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Effect of Manganese on haematological parameters of fish, *Garra gotyla gotyla*

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ABSTRACT
In the present study, an attempt has been made to study the effect of heavy metal Manganese ($MnSO_4$) on the haematological parameters of fish *Garra gotyla gotyla* for 9 weeks experimental duration. 96 hours LC_{50} value of Manganese for *G. gotyla gotyla* was found to be 3.2 mg/l and three concentrations viz. 20%, 40% and 60% of LC_{50} were employed during experimental period. The parameters evaluated include total erythrocyte count (TEC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total leucocyte count (TLC) and differential leucocyte count (DLC). Compared to control groups, TEC, Hb and Hct were found to exhibit significant decline whereas MCV and MCH exhibited overall increase in all concentration groups while MCHC contrarily exhibited fluctuating pattern. TLC depicted significant increase in all fishes following exposure to Manganese. DLC was observed to register an increase in number of lymphocytes, monocytes and eosinophils but a decrease in neutrophils, basophils and thrombocyte population.

Keywords: Manganese, *Garra gotyla gotyla*, Haematological parameters.

1. Introduction
Haematological parameters are important in diagnosing the structural and functional status of fish exposed to toxicants. They are considered to be reliable approach in the assessment of toxicity of different chemicals [1] either singly or in combination on fish health [2]. Changes in haematological parameters depend on the magnitude of impact of contaminants (concentration), the duration of exposure besides fish species, their age and health status [1, 3, 4 , 5]. Alteration in white blood cells may be regarded as a prognostic tool as well as early warning signal of the disturbance in homeostatic defense abilities of fish [6]. Haematological indices are therefore, ready tools used by fish biologists and researchers in many parts of the world in diagnosing stress. This is so because fish are closely associated with aquatic environment and the blood becomes an indicator of slightest change within the body of fish, well before there is any visible sign of disease [7].
Present study, therefore has been undertaken to evaluate variations in haematological parameters of fish *G. gotyla gotyla* exposed to different sublethal concentrations (viz. 20%, 40% and 60%) of heavy metal Manganese (Mn).

2. Materials and Methods
G. gotyla gotyla were collected with the help of cast net from the Jhajjar stream of Jhajjar Kotli region of Jammu, J & K, India. After acclimatization, the 96 hours LC_{50} value of $MnSO_4$ was determined as 3.2 mg/l. Fishes were exposed to three sublethal concentrations of $MnSO_4$ i.e. 0.64 mg/l, 1.28 mg/l and 1.96 mg/l (20%, 40% and 60% respectively of LC_{50} value). The haematological parameters viz. TEC, Hb, Hct, TLC and DLC of control and treated fishes were studied by collecting blood samples with the help of disposable insulin syringes by making an incision through the heart of fish. TEC and TLC were counted with the help of improved Neubauer haemocytometer [8]. DLC was counted by methodology adopted by Anderson [9]. Hb was estimated by using Sahli's haemoglobinometer [10]. Hct was determined by centrifugation method [11]. The RBC calculated indices viz. MCV, MCH and MCHC were calculated using standard formulae. The results obtained were analyzed statistically by one way analysis of variance (ANOVA) by SPSS software for determining the significance of change from control.

3. Results

The mean/ average values of haematological parameters of control and experimental fish are depicted in Tables (1 and 2). Compared to control groups, RBC dependent parameters (TEC, Hb and Hct) were found to exhibit significant decline (P<0.01) whereas MCV and MCH exhibited overall increase in all concentration groups while MCHC contrarily exhibited fluctuating pattern (Table 1). Contrary to RBC dependent parameters TLC depicted significant Increase in all fishes following an exposure to manganese. Differential leucocyte count (DLC) depicts an increase in number of lymphocytes, monocytes and eosinophils (Table 2) but a decrease in neutrophils and basophils (Table 2). Thrombocytes observed a decrease in their number in all treated fish (Table 2 and Figure 6).

4. Discussion

Comparison of data of control with that of treated groups very clearly indicates that there is a marked decline in TEC, Hb and Hct at the end of experimental period in Manganese treated fish (Table 1).

Similar to present findings [12, 13, 14, 15, 3, 4, 5] have also reported declining trend in TEC, Hb and Hct of fishes following subjection to different metals.

Table 1: Haematological parameters of *G. gotyla gotyla* subjected to Manganese treatments.

