Pakistan J. Zool., pp 1-4, 2022. DOI: https://dx.doi.org/10.17582/journal.pjz/20210414060434

Short Communication

Toxicity of Iron-Oxide Nano-Particles on the Hematology of *Cyprinus carpio*

Khalid Javed Iqbal[1], Irfan Baboo[2]*, Arshad Javid[3], Noor Khan[4], Hamid Majeed[5], Azra Anwer[1], Zahid Farooq[2], Muhammad Altaf[6] and Moazam Jalees[7]

[1]*Department of Zoology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan*
[2]*Department of Zoology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*
[3]*Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore*
[4]*Department of Fisheries and Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan*
[5]*Department of Food Science and Technology, Cholistan University of Veterinary and Animal Sciences Bahawalpur, Pakistan*
[6]*Department of Forestry, Range and Wildlife Management, The Islamia University of Bahawalpur, Bahawalpur, Pakistan*
[7]*Department of Applied Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan*

Article Information
Received 14 April 2021
Revised 26 September 2021
Accepted 07 October 2021
Available online 21 January 2022 (early access)

Authors' Contribution
KJI collected and analyzed the data and prepared the manuscript. IB helped in write up and corresponded the manuscript. AJ supervised the work, helped in the analysis and refined the manuscript. NK planned the experiment. HM provided facilities for the conduction of experiment and basic protocols. AA helped in preparation of nano-particles. ZF helped in biological synthesis of IONPs. MA and MJ helped with hematological parameters.

Key words
Iron oxide nano-particles, C. carpio, Aquatic environment, Aquatic toxicity, Hematological parameters

ABSTRACT

Owing to growing exploration in nanotechnology, nano-particles application in various fields of industries and medicine is growing. However, limited study is available on their toxicological impacts which can adversely affect the organism's health and their environment. Current research was designed to study the possible toxicity of iron oxide nano-particles on common carp, *C. carpio* after exposure period of 96 hours by the analysis of their haematological parameters. Fish were segregated into three groups (10fish/treatment). The iron oxide nano-particles at 0.25g/l doze were administered orally to treatment T_1 and intravenously from caudal vein of fish to treatment T_2. After 96h of exposure period, the blood was collected from fishes by the caudal vein puncture method into tubes containing anticoagulants EDTA and transferred immediately to the laboratory for the assessment of their blood profile. There was recorded no mortality after 96h of exposure period. IONPs significantly affected the counts of WBCs, Hb, MCH and MCHC of experimental and control groups. The analysis of blood profile demonstrated that haematological parameters; the number of WBCs and Hb were increased in T_1 and decreased in T_2 in relation to T_0. The counts of RBCs, HCT, MCV and PLT were found to be increased in both experimental groups as compared to control one. It is inferred from current results that IONPs have toxic effect on the haematology of *C. carpio*. Moreover, oral route of administering IONPs was found to be more toxic than intravenous one. Present study can be beneficial for the safety of aquatic environment and management of aquatic toxicity.

Hematological parameters are commonly used in aquatic toxicological studies as potent bio-indicators indicating environmental stress on fish due to their sensitivity to some toxicants (Singh and Srivastava, 2010). Fish are extensively used to assess health of aquatic environments by metal level determination in food chain (Farkas *et al*., 2002). The bioaccumulation of heavy metals points out toxicity in biological tissues (Yi *et al*., 2011). Nano-particles of metal oxide with their increasing exposure may impose toxicity in biological tissues, greatly enforcing the evaluation of toxic level and toxicological aspects of these nano-materials (Hafiz *et al*., 2018). Nanoparticles having excellent chemical, optical and magnetic properties and diverse forms and diameter in nanoscale range of 1-100 nm are extensively used in various fields of medicine, pharmacy, science and industry (Channappa *et al*., 2018).

Modern chemical methods for production of nanoparticles use toxic chemicals which yield hazardous products; precluding them from any biomedical

* Corresponding author: irfan@cuvas.edu.pk
0030-9923/2022/0001-0001 $ 9.00/0

application. Consequently, biological method using i.e. bacteria, fungi, yeast, plants etc., have become a necessary requirement to apply as a non-toxic, bio-compatible, cost-effective, eco-friendly, safe and ecologically sound method for the synthesis of nanoparticles (Zhu *et al*., 2012). The various parts of a plant and their extracts offer an excellent substitute for nanoparticles synthesis in their ease of management and accessibility because they possess many metabolites (Kumar *et al*., 2017).

