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Comparative Toxicological Studies of a Carbamate (Carbaryl) and Herbicide (2,4-d Sodium Salt) On Haematological Parameters on a Freshwater Catfish, *Heteropneustes fossilis* (Bloch)

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The aim of the present work to study the comparative effects of a carbaryl (carbamate) and 2,4-D sodium salt (Herbicide) on haematological parameters of a freshwater catfish, *H.fossilis,* at different concentrations and time intervals. The catfish shows significance differences in their haematological parameters like RBC, WBC, Hb g%, haematocrit and clotting time at acute and subacute concentration for acute (96h) and short (10-20 days) time intervals but no remarkable changes were observed in sublethal concentration at long (30-60 days) term to both the toxicant. It is also observed that carbaryl is more toxic to fish then 2,4-D sodium salt.

Key words: Carbaryl, 2,4-D sodium salt, Haematological parameters, *Heteropneustes fossilis*.

The environmental pollution due to extensive usage of the pesticides without proper management has toxic effects on survival of aquatic animals because some of these toxic pesticides like organochlorine and organophosphorus may present in the environment for long periods, often unchanged. They accumulate in liver and fatty tissues [1]. Poisoning risk depend on dose, toxicity, duration of exposure and sensitivity. Organochlorine and organophosphorus are most widely used pesticides. The fishes exposed by these pesticides are reach to human being by food chain which has great nutritive values. Now a days these highly toxic pesticides are replaced by less toxic pesticides like carbamate and herbicides.

Carbaryl (1-naphthyl 1-N-methyl carbamate; sevin) is a carbamate insecticide introduced in 1956. It is a good substitute of some organochlorine pesticides [2]. Small amount of carbaryl have caused adverse effect on birds, fish, tadpoles, salamenders, shrimp, bees and other animals. The main effect of carbaryl include reduced production of eggs, reduced ability to run, deformed legs, reduced swimming speed and mortality.

A herbicide 2,4-D sodium salt (sodium salt of 2,4-dichlorophenoxyacetic acid) used against pest, undesirable herbs and agricultural diseases were found to have adverse effects in the non target environmental animals and plants [3]. The 2,4-D is the most widely used herbicide in the world (Industry Task Force Research Data). It is low toxicity for microorganism, fish, insects, domastic and wild

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animals and humans at low concentrations but acute poisoning causes the toxic effect in fish, domastic and wild animals causing apathy, anaemia of mucous membranes and drop of body temperature[4]. High does also causes changes in blood parameters[5], necrosis of liver, hyperplasia of Bowman's capsule and adenomas [4]. The fish showed marked depletion in liver glycogen, collection of glycoproteins in the blood. The 2,4-D sodium salt also effects can lead to gradual decreases in fish population through increased predation, reduced reproduction, diseases and starvation and morphological and histochemical changes in tissues and organs has been also demonstrated on exposure of 2,4-D sodium salt [6]. The aim of present work to study the effect of carbamate (carbaryl) and herbicide (2,4-D sodium salt) on haematological parameters on a catfish, *Heteropneustes fossilis,* at different dose and time intervals.

MATERIAL AND METHODS

Live specimen of fish, *H. fossilis* (Weight 16.32±1.30 gm, length 11.30±1.30 cm) were procurred from the local market and brought to the laboratory in 15 litre plastic bucket. They were acclimatized to the laboratory condition for 10 days in dechlorinated water. The water used during experiments was analysed as per the standard method [7]. The methods [8] were used to calculate 24, 48, 72 and 96h LC_{50} values and 95% confidence limits. The presumably harmless (safe) concentrations of both the toxicants were estimated by the formula of [9]. The stock solution of toxicants were prepared in distril water and the toxicants used were of technical grade (95 to 98% pure) and purchased from Sigma Chemical Company, Mumbai. The fish were exposed to acute ($1/5^{th}$ of 96h LC_{50} value), subacute ($1/10^{th}$ of 96h LC_{50} value) and sublethal ($1/15^{th}$ of 96h LC_{50} value) concentrations for the acute (96h), subacute (10-20 days) and sublethal (30-60 days) term. A parallel group of control fish was maintain in the toxicant free tapwater. The physico-chemical characteristics of tapwater were (pH 7.8, dissolve oxygen 8.40 mg/L^{-1}, total hardness 270.50 mg/L^{-1} as $CaCO_3$ and BOD 16.20 mg/L^{-1}). On completion of fixed exposure periods, caudal peduncle of fish were cut off. The free flowing blood from the caudal artery was directly collected for the estimation of haematological parameters.

The total RBC and WBC counts were made by improved Neubaurer hemocytometer and haemoglobin content (g%) determined by Sahlin-Hellige method using 0.1 N HCl. Packed cell volume (PVC) was measured by the method using 75x1.00-1.25 mm capillary tubes. Clotting time of blood was determined by the capillary tube method as used in clinical haematology . The results were subjected to statistical analysis by student's 't' test [10].

