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Effects of Mustard Oil Cake on Haematological Parameters of the Freshwater Fish *Channa punctatus*

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Abstract

MOC, a widely used bio-fertilizer in agriculture and fish cultivation in India, was considered to observe the changes in haematological parameters of *Channa punctatus* (Bloch). The present study revealed a decreasing trend of haemoglobin volume and erythrocytes count and increasing value for MCH when this fish was exposed to 0.42 mg l^{-1} concentration of mustard oil cake for 4,7,14,21 and 28 days of exposure respectively in laboratory conditions. Study also showed variation of DCL with prolong period of exposure.

Keywords: Mustard oil cake, fish, haemoglobin, erythrocytes, Differential count, MCH.

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INTRODUCTION

Indiscriminate and injudicious use of different agro-chemicals (i.e. fertilizers, pesticides etc.) to increase crop production, is one of the most important causes of continuous pollution of water bodies, like ponds, lakes, river and low laying water areas [1]. The process of contamination of these agro-chemicals into the aquatic environments mostly occurred by surface run off, direct application into the field and sediment transport. In water, fish acquire fertilizer through the gills, as well as by the digestive tract [2]. As a path of physiological reflector of the whole body, haematological assessment are considered as an important screening tool to investigate the well-being of fish exposed to contaminants [3]. For example, Singh *et al.* evaluated the effects of copper on hematological profile of *Channa punctatus* and observed significant increase in Mean Corpuscular Haemoglobin (MCH) values after 15 and 30 days of exposure[4]. In the same fish species haematological profile was considered in malathion-exposed animals[5]. The Erythrocyte Sedimentation Rate (ESR) in *Channa punctatus* treated with chlorpyrifos pesticide [6] whereas, Maitra and Nath has recently investigated the changes in blood parameters of *Heteropneustes fossilis* induced by urea [7].At the same time, changes in blood parameters of *Heteropneustes fossilis* during environmental stress conditions has been reported [8]. Sublethal concentrations of quaternium-15 have significant effect on haemolymphatic parameters of *Mytilus provincialis* [9].

Cellular and nuclear hypertrophy along with agglutination and bursting of erythrocytes in *Cirrhinus mrigala* in presence of organic fertilizer like urea was observed [10]. Toxicants were found to be carried by different agents like air, water, soil etc., after entering into the ecosystem and got their way into the food chains [11,12]. In India, regular increase in the use of organic and the bio-fertilizer in agriculture in the name of the production of healthy and artificial chemical free crop, leads to the accumulation of this organic substances in the fresh water bodies causing pollution at various level. MOC is one of the randomly used fertilizer in agriculture and fish cultivation, contains 43% protein, 2.05% oil, 1.22% AITA and 2.75% phytic acid [13].

The present study was performed to establish the effects of a sub-lethal dose of MOC on fresh water *Channa punctatus*, as very few works has been done so far on this aspect in the area of toxicological research.

MATERIAL AND METHODS

MOC Dose Selection :

On the basis of the LC_{50}-96 h value of MOC on *Channa punctatus* (Bloch) calculated using the method of Finney [14], a sub-lethal dose of 0.42g $l^{-1}$was chosen.

***Experimental Design*:**

Fish of about 51.68 ± 0.634 g of weight were collected from the local market near the college campus. The fishes were cleaned by tap water and washed with 0.02% $KMnO_4$ for removing external infections of fungi, algae etc. Fish were then put into the aquarium under laboratory conditions after for acclimatization to 7 till days.The dead fishes were removed immediately.

In the experiment, 60 healthy fishes were selected for experiments. Fishes were divided into two batches of 30 animals each and kept in two separate aquaria (60cm x 48 cm x 48 cm) each containing 60 l of water. Aquaria were marked as A and B respectively. Aquarium "A" was used as control, that is without any treatment. Fishes in aquarium "B" were exposed to 0.42 g l^{-1} of MOC. Water was changed every 48 hrs during the experiment. Every effort was made to provide optimal condition for fish and no mortality occurred during conditioning period. A special floating type of fish food marketed under the trade name "Tokyu" (46% crude protein) was provided at regular intervals of 24 hours in each aquarium, at the rate of 5 % of the body weight [15].Five fishes from both aquaria (control and treated) were collected after 4, 7, 14, 21 and 28 days respectively and blood was collected by tail ablation from each unanesthesized fish.