Parameters	Manganese Treatment (Dose)			
	Untreated (Control)	20%	40%	60%
TEC($\times 10^6$/cmm)	2.72±0.24	1.46±0.12	1.38±0.36	1.27±0.74
Hb (gm%)	8.5±0.33	5.7±0.09	5.5±0.48	5.2±0.67
Hct (%)	42.5±0.20	32.4±0.49	28.4±0.24	24.8±0.46
MCV (fl)	156.2±1.23	221.9±1.76	205.7±2.22	195.2±1.24
MCH (pg)	31.2±0.12	39.0±0.73	39.8±0.55	40.9±0.77
MCHC (%)	20.0±0.32	16.3±1.89	19.3±0.14	20.9±0.59
TLC ($\times 10^3$/cmm)	12.34±0.20	16.14±0.54	16.22±0.14	16.35±0.19

Present authors propose that due to metal toxicity haemopoietic organs get affected and became unable to release normal RBCs in general circulation and thus can be held responsible for drastic decline in TEC. Present viewpoint that haemopoietic organs get impaired by xenobiotics toxicity and thereby lead to reduction in TEC get an added support from the work of [16, 15, 5] who also have observed a similar trend [17], however, advocated reduced erythrocyte life span as well as slower erythropoiesis to be responsible for reduction in RBC number as a result of metal toxicity.

Presently, besides affecting erythrocyte number (Table 1) metal toxicity has also been found to result in marked anomalies in shape of RBCs as well as nucleus compared to that of control (Figures 1,2,3 & 5). The distorted RBCs which make their appearance during the first week of experimental period in very few number register an increase with the advancement of experiment indicating clearly that TEC not only decline quantitatively but qualitatively also (Figures 1-6). Distorted/ abnormal shape of RBCs can lead to tissue hypoxia by reducing the oxygen carrying capacity of RBCs and same has also been earlier reported by workers viz [18, 19, 20]. These morphological changes in erythrocytes initiate the process of RBC destruction and ultimately lead to their complete degeneration.

It is clearly depicted in Table 1 that MCV exhibited significant (P<0.01) increase in its values in all the metal treated groups. Increase in MCV values is a clear reflection of reduction in TEC values because MCV and TEC have an inverse relationship with each other. Increase in MCV may be due to swelling of RBCs under Manganese toxicity and it gets evident from the prevalence of a number of swollen erythrocytes (Figure 5) in microphotographs of metal treated fish whose increased volume in turn increases the mean corpuscular volume. MCH exhibited significant increase in its values and MCHC depicted fluctuating pattern in all the metal treated groups compared to control groups. Such change in MCH and MCHC may be due to increased lysis of RBCs and reduction in cellular blood iron which simply reflects decline in Hb content due to metal toxicity. Presently, fish *G. gotyla gotyla* has been diagnosed to suffer from megaloblastic anaemia.

White blood cells or leucocytes are the cells of immune system which defend the body against infectious as well as foreign materials. Review of literature reveals that there are two schools of thought regarding the response of leucocytes to various stressors and xenobiotics. According to workers of first school [21, 22, 23, 24, 25] there is a decrease in TLC whereas workers of second school viz. [26, 27, 28, 29] advocated increase in their number in response to metal toxicity. Presently our results are in accordance with second group of workers who have observed an increase in total leucocyte count of fish on exposure to different xenobiotics. This increase in TLC can be attributed to a stimulation of the immune system in response to tissue damage caused by Manganese.

Leucocytes, which play a major role in the defense mechanism of fish, consist of granulocytes (basophils, neutrophils and eosinophils) and agranulocytes (monocytes and lymphocytes). In depth study of the DLC (Table 2) further reveals that increase in TLC upon Manganese exposure in all the treated groups can be very safely attributed to an increase in lymphocytes, monocytes and eosinophils. Neutrophils, basophils and thrombocytes however, have been observed to witness a decline in their number (Table 2).

Table 2: Differential leucocyte count of *G. gotyla gotyla* subjected to Manganese treatments.

Parameters	Manganese Treatment (Dose)			
	Untreated (Control)	20%	40%	60%
Lymphocytes (%)	40.2±0.63	53.2±1.76	55.0±0.69	55.8±0.39
Neutrophils (%)	28.4±0.0.35	19.3±0.32	18.6±0.81	17.3±0.14
Monocytes (%)	4.1±0.17	6.5±0.12	6.7±0.85	6.9±0.80
Basophils (%)	1.7±0.19	0.1±0.89	0.2±0.27	0.1±0.61
Eosinophils (%)	0.1±0.54	1.6±0.32	1.4±0.19	1.7±0.18
Thrombocytes (%)	25.5±0.56	19.3±0.09	18.1±0.29	18.2±0.29

Lymphocytes, which account for major contribution to TLC (14.25-17.25×10^3/cmm) are the blood cell constituents usually responsible for specific type of immune response. It appears that by stimulating lymphopoiesis (release of more and more lymphocytes) from the lymphomyeloid tissues into general circulation, fish responds against metal toxicity. Such stimulatory response of lymphocytes has also been reported by [30, 5] who further added that increased availability of lymphocytes under xenobiotic intoxication possibly results in increased antibody production. [31, 32] too held that increased lymphocytes result in production of antibodies. Present authors propose that Manganese toxicity stimulate the immune system of fish by releasing more and more antibodies from increased lymphocytes and help to combat the stress of metal toxicity.

