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Toxicological studies of methanol on hematological profile of freshwater fish *Cirrhinus mrigala*

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ABSTRACT

Hematological profile of fishes gives the physiological status of fish's wellbeing. When the fish is exposed to any toxicant, it affects the blood and then consequently other vital organs are deformed. Hence in the present study hematological profile of freshwater fish Cirrhinus mrigala is studied after acute exposure to methanol. The fishes were exposed to lethal and sub lethal concentrations for 96 hrs. The results reviled that values of Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and Hemoglobin (Hb) decreased significantly (P < 0.05) in the treated group as compared to the control group. Whereas the values of Total Leucocytes Count (TLC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Hemoglobin (MCH) increased significantly (P < 0.05) in all exposed group than the control.

Keywords: Methanol, Cirrhinus mrigala, hematological profile

INTRODUCTION

Methanol is an organic solvent used in different industries for many purposes (Sitting 1980., Walden *et al.*, 1986). It is used for manufacturing in paints, varnish removers and spirits (Budavari 1989). The large amount of methanol is found in the industrial wastewater (Kaviraj *et al.*, 2004). Indiscriminate use of such chemicals alters structural and functional status of fish. The changes in hematological profile induced by different toxicants have been investigated by many authors (Ahmadivand *et al.*,2014, Christobher *et al.*,2016,. Scanty information is available regarding effects of methanol on the hematological profile in fishes. Therefore the present study was carried out to observe the alterations in hematological profile of freshwater fish *Cirrhinus mrigala* after acute exposure to methanol.

METHODOLIGY

Freshwater fish *Cirrhinus mrigala* (Order: *Cypriniformes* and Family: *Cyprinidae*), weighing 8-10g and 10-12cm in length, were obtained from Government fish seed production center, Dhom, Tal -Wai, Dist- Satara.

The fishes were acclimatized for two weeks in laboratory condition. For experimentation, healthy fishes were exposed to lethal concentrations of methanol (LC₀-11ml/l, and LC₅₀-13.05ml/l) for 96 hrs. After exposure period, the hematological profile such as PCV, TEC, TLC, Hb, MCV, MCH and MCHC were studied by collecting blood samples from fishes using an insulin syringe with cardinal vein puncture technique (Remya2010). TEC and TLC were counted with a Neubauer haemocytometer (Rusia and Sood1992). Hb was estimated by using Sahli's haemoglobinometer (Dethloff *et al.*1990). HCT was determined by the centrifugation method. MCV, MCH and MCHC were calculated using standard formulae (Kang *et al.*, 2005).

Obtained data were expressed as a means \pm SE. The values were statically analyzed by the student t test (Milton *et al.*, 1983) and result were considered significant at (P<0.05), (P<0.01) and (P<0.001) level.

RESULTS AND DISCUSSION

Changes in the hematological profile of freshwater fish *Cirrhinus mrigala* after acute exposure to methanol are presented in Table 1 and 2; graphically represented in Figure 1 and 2.

Table 1: Alteration in hematological profile PCV, TEC and Hb of *Cirrhinus mrigala* after acute exposure to Methanol

Profile	Control	LC ₀	% change over	LC ₅₀	% change over
			control		control
PCV (%)	22.2±2.39	16.8±2.64*	(-24.32)	15±2.19**	(-32.43)
TEC (10 ⁶ /mm ³)	0.93±0.033	0.86±0.03*	(-7.52)	0.84±0.016**	(-9.68)
Hb(g/dl)	2.86±0.11	2.56±0.18*	(-10.48)	2.3±0.25**	(-19.58)

Values are the mean of (n=5) ± SD

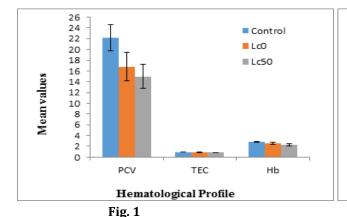
*= P < 0.05; ** = P < 0.01; ***= P < 0.001

Table 2: Alteration in hematological profile MCV, MCHC, MCH and TLC of *Cirrhinus mrigala* after acute exposure to Methanol

Profile	Control	LC ₀	% change over control	LC50	% change over control
MCV (fl)	212.8±5.97	221.8±5.04*	(4.23)	226.8±5.27**	(6.59)
MCHC (%)	10.45±0.32	11.07±0.37*	(5.93)	12.16±0.79**	(16.36)
MCH (pg)	24.6±0.31	26.2±0.97*	(6.50)	28.5±2.29**	(15.85)
TLC $(10^3/\text{mm}^3)$	5.6±0.30	6±0.12*	(7.14)	6.56±0.29**	(17.14)

Values are the mean of $(n=5) \pm SD$

*= P < 0.05; ** = P < 0.01; ***= P < 0.001



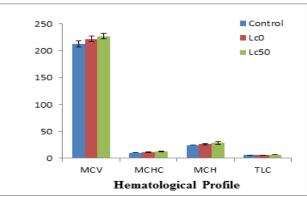


Fig.2

Figure 1: Effect on hematological profile PCV, TEC and Hb of freshwater fish *Cirrhinus mrigala* after acute exposure to Methanol

Figure 2: Effect on hematological profile MCV, MCHC, MCH and TLC of freshwater fish *Cirrhinus mrigala* after acute exposure to Methanol.

