and T2 compared to control group, beside non- significant change in WBC, PCV and Hb concentration, after 10th day of the experiment in all studied treatments. There are no data can be obtained on the effects of graphene nanoparticles on hematological parameters in fish. Several authors proposed that in toxicological investigates the reduction in RBC count; PCV and Hb levels could be linked to the conditions of quarantine or stress induced by the lack of food (John, 2007). Decrease in RBC count, Hb and Hct levels in fish exposed to toxicant materials may be due to erythropoiesis disorder and the formation of RBCs (Shaluei et al, 2013). In fact, the immune cells of the body may be triggered by the foreign molecules and cause a injurious inflammatory response (Orecchioni et al, 2014). Results of hematological parameters of rain-bow trout exposed to CuO NPs (96 hr.) at 1, 5, 20 and 100 mg/l showed significant decrease ($P \leq 0.05$) of RBC, WBC and PCV compared to control group and showed no significant effects $P > 0.05$ on Hb (Khabbazi et al., 2015). *Oreochromis mossambicus* exposed to ZnO nanoparticles (96 hr.) at concentrations 30, 50 and 70 mg/l exhibited a significant decline in hematological parameters (RBC, WBC, PCV and Hb) compared to control group ($P \leq 0.05$) (Suganthi et al., 2015). Rainbow trout exposed to Ag nanoparticles (8 days) at 0.1 (T1), 0.2 (T2) and 0.4 (T3) mg/l showed signifycant increases ($P \leq 0.05$) in RBC and WBC values compared to the control group, PCV in the T3 group showed lower values than control group, although the Hb values were higher in the T2 and T3 than those in control group (Imani *et al*., 2014). Shaluei et al, (2013) noticed that effect of silver nanoparticle (3, 7 and 14 days) on silver carp at sub-lethal concentration 0.04 and 0.02 mg/l was led to significant reduced ($P \leq 0.05$) in RBC, PCV and Hb values compared to control group, whereas exhibited significant increases ($P \leq 0.05$) in WBC compared to control group. The toxicity of CuO nanoparticles on *Rutilus rutilus* at 50% and 70 % of LC50 of concentration exhibited significantly ($P \leq 0.05$) lower of RBC count, PCV and Hb values and a significant increase in the WBC numbers ($P \leq 0.05$) (Jahanbakhshi, 2015). By investigating effects of chromium nanoparticles (14 days) on *Labeo rohita* at concentration 25 mg/l showed that chromium nanoparticle caused reduced of WBC , but no effect on RBC count (Kanwal et al., 2016). Rajkumar (2016) noticed that exposure of *Labeo rohita* to silver nanoparticles (7 days) lead to reduced RBC, WBC and Hb at 25 mg/ kg compared to control samples. The results of chronic exposure of tilapia to iron oxide nanoparticle (60 days) at concentrations 0.1, 0.5, and 1.0 mg/l showed significant $P > 0.05$ differences in RBC, WBC, PCV and Hb values between all treatments after exposure and recovery periods (Ates et al., 2016). *Oreochromis niloticus* exposed to zinc oxide nanoparticles (7 days and 15 days) at concentration 1 and 2 mg/l and after treated with vitamin E and C showed significant decrease ($P \leq 0.05$) in the red blood 15 days) at concentration 1 and 2 mg/l and after treated with vitamin E and C showed significant decrease ($P \leq 0.05$) in the red blood cells count, PCV and Hb concentration (Alkaladi et al., 2015). It has been demonstrated that graphene-based nanomaterials were compatible with blood and did not cause hemolysis, platelet activation and changes in coagulation or abnormalities in hematological parameters (Pinto et al, 2013).

5. Conclusion

The current study compacts with the toxicity of graphene nanoparticles in common carp. Graphene exposure led to significant decrease in RBC count and no significant effect on WBC, PCV and Hb. These results showed the harmful impacts of graphene nanoparticles on fish and regarded as alert for future toxicity of these substances for aquatic organism.

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