In addition to lymphocytes, neutrophils and monocytes also provide protection to fish against toxicants through their elevated phagocytic activity [33]. Presently, monocytes and eosinophils have also been observed to exhibit an increase in number while neutrophils and basophils rather were observed to fall appreciably during entire course of experimental period (Table 2). It appears that in a bid to overcome the stress of metal toxicity fish *G. gotyla gotyla* tends to increase monocytes for phagocytic function. It may be added at this juncture that increase in monocytes may be in view of the fact that neutrophils and basophils which too are the other members of phagocytic machinery show a dip under the stress of metal toxicity. Increase in monocytes and decrease in neutrophil count in fish following subjection to heavy metals has also been reported by [34, 35].

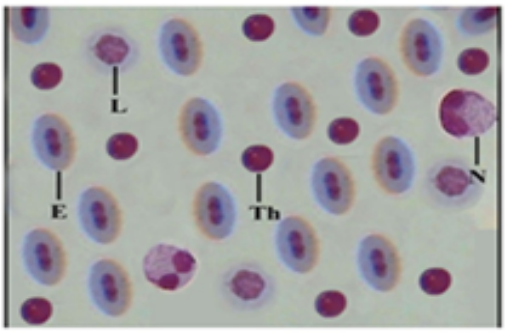

Fig 1

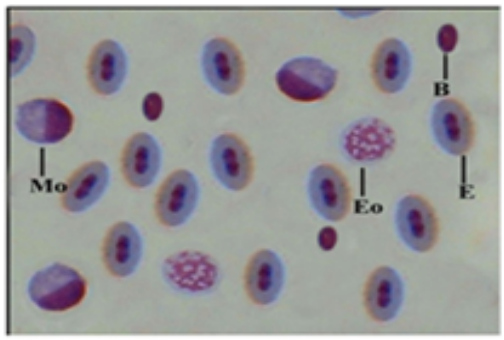

Fig 2

Fig 1: Microphotograph of blood smear of control *G. gotyla gotyla* showing erythrocytes (E), neutrophils (N), lymphocytes (L) and thrombocytes (Th) (100x).

Fig 2: Microphotograph of blood smear of control *G. gotyla gotyla* showing basophils (B), eosinophils (Eo) and monocytes (M) (100x).

Thrombocytes comparable to mammalian blood platelets play an important role in blood clotting [36] and prevent blood loss from hemorrhaging. Thrombocytes are also known to be phagocytic in action [9] .Thrombocytes were found to exhibit significant decrease in their number in all metal treated groups (Table 2). Decreased thrombocytes, it appears are incapable to contribute to the phagocytic activity of the fish under Manganese toxicity but remain available for their primary function of blood clotting during any emergencies of tissue injury related bleeding during metal intoxication.

Macrophages which appeared during 3rd week recorded an increase in their number with the advancement of experimental period. Present authors propose that their increase is an attempt on the part of fish to strengthen the phagocytic function to combat the Manganese mediated stress.

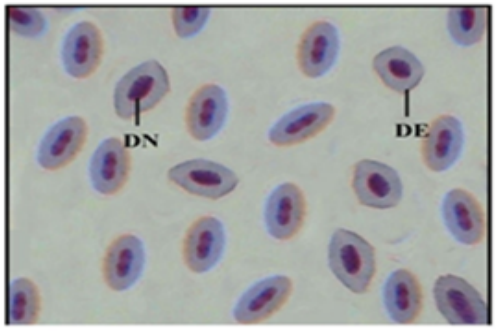

Fig 3

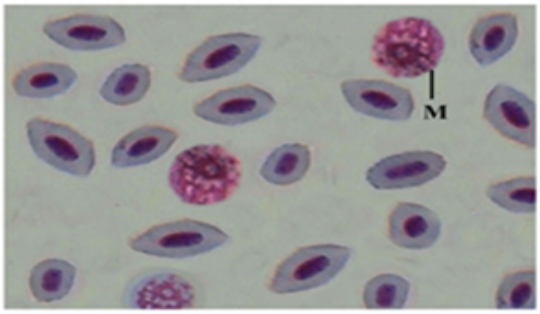

Fig 4

Fig 3: Microphotograph of blood smear of *G. gotyla gotyla* treated with manganese showing distorted erythrocytes (DE) with distorted nucleus (DN) after 1st week of the treatment (100x).