***Blood Collection* :**

Tail ablation (Herbing, 2004) was done and blood was arranged to study different parameters such as red blood corpuscle (RBC) count (10^6 mm^{-3}), haemoglobin, MHC and differential leucocyte count (DLC) of *C. punctatus* [16].

***Total Count of Red Blood Corpuscle* :**

RBC was determined by using Neubauer's haemocytometer. Blood was smeared by RBC pipette i.e. the blood stream was a continuous one R.B.C.

Cells were counted under a light microscope at 40x resolution. Out of 25 squares only 5 were considered (4 small squares at the corner and the central one) for this purpose.

The formula to calculate RBC number was :

No. of Red Blood Cells per cubic mm of Blood

$$= \frac{\text{Total No. of cells counted x dilution x 4000}}{\text{No. of small squares in which counting has been done}}$$

***Haemoglobin percentage estimation*:**

Using Sahli's haemoglobinometer pipette consist of capillary tube rinsed with anticoagulant (1% sodium citrate solution) blood was collected and blown out into the haemoglobin tube containing N/10 HCl. Stirring of the content of haemoglobin tube was done by a glass stirrer and allowed it to stand for 10 to 20m By adding N/10 HCl drop by drop to the tube while stirring with rod, till the colour in the tube matched exactly with that of the standard brown plates.

***Mean Corpuscular Haemoglobin (MCH)* :**

M.C.H. is the average amount (weight) of haemoglobin contained in a single red cell and was calculated as follows :

$$\text{MCH } (\rho g) = \frac{\text{Haemoglobin (g\%) x 10}}{\text{Erythrocyte count (per / L)}}$$

***Differential Count of Leukocyte (DCL)* :**

The standard method of Human Blood film preparation with the Leishman's stain was applied for DCL determination. The counting was replicated three times.

Statistical Analysis: Regression, Co-relation co-efficient and its significant t-value, ANOVA : Single Factor were computed by using Origin 6.0 Software.

RESULTS

Exposure of fish to sub-lethal concentration of MOC for various days caused considerable alterations in the haematological parameters of *C. punctatus*. These changes were observed to be significant in the treated specimens at 4, 7,14,21 and 28 days of exposure respectively. However, comparison revealed an increasing value of RBC and Hb after 4,7,14 and 21 days exposure, and a decreasing trend at 28th day.

In the case of RBC, the study revealed that total count of RBC was $3.926 \pm 7.65\ 10^6\ mm^{-3}$ in controlfish and $3.068 \pm 1.5\ 10^6\ mm^{-3}$ in treated fish for 4 days exposure (Table-1). It was seen that the count was $4.844 \pm 5.81\ 10^6\ mm^{-3}$ and $5.354 \pm 7.76\ 10^6\ mm^{-3}$ in control and 3.352 ± 4.66 and $3.776 \pm 0.93\ 10^6\ mm^{-3}$ in exposed fish for 7 days and 14 days respectively. The count of RBC reached the value of 6.198 ± 13.28 and $7.058 \pm 7.22\ 10^6\ mm^{-3}$ in control animals and 5.094 ± 16.81 and $2.588 \pm 4.01\ 10^6\ mm^{-3}$ in MOC-treated fish for 21 days and 28 days respectively.

Table 1: Total Count of RBC, Hb %, MCH in Control (C) and Treated (T) fish after 4, 7, 14, 21, 28 days of exposure with 0.42 $g.l^{-1}$ concentration of mustered oil cake.