A plant *Withania coagulans* have anti-inflammatory, antimicrobial, antitumor, anti-hyperglycemic, hepatoprotective, free radical scavenging, cardiovascular, central nervous system depressant and immuno-suppressive activities (Gupta, 2012), has been frequently used for preparation of nanoparticles of diverse properties i.e. enhanced catalytic, mechanical, magnetic, optical (Ferrari, 2005). The iron oxide nano-particles (IONPs) in different fields of biomedical science and industries have attracted much attention because of their excellent magnetic, catalytic and electric properties (Zhu *et al*., 2012; Chen *et al*., 2013). Iron concentration above an optimal level is unsafe for the life of an organism (Wood *et al*., 2012), hence limited explorations are available so far on the effects of IONPs (Li *et al*., 2009).

The potential biomarkers to evaluate toxicological effects of nanoparticles in living organisms are the alterations in biochemical, histopathological, oxidative enzyme and hematological parameters (Kanwal *et al*., 2019). Fish can mimic well biological reactions in the same way as they happen in humans because their immune system has evolutionary resemblances with humans (Kanwal *et al*., 2019), it is considered as an appropriate biological model and ideal sentinel to study toxicological effects of nanoparticles. *Cyprinus carpio,* a freshwater teleost, is one of the chief edible fish and has been described very sensitive to environmental pollutants (Channappa *et al*., 2018). Current research was conducted to study the potential toxicity of IONPs in *C. carpio* and its impact on their haematological parameters. Present study will be beneficial for environmental safety and management of aquatic toxicity.

Materials and methods

For preparation of nanoparticles, the seeds of *Withania coagulans* were washed, dried, pulverized into coarse powder, 10g mixed with 100ml of distilled water and boiled on hot plate stirrer for 45 min. This extract was filtered and then stored at 4°C until further processing. The seed extract (20ml) was mixed with 100ml of $FeCl_2.4H_2O$ and $FeCl_3.6H_2O$ (2:1 molar ratios) solution heated at 90°C and stirred for 2 min. After 15 min, 20ml of aqueous solution of sodium hydroxide with ratios of 3 ml per min was added in the mixture and change in color was observed.

Experimental fish (*C. carpio)* of average weight of 115±7g and length of 19±4cm were purchased from Punjab Government Fish Hatchery Bahawalpur, were stocked in well aerated water glass tanks of 92×46×46 cm and dissolved oxygen at 7.0 ± 0.5 mg/L. Fish were fed twice a day with commercial fish food at the rate of 3% of their body weight and acclimatized for 15 days. Fish were divided into 3 groups (T_0, T_1, T_2) each of 10 fish. Fishes were exposed to sonicated iron oxide nanoparticles for 30 min at room temperature orally (T_1) at 0.25g/l doze and intravenously from the caudal vein (T_2) for 96 h. The blood was collected from caudal vein of fish and transferred immediately into tubes containing anticoagulants ethylene diaminetetra acetic acid (EDTA) to prevent blood coagulation. Fish blood hematological analysis was carried out by using digital analyzer from a local laboratory.

The collected data achieved from experimental and control groups were analyzed using SPSS. The difference of $p<0.05$ was exhibited as statistically significant between treatment groups and data was presented as mean ± standard error of mean (SEM).

Results and discussion

Table I shows the effect of nanoparticles administrated orally and intravenously for 96 h on hematological parameters of fish. The maximum immunity response with increased number of WBCs was revealed in T_1 (282.32±9.32), followed by T_0 (275.55±13.64) and T_2 (258.62±2.92) groups. The mean number of RBCs (0.66±0.36) was found to be highest in T_1 and lowest in T_0 (0.38±0.05). T_1 demonstrated peak Hb level of 9.64±0.99 as compared to 7.95±0.41 in T_2. There was observed extreme Hct and MCV value of 11.42±6.94 and 168.98±7.74, respectively in T_1 of treatment groups followed by T_2 and T_0. The level of MCH and MCHC was recorded highest in control group and lowest in T_1. Unlike other heamotological parameters, there was found uppermost increase in platelets of 17.25±13.91 in T_2, 15.20±8.29 in T_1 and 13.00±10.03 in T_0.

In relation to control group, the number of WBCs and Hb were increased in T_1 and decreased in T_2. The counts of RBCs, Hct, MCV and PLT were found to be increased in both experimental groups as compared to control one. However, reversed was recorded in case of MCH and MCHC. The oral route of administering IONPs was found to be more toxic than intravenous one.