Fig 4: Microphotograph of blood smear of *G. gotyla gotyla* treated with manganese showing appearance of macrophages (M) after 3rd week of the treatment (100x).

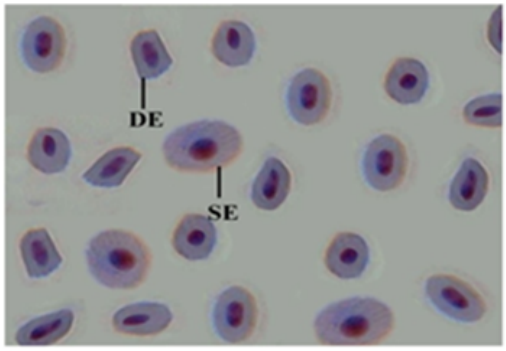

Fig 5

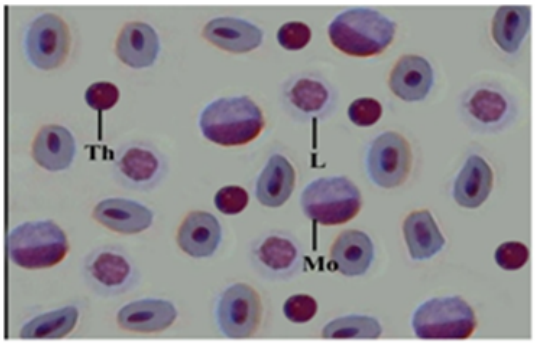

Fig 6

Fig 5: Microphotograph of blood smear of *G. gotyla gotyla* treated with manganese showing swelled erythrocytes (SE) and deformed erythrocytes (DE) after 5th week of the treatment (100x).

Fig 6: Microphotograph of blood smear of *G. gotyla gotyla* treated with manganese showing marked increase in lymphocytes and monocytes and decrease in thrombocytes after 9th week of the treatments (100x**).**

5. Conclusion

Thus, from above results and discussion it can be inferred that Manganese toxicity affect haematological parameters of fish, *G. gotyla gotyla* and changes were dose and time dependent.

6. References

1. Jezierska B, Witeska M. Metal toxicity to fish. Reviews in Fish Biology and Fisheries 2001; 11(3):279–279.
2. Vosyliene MZ, Kazlauskiene N, Svecevicius G. Effect of heavy metal model mixture on biological parameters of rainbow trout *Oncorhynchus mykiss*. Environ Sci & Pollut Res 2003; 10:103–107.
3. Raina S. Effect of environmental stress on haematology and immune organs of *Labeo* species. Ph D Thesis University of Jammu 2011.
4. Sachar A. Studies on effect of organic and inorganic pollutants on haematology blood biochemistry and immune organs in some fishes of Jammu region. Ph D Thesis University of Jammu 2011.
5. Gupta K. Studies on effect of heavy metal toxicity on the histophysiology of blood and haemopoietic tissues of some fish species. Ph D Thesis University of Jammu 2012.
6. Brucka-Jastrzebska E, Protasowiecki M. Effects of cadmium and nickel exposure on haematological parameters of common carp *Cyprinus carpio* L. Acta Ichthyol Piscat 2005; 35:29–38.
7. Fernandes MN, Mazon AF. Environmental pollution and fish gill morphology. In: Val, A. L. & B. G. Kapoor (Eds.). Fish adaptations. Enfield, Science Publishers, 2003; 203-231.
8. Maule AG, Schreck CB. Changes in the number of leucocytes in immune organs of juvenile Coho after acute stress or cortisol treatment. Journal of Aquatic Animal Health 1990; 2:298-304.
9. Anderson DP. Text book of fish immunology. Narendra Publishing House 2003; 1-177.
10. Dethloff GM, Schlenk D, Khan S, Bailey HC. The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Arch Environ Contam Toxicol 1990; 36:415-423.
11. Wintrobe MM. Clinical Haematology VI ED.