Para--meters	Exposure period (days)									
	4 DAYS		7 DAYS		14 DAYS		21 DAYS		28 DAYS	
	C	T	C	T	C	T	C	T	C	T
TC of RBC (10^6 mm^{-3})	3.926 ± 7.65	3.068 ± 1.5 r = 0.90 t = 3.6* F = 121.28**	4.844 ± 5.81	3.352 ± 4.66 r = 0.96 t = 5.65* F =401.09**	5.354 ± 7.76	3.776 ± 0.93 r = 0.96 t = 6.0* F = 407.41**	6.198 ± 13.28	5.094 ± 16.81 r = 0.91 t = 3.96* F = 31.75**	7.058 ± 7.22	2.588 ± 4.01 r = 0.91 t = 3.79* F = 15.06**
Hb(g%)	8.14 ± 0.2	7.44 ± 0.05 r = 0.98 t = 7.54* F = 11.61**	9.92 ± 0.09	7.76 ± 0.09 r = 0.92 t = 4.0* F = 291.6**	10.62 ± 0.07	8.48 ± 0.08 r = 0.87 t = 3.0* F = 424.04**	11.26 ± 0.12	9.56 ± 0.10 r = 0.89 t = 3.42* F = 114.68**	12.06 ± 0.09	4.94 ± 0.09 r = 0.88 t = 3.3* F = 2930.81**
MCH(ρg)	20.73 ± 0.32	24.26 ± 0.27	20.50 ± 0.39	23.16 ± 0.26	19.85 ± 0.22	22.46 ± 0.25	18.41 ± 0.12	18.82 ±0.42	17.09 ± 0.09	19.17 ± 0.28

* - Experimental value is significantly different from control with statistical significant ($p<0.05$) ;

** - Experimental value is significantly different from control with statistical significant ($p<0.01$) ;

Each value is mean of five observations ± SE ; TC – Total count OF RBC; Hb – haemoglobin; MCH – Mean Corpuscular Haemoglobin.

A trend similar to that of RBC count was recorded for in case of haemoglobin (Table-1), the values resulting lower in treated fish when compared with controls at each tissue sampling time.

Differential counts of lymphocytes and neutrophils were found to increase during exposure Table 2).

Table 2: Differential count (%) showing variations in control (C) and MOC- treated (T) fish.

WBC	***4 DAYS***		***7 DAYS***		***14 DAYS***		***21 DAYS***		***28 DAYS***	
	C	*T*	*C*	*T*	*C*	*T*	*C*	*T*	*C*	*T*
LYMPHOCYTES	*38*	*38*	*38*	*39*	*38*	*41*	*39*	*39*	*39*	*43*
EOSINOPHILS	*10*	*9*	*10*	*10*	*12*	*10*	*12*	*11*	*13*	*9*
MONOCYTES	*17*	*17*	*16*	*15*	*13*	*13*	*10*	*10*	*9*	*14*
NEUTROPHILS	*6*	*5*	*7*	*6*	*9*	*9*	*11*	*11*	*11*	*12*
BASOPHILS	*16*	*16*	*15*	*15*	*11*	*11*	*8*	*9*	*6*	*9*
HETEROPHILS	*9*	*10*	*9*	*10*	*11*	*10*	*12*	*12*	*13*	*7*
THROMBOCYTES	*4*	*5*	*5*	*5*	*6*	*6*	*8*	*8*	*9*	*6*

However the number of eosinophils increased initially, but then decreased. Monocyte, Heterophil and Basophil were found to decrease on 28th day. Thrombocyte count was found almost similar in both untreated and treated fish (Fig.1).