RESULTS AND DISCUSSION

The LC_o, LC_{50} and LC_{100} values with 95% confidence limits for 24, 48, 72 and 96h for both the toxicants are presented in Table Ia and Ib . The comparative values of 96h $LC_{50,}$ safe concentration and fraction of 96h LC_{50} value of these toxicants are also given in table Ic. On exposure at acute, subacute and sublethal concentrations of both the toxicants for acute (96h) and both short (10-20 days) and long (30-60 days) terms, noticeable changes were observed is haematological parameters of the exposed fish (Tables 2, 3 and 4). The haematological parameters play an important role in diagnosis of diseases in fish following different stress condition. Thus haematology can be considered as an essential index to general health status [11]. The results also showes that carbaryl is more toxic than 2,4-D sodium salt.

Total RBC Counts

The decrease in erythrocytes in the catfish during carbaryl and 2,4-D sodium salt exposure indicates an inhibited production of red blood cells caused by increased erythrocytes destruction [12]. The development of erythropenia in the catfish could be due to toxicants interference with haemopoesis and/or alteration of cell membrances by hydrolysis of acetylcholine in the body fluids by cholinesterase of erythrocytes [13]. The carbaryl and 2,4-D sodium salt induced erythropenia were also reflected by reduced hemoglobin content in this study. The reduction in total RBC counts may be due to microcyctic or normocytic anaemia as suggested by Dutta[14] , Tuschiya[15]. These observations are in close agreement with the findings of other workers [16,17,18] who reported similar changes in *H. fossilis* after exposure to different water pollutant and GOAW effluent.

Total WBC counts

The studies of total and different leucocyte counts have been suggested as an indicator of stress in fish [11]. Significant increase was observed in total WBC counts of the catfish at acute, subacute and sublethal concentrations of carbaryl and 2,4-D sodium salt at acute (96h) and short (10-20 days) term but no remarkable changes were observed at long (30-60 days) term exposure to 2,4-D sodium salt.

Leucocytosis was also observed in the other teleostean fishes at post exposure to toxicants [12,16,17,18,19,20]. The WBCs are inextricably involved in the regulation of immunological function[14] and a prolonged exposure of *H. fossilis* to a toxicant may inflict immunological deficiencies where the toxicant may work as an antigen. The rise of total WBC counts at different cocentrations of carbaryl and 2,4-D sodium salt may be due to malfunctioning of haematopoeitic system caused

Table 1(a). LCO, LC50 AND LC100 value (mg/l) of carbaryl (carbamate) for the catfish, Heteropneustes fossilis; 95% confidence limits are given in parentheses

h	LCO	LC50	LC100
24	60.33	68.60 (60.75-72.45)	72.45
48	21.35	24.75 (22.65-26.75)	28.65
72	17.85	18.30 (16.85-19.45)	20.55
96	15.45	16.40 (14.20-17.50)	18.50

Table 1(b): LCO, LC50 and LC100 values (mg/l) of 2,4-D sodium salt (herbicide) for the catfish, *Heteropneustes fossilis*; 95% confidence limits are given in parentheses

h	LCO	LC50	LC100
24	3800	7500 (6400-9300)	9000
48	1825	2150 (2000-2325)	4025
72	1650	1800 (1570-1990)	2050
96	1400	1500 (1200-1800)	1625

Table 1(c). Comparative chart of LC50 values for 96h, safe concentrations and fractions of 96h LC50 values (mgL^{-1}) for the catfish, *Heteropneustes fossilis,* exposed tocarbaryl (carbamate) and 2,4-D sodium salt (herbicide)

Name of Toxicants	96h LC50 values	Safe concentrations	Fraction of 96h LC50 values
Carbaryl (Carbamate)	16.40	0.96	$1/17^{th}$
2,4-D sodium salt (Herbicide)	1500	53.12	$1/28^{th}$

Table 2: Hematological parameters for the catfish, *Heteropneustes fossilis* following exposure to acute concentrations of carbaryl (3.28 mgL^{-1}) and 2,4-D sodium salt (300 mgL^{-1}) for 96h

Parameters	Control	Experimental	
		Carbaryl	2,4-D sodium salt
Total RBC count ($\times 10^6$ mm^3)	3.55±0.75	2.10±0.15**	2.50±0.25
Total WBC count ($\times 10^5$ /mm^3)	2.65±0.35	3.75±0.45**	3.00±0.15*
Hemoglobin(g%)	11.40±0.40	10.30±0.20**	10.50±0.30*
Hematocrit(%)	44.60±1.50	35.50±1.75**	40.15±1.25*
Clotting Time (sec.)	130±2	140±3**	135±2*

Note: Values are mean ± SE (n=6). Significant differences are indicated by asterisks

Table 3. Hematological parameters for the catfish, *Heteropneustes fossilis* following exposure to subacute concentration of carbaryl (1.64 mgL-¹) and 2,4-D sodium salt(150 mgL⁻¹) for short (10-20 days) and long (30-60 days) terms