Current study exhibited a substantial increase in the value of total RBCs, Hct, MCV and PLT in both experimental groups; while WBCs and Hb counts were high in T_1 and low in T_2 in relation to control one. In previous toxicological studies, significant rise of RBCs, Hb and Hct was explored in *C. carpio* on exposure to copper NPs by Sevcikova *et al.* (2016) and in *Labeo rohita* treated with IONPs (Remya *et al*., 2014), While authors found reduction in value of total WBCs, MCHC, MCV and MCH. Channappa *et al.* (2018) recorded a considerable rise of WBCs and fall in number of RBCs and Hb in *C. carpio* on exposure to calcium zincate nanoparticles at the dose of 3.53 mg/L and 7.05mg/L in 14 and 21 days. Similarly, WBCs increase and RBCs, Hb, Hct and PLT decline in fish *Labeo rohita* was studied by Keerthika *et al.* (2017). The value of RBCs, WBCs, Hb and Hct was increased and MCH, MCV, MCHC counts were declined in *Oreochromis mossambicus* when exposed to IONPs (Karthikeyeni *et al*., 2013).

Table I. The effect of biologically synthesized IONPs administered orally at 0.25g/l (T_1) and intravenously in caudal vein (T_2) for 96 h on hematological parameters of *C. carpio*.

Parameters	T_0	T_1	T_2
WBCs (10^3/ul)	275.55±13.64^{a}	282.32±9.32^{a}	258.62±2.92^{b}
RBCs (10^3/ul)	0.38±0.05^{a}	0.66±0.36^{a}	0.43±0.05^{a}
Hb (g/dl)	9.43±1.18^{a}	9.64±0.99^{a}	7.95±0.4^{b}
HCT (%)	5.78±1.20^{a}	11.42±6.94^{a}	7.05±1.11^{a}
MCV (fl)	157.17±10.35^{a}	168.98±7.74^{a}	163.50±6.92^{a}
MCH (pg)	249.97±50.60^{a}	167.16±52.89^{b}	186.53±18.90^{ab}
MCHC (g/dl)	171.15±55.37^{a}	99.94±33.21^{b}	114.55±15.99^{ab}
PLT (10^3/ul)	13.00±10.03^{a}	15.20±8.29^{a}	17.25±13.91^{a}

WBSs, white blood cells; RBCs, red blood cells; Hb, hemoglobin; HCT, hematocrit, MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelets.

The changes in RBCs indicate variation in all red cell indices; Hb, MCV, Hct, MCH, MCHC. The variation in RBCs counts in metal exposed gills is an adaptive response of a fish towards the impairment of gaseous exchange prompting an increased energetic demand (Witeska *et al*., 2010). In response to chronic metal exposure in the blood of fish facing stress condition, an increase in number of total RBCs, Hb concentrations and Hct values is frequently observed due to enhanced erythropoiesis (Witeska and Kondera, 2013), as has been studied in present research. The increased concentration of Hb reflects the increased oxygen supply to the tissues leading to enhanced physical activities (Nussey *et al*., 1995). The decline in RBCs counts is an indication of severe anemia and decreased Hb and Hct level demonstrates the shrinkage of RBCs due to microcytic anemia or hypoxia or some toxicant stress on the erythropoietic tissues (Zorriehzahra *et al*., 2010).

WBCs counts in all of the vertebrates including fish may rise or fall in response to many stressors i.e. chemical pollutants and infections (Olurin *et al*., 2012). There is recorded rise of WBCs in T_1 and fall of these in T_2 in comparison to T_0 (Table I). The high level of WBCs reflects immune system response against nanoparticles as a foreign-agent and their decline might be a response against various infectious diseases or can be attributed to unhealthy functioning of blood forming tissues (kidney and spleen) (Al-Bairuty et al., 2013). WBCs reduction (leucopenia) can also be a response of toxic metals accumulation in different biological tissues and its adverse effect on cell manufacturing from spleen due to the rise in the level of corticosteroid hormones which play a great role for preventing and healing inflammation (Kori-Siakpere *et al*., 2008).

The values of erythrocyte indices i.e. MCH, MCV and MCHC exhibit a wide range of physiological changes as a result of exposure of fish to different inorganic form of supplemented diet. There is recorded a significant decrease in the values of MCV, MCH and MCHC in experimental fishes as compared to control one (Table I). Kori-Siakpere *et al.* (2008) demonstrated that change in the level of RBCs, Hb and HCT affect other hematological indices; MCV, MCH and MCHC. The decline in the level of Hb and MCH cause hypocromia (Kandeepan, 2014) as revealed in current study. The increase level of MCH and MCHC results from spherocytosis (Sobecka, 2001).

Present research confirmed that IONPs have toxic impacts on the hematology of fish, *C. carpio* which can adversely disturb the survival of fish. Therefore, there is a need of proper and safe handling of IONPs in research laboratories and their proper disposal should be carried out while promoting their application in various fields.

References

Al-Bairuty, G.A., Shaw, B.J., Handy, R.D. and Henry, T.B., 2013. *Aquat. Toxicol.,* **126**: 104-115.