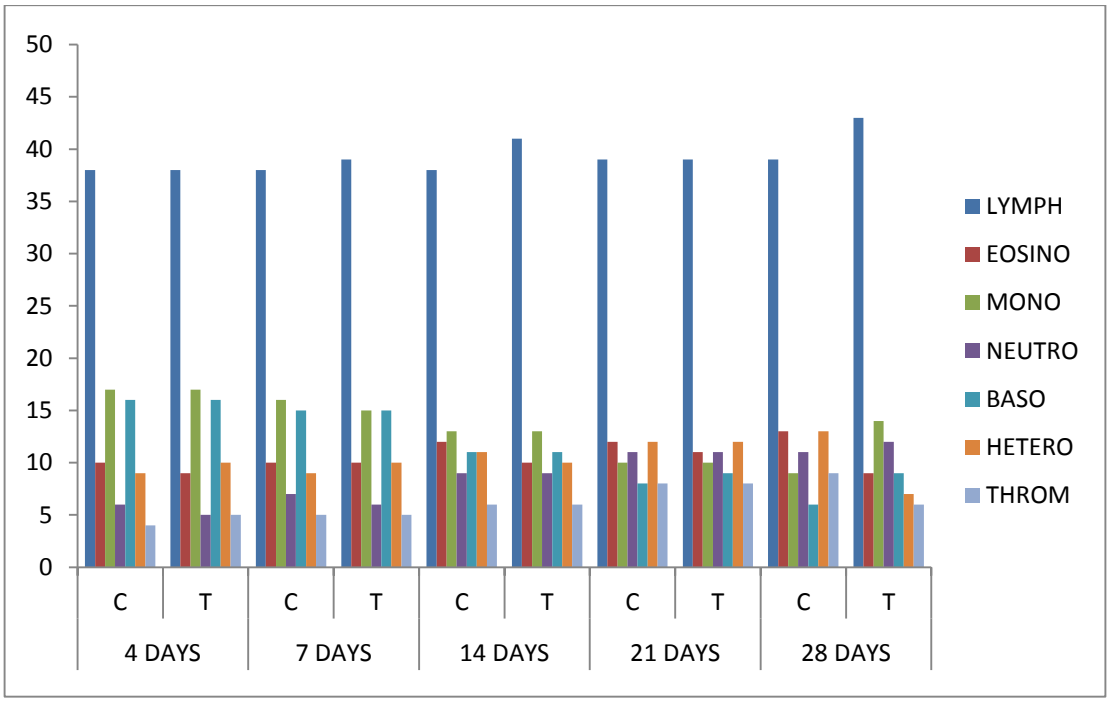

Fig.1: Differential count (%) showing variation in control(C) & MOC treated (T)fish

DISCUSSION

Measurement of haematological parameters are routinely applied in determining the physiological state of animals, which are known to be affected by different environmental factors. Such indices are considered as guide in the diagnosis of many diseases and in evaluating the responses to therapy in both animals and human [17, 18]. Like the pollutants, bio-fertilizer also produce relatively rapid changes in blood [19, 20]. In aquatic ecosystems, changes in haematological parameters in response to environmental hazards usually depend on fish species, age, the cycle of sexual maturity parameters of fish. In spite of bio-fertilizer, water quality is one of the major factors responsible for variation in fish haematology since, they live in close association with their environment and are sensitive to slight fluctuation that may occur within their milieu [21].

In the present study on *C. punctatus,* the RBC count and Hb content decreased at different exposure periods when compared to controls. The destruction of mature RBCs or inhibition of erythropoiesis was probably due to degeneration of erythopoietic tissue in kidney and spleen [22]. The anaemic response as observed in the present study could be as a result of haemodilution[23]. It was postulated that the

exposure of *C. punctatus* to MOC, reduced the erythrocytes production as well as erythrocyte counts which was the probable cause of reduction in Hb concentration as was observed in the Pyrazosulfur ethyl treated fish [24]. Under the stress condition, fish exhibits asphyxiation due to respiratory failure and anaerobic glycolysis is enhanced[25]. The decrease in haematological parameters (i.e. RBC count, Hb content) could also be due to the impairment in iron synthesizing machinery and defective uptake and absorption of iron, caused anemia as seen in *Channa striatus* exposed to metasystox[26].

Along with protein and oil, MOC also composed of allylisothiocyante (AITA) and phytic acid [13]. AITA serves on the plant as a defense against herbivores and phytic acid has the strong ability to chelate multivalent metal ions like iron. The binding can result in very insoluble salt that are poorly absorbed from the gastrointestinal tract which results a poor bio-availability of minerals [27]. In the present investigation, the decreased level of haemoglobin in comparison with non-treated fish, was formed probably due to less bio-availability of iron in *C. punctatus* exposed to MOC.

A significant decrease in haematological indices like MCH was observed in *Carassius auratus* when injected with extracted microsystins to compensate impaired oxygen uptake due to gill damage [28]. Alteration of MCH appeared in this experiment might be due to swelling of RBC or release of young erythrocytes containing less haemoglobin into the blood circulation [29].