Philadelphia, Lea and Febiger, 1967.
12. Jenkins F, Smith. Effect of sublethal concentration of endosulphan on haematological and serum biochemical parameters in the carp *C carpio* Bull. Environ Contam Toxicol 2003; 70:993-947.
13. Tyagi A, Srivastava N. Haematological changes of fish *Channa punctatus* (Bloch) to chronic zinc exposure. J Environ Biol 2005; 26(2):429-432.
14. Gupta K. Copper induced toxicity on haematological and haemopoietic profile of Puntius sophore (Ham.). M Phil Thesis University of Jammu, 2008.
15. Gupta K, Sachar A, Gupta K, Raina S. Effect of lindane on haematological parameters of minor carp *Labeo boga* (Ham.). Biosci Biotechnol Res Asia 2009; 6(2):695-702.
16. Santhakumara M, Balaji M, Amudu K. Adaptive changes in respiratory movements of an air breathing fish *Anabas testudineus* exposed to organophosphate pesticide monocroptophos. Eco Env Conserv 2000; 6(1):67-69.
17. Senczuk W. Toxykologia Wydanie III. [Toxicology Third Edition] PZWL, Warszawa. (In Polish), 1999.
18. Das BK. Studies on the effect of some pesticide and commonly used chemicals on Indian major carps and their ecosystem M Phil. Thesis Orissa University of Agriculture and Technology Bhubaneshwar (India) 1998, 139-162.
19. Yang JL, Chen HC. Effects of gallium on common carp (*Cyprinus carpio*) acute test serum biochemistry and erythrocyte morphology. Chemosphere 2003; 53:877-882.
20. Verma G. Lindane (an insecticide) induced haematological changes in a minor carp *Puntius sophore* (Ham.) M Phil. Thesis University of Jammu, 2007.
21. Iwama GK, Greer GL, Arkin PA. Changes in some haematological characteristics of Coho Salmon (*Onchorhynchus kisutch*) in response to acute exposure to dehydroabieticacid (DHAA) at different exposure levels. J Fish Res Board Can 1976; 33:285-289.
22. Mishra S, Srivastava AK. Haematology as index of sublethal toxicity of zinc in a freshwater teleost. Bull Environm Contam Toxicol 1979; 22:695-698.
23. Ellis AE. Stress and the modulation of defense mechanism in fish. In: Stress and Fish Pickering AD (Ed.). Academic Press London, 1981; 3:147-171.
24. Sharma RC, Gupta N. Carbon Tetrachloride induces haematological alterations in *Clarias batrachus* (L). J Environ Biol 1984; 3:127-131.
25. Adeyemo OK. Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. Turkish Journal of Fisheries and Aquatic Sciences 2007; 7:163-169.
26. Torres P, Tort L, Planas J, Flos R. Effects of confinement stress and additional zinc treatment on some blood parameters in the dogdish (*Scyliorhinus canicula*). Comp Biochem Physiol 1984; 83:89-92.
27. Garg VK, Garg SK, Tyagi SK. Manganese induced haematological and biochemical anomalies in *Heteropneustes fossilis.* J Environ Biol 1989; 10(4):349-353.
28. Singh BP, Tandon PK. Effect of river pollution on haematological parameters of fish *Wallago attu*. Res Environ Life Sci 2009; 2(4):211-214.
29. Buthelezi PP, Wepener V, Cyrus DP. The sublethal effect of zinc at different water temperatures on selected haematological variables in *Oreochromis mossambicus*. American Journal of Aquatic Sciences 2000; 25:146-151.
30. Gill TS, Pant JC. Erythrocytic and leucocytic response to cadmium in fresh water fish *Puntius conchonius*. Environ Res 1985; 36:327-335.
31. Fink NE, Salibian A. Toxicological studies in adult amphibians: Effects of lead Appl Herpetol 2005; 2(3):311-333.
32. Tomaszewski JJ. Diagnisyka laboratoryina (laboratory diagnostics). PZWL Warszawa 1997; 36(4):73-76.
33. Safahieh A, Hedayati A, Savari A, Movahedinia. Experimental approaches of hematoxix and immunotoxic effects of mercury chloride on yellowfin sea bream (*Acanthopagrus latus*). American-Eurasian Journal of Toxocological Sciences 2010; 2(3):169-176.
34. Ishikawa NM, Ranzani-Paiva MJT, Lombardi JV, Ferreira CM. Haematological parameters in Nile Tilapia *Oreochromis niloticus* exposed to sublethal concentrations of mercury. Braz J Zool 2007; 50(4):619-626.
35. Devi P, Baruah D, Baruh BK, Borkotok A. Impact of endosulphan on some haematological parameters of *Channa punctatus* (Bloch). Poll Res 2008; 27(3):485-488.
36. Atamanalp M, Kocaman EM, Ucar A, Alak G. The alterations in the haematological parameters of brown trout *Salmo trutta fario*, exposed to cobalt chloride. Journal of Animal and Veterinary Advances 2010; 9(16):2167-2170.