Channappa, B., Kambalagere, Y. and Kittappa, M.K., 2018. *Int. J. Fish. aquat. Res.,* **3**: 42-49.

Chen, P.J., Wu, W.L. and Wu, K.C.W., 2013. *Water Res.,* **47**: 3899-909. https://doi.org/10.1016/j.watres.2012.12.043

Farkas, A., Salanki, J. and Specziar, A., 2002. *Arch. environ. Contam. Toxicol.,* **43**: 236-243. https://doi.org/10.1007/s00244-002-1123-5

Ferrari, M., 2005. *Natl. Rev. Cancer*, **5**: 161-171. https://doi.org/10.1038/nrc1566

Gupta, P.C., 2012. *Int. J. Pharm. Sci. Rev. Res.,* **12**: 68-71.

Hafiz, S.M., Kulkarni, S.S. and Thakur, M.K., 2018. *Biosci. Biotech. Res. Asia,* **15**: 419-425. https://doi.org/10.13005/bbra/2645

Kandeepan, C., 2014. *Int. J. Curr. Microb. appl. Sci.,* **3**: 1015-1022.

Kanwal, Z., Raza, M.A., Manzoor, F., Riaz, S., Jabeen, G., Fatima, S. and Naseem, S., 2019. *Nanomaterials*, **9**: 309. https://doi.org/10.3390/nano9020309

Karthikeyeni, S., Vijayakumar, T.S., Vasanth, S., Ganesh, A., Manimegalai. M. and Subramanian, P., 2013. *J. Acad. indust. Res.,* **1**: 645-649. https://doi.org/10.1155/2013/785064

Keerthika, V., Ramesh, R. and Rajan, M.R., 2017. *Int. J. Fish. aquat. Stud.,* **5**: 01-06.

Kori-Siakpere, O., Ubogu, E.O. and Oghoghene, E., 2008. *Afr. J. Biotech.,* **7**: 2068-2073. https://doi.org/10.5897/AJB07.706

Kumar, V., Sharma, N. and Maitra, S.S., 2017. *Int. Nano Lett.*, **7**: 243-256. https://doi.org/10.1007/s40089-017-0221-3

Li, H., Zhou, Q., Wu, Y., Fu, J., Wang, T. and Jiang, G., 2009. *Ecotoxicol. environ. Safe.,* **72**: 684-692. https://doi.org/10.1016/j.ecoenv.2008.09.027

Nussey, G., Van-Vuren, J.H.J. and Du-Preez, H.H., 1995. *Comp. Biochem. Physiol.*, **111**: 369-380. https://doi.org/10.1016/0742-8413(95)00063-1

Olurin, K.B., Olojo, E.A.A. and Tijani, O.B., 2012. *Asian J. Pharm. Hlth. Sci.*, **2**: 266-272.

Remya, A.S., Ramesh, M., Saravanan, M. and Poopal, R.K., Bharathi, S. and Nataraj, D., 2014. *J. King Saud Univ. Sci.,* **27**:1018-3647.

Sevcikova, M., Modra, H., Blahova, J., Dobsikova, R., Plhalova, L., Zitka, O., Hynek, D., Kizek, R., Skoric, M. and Svobodova, Z., 2016. *Vet. Med.,* **61**: 35–50. https://doi.org/10.17221/8681-VETMED

Singh, N.N. and Srivastava, A.K., 2010. *Ecotoxicology*, **19**: 838-854. https://doi.org/10.1007/s10646-010-0465-4

Sobecka, E., 2001. *Acta Ichthyol. Piscat.,* **31**: 127-143. https://doi.org/10.3750/AIP2001.31.2.10

Witeska, M. and Kondera, E., 2013. *Fish Physiol. Biochem.,* **39**: 755-764. https://doi.org/10.1007/s10695-012-9738-6

Witeska, M., Kondera, E., Lipionoga, J. and Jastrzebska, A., 2010. *Fresen. Environ. Bull.,* **19**: 115-122.

Wood, C.M., Farrell, A.P., Brauner, C.J., 2012. *Fish physiology: Homeostasis and toxicology of essential metals.* Canada Academic Press., pp.520.

Yi, Y., Yang, Z. and Zhang, S., 2011. *Environ. Pollut.*, **159**: 2575-2585. https://doi.org/10.1016/j.envpol.2011.06.011

Zhu, X., Tian, S. and Cai, Z., 2012. *Publ. Lib. Sci. One*, **7**: e46286. https://doi.org/10.1371/journal.pone.0046286

Zorriehzahra, M.J., Hassan, M.D., Gholizadeh, M. and Saidi, A.A., 2010. *Iran. J. Fish. Sci.,* **9**: 185-198.