An increase in lymphocyte counts with the advancement of exposure may be characterized to more production of antibodies to combat the stressor [30]. Heterophils are analogous, but not identical, to the mammalian neutrophil have high phagocytic and antibacterial property and also responsible for inflammatory tissue damage [31,32].The present study revealed that the number of both heterophils and thrombocytes in treated fish were almost similar to that of controlledfish indicating no impact of MOC on these cells. Moreover, rate of increase in lymphocytes and neutrophils was not much higher than that of controlled fish, probably designated less damage of body tissues and no severe physical stress in MOC. Whereas, lymphocytes and monocytes showed higher count in treated than untreated on 28^{th} days was probable indication of resurgence from this fertilizer stress.

REFERENCES

[1] Joshi,P.K., Bose, M. and Harish, D.,2002, "Haematological Changes in the Blood of *Clarias batrachus* Exposed to Mercuric Chloride," J. Ecotoxicol. Environ. Monit., 12,119-122.

[2] Kamunde, C., Clayton, C. and Wood, C. M., 2002, "Waterborne Vs. Dietary Cu Uptake in Rainbow Trout and the Effects of previous Waterborne Cu Exposure," Am. J. Physiol., 283(1), 9-78.

[3] Adhikari, S., Sarkar, B.,Chatterjee, A., Mahapatra C.T. and Ayyappan, S., 2004,"Effects of Cypermethrin and carboforan on certain haematological

parameters and prediction of their recovery in a fresh water teleost, *Labeo rohita* (Hamilton**),"**Ecotox. Environ.Safe.,58, 220-226.

[4] Singh, D., Nath, K., Trivedi, S.P. and Sharma Y.K., 2008, "Impact of Copper on Hematological Profile of Freshwater Fish, *Channa punctatus,"* J. Env. Biol., 29(2), 253-257.

[5] Parveen, N. and Shadab, G.G.H.A., 2011, "Evaluation of Micronuclei and Hematological Profiles as Genotoxic Assays in *Channa punctatus* Exposed to Malathion," Int. J. Sci. Nat., 2 (3), 625-631.

[6] Malla, F.A., Sharma, G. and Singh, S., 2009 "Chlorpyrifos Pesticide Toxicity on Erythrocyte Sedimentation Rate in Fish, *Channa punctatus*(Bloch)," Biol. Med., 1 (2), 54-55.

[7] Maitra, S. and Nath, S., 2014, "Toxic Impacts of Urea on the Hematological Parameters of Air Breathing Fish, *Heteropneustes fossilis*(Bloch)," Am. Eurasian. J. Agric. Environ. Sci., 14(4), 336-342.

[8] Goel, K.A., Awasthi, A.K. and Tyagi, S.K.,1981, "Haematoenzymology of *Heteropneustes* under Chemicoazo Stress of Bismark Brown,"Curr. Sci., 50(19), 875-876.

[9] Faggio, C., Pagano, M., Alampi, R., Vazzana, I., Felice, M.R., 2016, "Cytotoxicity, haemolymphatic parameters, and oxidative stress following exposure to sub-lethal concentrations of quaternium-15 in *Mytilus galloprovincialis*," Aquat. Toxicol., 180, 258-265.

[10] Shrivastava, D.K. and Sriwastva, V.M.S., 1980, "Erythrocytic Abnormalities Due to Urea Stress in*Cirrhinus mrigala* (Ham.)Fingerlings," Curr. Sci., 549(20), 799-800.

[11] Weber, J.B., 1977, "The Pesticides Score Card," J. Environ. Sci. Technol., *11*, 756.

[12] Farmer, W.J., Igue, K., Spencer, W.F. and Martin, J.P., 1972, "Volatility of Organochlorine Insecticides from Soil: I. Effect of Concentration, Temperature, Air Flow Rate and Vapour Pressure," Soil Sci. Soc. Amer. Proc., 36, 443-447.

[13] Khan Niazi, A.H., 1986, "Improvement in the Nutritive Value of Mustard seed cake," Doctoral thesis, University of Punjub, Lahor, Pakistan.

[14] Finney, D.J., 1978,Statistical Methods in Biological Assay,3rd ed., Charles Griffin& Co. Ltd, London.