Packed Cell Volume (%)

The average packed cell volume was recorded as 22.2±2.39 in control fish, whereas a dose dependent significant reduction was observed in the value of packed cell volume as 16.8±2.64 (p<0.05), 15±2.19 (p<0.01) after 96 hours of exposure (Table 1 and Fig. 1).

Total Erythrocyte Count (106/mm³)

Total erythrocyte count in control fish was observed as 0.93 ± 0.033 . In methanol treated fishes, TEC values were recorded as 0.86 ± 0.03 (p<0.05) at LC₀ and 0.84 ± 0.016 (p<0.01) at LC₅₀ group. The erythrocyte count decreased in the exposed group as compared to control. The decreased percentages was (-7.52) and (-9.68) at LC₀ and LC₅₀ concentrations respectively (Table 1 and Figure 1).

Hemoglobin (g/dl)

The average hemoglobin value recorded was 2.86 ± 0.11 in control group. In treated fish, it decreased and was recorded as 2.56 ± 0.18 (P<0.05), 2.3 ± 0.25 (P<0.01) for 96hours exposure to LC₀, LC₅₀ concentrations of methanol respectively (Table 1 and Figure 1). The decrease percentages were (-10.48) and (-19.58) respectively.

Mean Corpuscular Volume (fl)

In the control group, MCV value recorded was 212.8 \pm 5.97. In treated fish, it increased and was observed as 221.8 \pm 5.04 (p<0.05) at LC₀ and 226.8 \pm 5.27 (p<0.01) at LC₅₀ group after 96 hours exposure. After the exposure maximum percentage change (6.59) was observed in LC₅₀ exposure group (Table 2 and Figure 2).

Mean Corpuscular Hemoglobin Concentration (%)

The MCHC value of control group was 10.45 ± 0.32 . In methanol treated fishes, the MCHC values were 11.07 ± 0.37 and 12.16 ± 0.79 for 96 hours of exposure. The MCHC values increased in the methanol exposed fingerlings. The increased percentages was (5.93) and (16.36) at LC₀ and LC₅₀ concentrations respectively (Table 2 and Figure 2).

Mean Corpuscular Hemoglobin (pg)

The value of Mean corpuscular hemoglobin of control group was (24.6 \pm 0.31). In methanol induced fishes, the MCH values were 26.2 \pm 0.97 and 28.5 \pm 2.29 for 96hrs exposure. The MCH values were increased in acute exposure with percentages (6.50) and (15.85) at LC₀ and LC₅₀ concentrations respectively (Table 2 and Figure 2).

Total Leukocyte Count (10³/mm³)

In control fish, the total leukocyte count was 5.6 ± 0.30 . In methanol treated group, the TLC values recorded were 6 ± 0.12 (P<0.05) and 6.56 ± 0.29 (P<0.01) at LC₀ and LC₅₀ concentrations respectively for 96hours exposure. The TLC values increased in both the exposure groups as compared to control. The percentage incline was observed as (7.14) and (17.14) to LC₀ and LC₅₀ concentrations respectively (Table 2 and Figure 2).

Results of present study reveals that hematological profile such as PCV, TEC, Hb level decreased significantly and an increased in MCH,MCHC, WBC,MCV in all the exposed fishes after acute exposure to methanol (p<0.05). A significant decrease in erythrocyte count, and leukocyte count was observed in freshwater fish Cyprinus carpio after 35 days of exposure to ammonia (Thangam et al., 2014). He suggested that the decrease in erythrocytes is due to the inhibition of erythropoietin (Parthipan and Muniyan 2013) observed decrease level of Hb, RBC, PCV level and increased level of MCH, MCHC in the Cirrhinus mrigala after 28 days exposure to nickel. The decrease in RBC and Hb levels was caused due to reduction in the oxygen carrying capacity of male rainbow trout (Oncorhynchus mykiss) after exposure to butachlor (Ahmadivand et al., 2014, Madhavan et al., 2016) reported increased WBC, MCH and MCHC in Clarias batrachus due to a compensatory erythropoisis after exposure to sub lethal concentration of chromium for 28 days. Prakash (2016) reported Zinc, Cadmium and Copper induced effects on Hematological profile of fresh water fish Channa punctatus. Rauf et al.,2013, reported decreased RBC, Hb, Hct, WBC count and increased MCV and MCH value in Indian Carp, Cirrhinus Mrigala (Hamilton) after acute exposure to diazinon. The levels of RBC, WBC and Hb decreased due to stress developed after Phosphamidon exposure in freshwater fish Labeo rohita (Christobher et al., 2016).

Tilak et al., (2007) observed that the effect of phenol on hematological profile of three Indian Major Carps and concluded that there is a reduction in Hb, RBC and PCV values due to haemolysis. (Moraes et al., 2015) reported increased RBC values as well as decrease in MCV, MCH value in Channel Catfish *Ictalurus Punctatus* exposed to phenol. A significant decrease in the hemoglobin and leukocytes content was observed when *Channa punctatus* (Bloch) was exposed to Carbofuran (Huda et al., 2016)

CONCLUSION

From the present investigation, it can be concluded that the lethal concentrations of methanol produce adverse effects on hematological profile of freshwater fish *Cirrhinus mrigala*. After acute exposure PCV, TEC, Hb level decreased whereas the values of TLC, MCV, MCHC and MCH increased in all exposed group as compared to control group due to alterations in various physiological responses and duration of exposure.

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