[15] Yaji, A.J. and Auta, J., 2007, "Sub-lethal Effect of Monocrotophoson Growth and Food Utilization of the African Cat Fish *Clarias gariepinus* (Teugels)," *J. Fisheries International, 2*(2), 127-129.

[16] Herbing, H.V. and Cashon, R., 2004, "Haemoglobin sickling in boreal fishes: An adaptation to the cold," Symposium Proceedings, International Congress on the Biology of Fish, Manaus Brazil, August 1-5, 49-54.

[17] Golovina, N.A., 1996,"Morpho-functional characteristics of the blood of fish as objects of aquaculture," PhD Thesis, University of Moscow, Moscow.

[18] Luskova, V., 1997, "Annual cycles and normal values of hematological parameters in fishes," Acta Sci. Naturalium Academiae Sci. Bohemicae Brno, 31(5), 70-78.

[19] Johansen, K., 1970, Air breathing in fishes..In: W.S. Hoar & D.J. Randall (ed.) Fish Physiology, Vol. 4, Academic Press, New York.

[20] Solomon, S.G. and OkomodaV.T., 2012, "Effect of Photoperiod on Some Biological Parameters of *Clarias gariepinus* Juvenile," J. Stress Physiol. Biochem., 8 (4), 47-54.

[21] Casillas, E. and Smith, L.S.,1997, "Effect of Stress on Blood Coagulation and Haematology in Rainbow Trout(*Salmo gairdneri*)," J. Fish Biol.,10(5), 481-491.

[22] Hota, S., 1995, "Toxic Effect of Arsenic on Haemato Biochemical Abnormalities in *Channa punctatus,"* J. Ecotoxicol. Environ. Monit., 5 (4), 249-255.

[23] Sampath, K.,Velamnical, A., Kennedy, I.J. and James, R., 1993, "Haematological Changes and Their Recovery in *Oreochromis mossambicus* as a Function of Exposure Period and Sub-lethal Level of Ekalus," ActaHydrobiol.,35, 73–83.

[24] Upadhyay, A.A. and Parikh, P.H., 2014, "Pyrazophosulfuron-ethyl induced Alternation Hematology and blood Biochemistry of *Oreochromis mossambicus*:Sub-acute Study," The Experiment ,18(3), 1245-1253.

[25] Bhatkar, N.V., 2011, "Chromium (III) induced haematological alterations in Indian carp ,*Labeo rohita* (Ham.)," J. Appl. and Natural Sc., 3(2), 258-268.

[26] Natarajan, G.M.1981. Changes in the Bi-model Gas Exchange and Some Blood Parameters in the Air Breathing Fish,*Channa striatus* Following Lethal(LC50/46hr.) Exposure to Metasystox. Curr. Sci.,50(1),40-41.

[27] Zhou, J.R. and Erdman Jr, J.W., 1995, "Phytic acid in health and disease," Crit. Rev. Food Sci., 35(6), 495-508.

[28] Zhang, X., Xie, P., Li, D. and Shi, Z., 2007, "Hematological and Plasma Biochemical Responses of Crucian Carp (*Carassius auratus*) to Intraperitoneal Injection of extracted Microcystins with the possible mechanisms of Anemia," Toxicon, 49 (8), 1150-1157.

[29] Sobecka, E., 2001, "Changes in the Iron Level in the Organs and Tissues of Wels Catfish, *Silurus glanis* L. caused by Nickel," ActaIchthyol. Piscat., 31 (2), 127-143.

[30] Spielman, B., 2004, "Structure and function of the blood," Petplace.com Ed. Rhea Morgan, 1-3.

[31] Harmon, B.G.,1998, "Avian heterophils in inflammation and disease resistance," Poultry Sci., 77(7), 972-977.

[32] Bluml, S., Rose, B.,Lorinez, A., Seryerl, M., Kirchberger, S., Oskolkova,O., Bochkov, V.N., Majdic, O., Ligeti, E. and Stockl, J., 2008, "The oxidative state of Phospholipids controls the oxidative burst in neutrophil granulocytes," J. Immunol.,181(6),4347-4353.