Parameters	Short Term						Long Term					
	10 days			20 days			30 days			60 days		
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt
Total RBC count (×10v /mm³)	3.50±o.78	2.15±0.16*	2.50±0.50*	3.58±0.74	2.19±0.18*	2.53±O.56*	3.60±0.64	2.20±0.19*	2.84±0.58*	3.60±0.68	2.20±0.30*	2.80±0.38*
Total WBC count (×10u /mm³)	2.25±0.38	3.75±0.44**	3.00±0.18*	2.80±0.37	3.82±0.48**	3.20±0.21*	2.70±0.41	3.50±0.72**	3.10±0.38*	2.75±0.48	3.20±0.78*	3.00±0.38*
Hemoglobin (g%)	11.42±0.42	10.30±0.21*	10.50±0.40*	11.48±0.37	10.34±0.33*	10.50±0.49*	11.50±0.29	10.80±0.29*	11.20±0.52	12.20±0.36	10.52±0.36*	12.10±0.70
Hematocrit (%)	44.64±1.52	35.50±1.78**	39.16±1.25*	44.66±1.38	36.01±1.68**	39.29±1.38*	45.01±1.69	40.66±1.78*	42.78±1.29*	46.03±1.78	40.01±1.38*	45.28±1.32
Clotting Time (sec)	131 ±5	140±4*	134±2*	133±2	141±2*	134±2	135±2	143±2*	136±3	138±2	142±2*	139±3

Note: Values are mean ± SE (n=6). Significant differences are indicated by asterisks.

Table 4: Hematological parameters for the catfish, *Heteropneustes fossilis* following exposure to sublethal concentrations of carbaryl (1.09 mgL^{-1}) and 2,4-D sodium salt (100 mgL^{-1}) for short (10-20 days) and long (30-60 days) terms

Parameters	Short Term						Long Term					
	10 days			20 days			30 days			60 days		
	Control	Experimental		Control	Experimental		Control	Experimental		Control	Experimental	
		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt		Carbaryl	2,4-D Sodium salt
TTotal RBC count ($\times10^6/mm^3$)	3.58±0.72	3.00±0.78*	3.56±0.36	3.59±0.66	2.72±0.68*	3.50±0.38	3.62±0.71	2.85±0.71*	3.50±0.48	3.64±0.78	2.48±0.68	3.58±0.28
Total WBC count ($\times10^6/mm^3$)	2.88±0.77	3.77±0.43*	3.60±0.19*	2.89±0.78	3.79±0.42*	3.01±0.18*	2.90±0.82	3.50±0.45*	3.02±0.19	2.96±0.86	3.42±0.43*	3.25±0.44
Hemoglobin (g%)	11.48±0.38	10.32±0.33*	10.54±0.43	11.52±0.28	11.15±0.29**	11.34±0.44	11.58±0.33	11.26±0.33	11.48±0.33	11.98±0.28	11.58±0.29	11.80±0.29
Hematocrit (%)	44.68±1.68	35.44±1.73**	39.01±1.33*	44.92±1.77	35.49±1.27**	39.22±1.22*	45.36±1.98	43.33±1.38	43.53±1.33	45.48±1.77	43.56±1.28	43.66±1.28
Cloting Time (Sec.)	132±2	140±3*	133±2	135±3	141±2*	136±3	136±2	141±3*	137±2	137±4	140±2	138±2

Note: Values are mean ± SE (n=6). Significant differences are indicated by asterisks.

by intoxication of carbaryl and 2,4-D sodium salt.

Haemoglobin content

Significant reduction in haemoglobin content of the catfish resulted post exposure to acute, subacute and sublethal concentrations of carbaryl and 2,4-D sodium salt for 96h and short (10-20 days) term but long term exposure to these toxicants at the subacute and sublethal levels did not reduce haemoglobin content.

The anaemia in *C.fasciatus* exposed to lead was identified as haemolytic on the basis observed lysis of the erythrocytes with concomitant decrease in haemoglobin content and haematocrit[21]. Anaemia can be caused by a number of pathological conditions and in this case it was similar to those noticed in *Coho Salmon, Oncorhynchus Kisutch* following exposure to sublethal levels of total residual chlorine [22] and in other teleosts exposed to pulp mill and GOAW effluents [16,23]. Erythropenia was also reflected by the reduced hemoglobin content of the blood as well as by marked increase in sedimentation of erythrocytes. The decrease in haemoglobin content of fish on exposure to carbaryl and 2,4-D sodium salt implicates haemodilution and resulting increase in cell size. This is accomplished by either cellular swelling or mortality of small immature cells. Therefore, anaemic state of *H. fossilis* after metal treatment may also be attributed to inhibitat ion of erythropoesis[24] coupled with enhanced rate of erythrocyte destruction[25], distrubed Hb sysnthesis and haemodilution[26].

Haematocrit (P.C.V.)

The catfish, *H. fossilis* in the present study showed significant decreased in haematocrit (PCV%) following acute (96h) and short (10-20 days) term exposure to acute, subacute and sublethal concentrations of carbaryl and 2,4-D sodium salt but no effect at long term to subacute and sublethal concentrations. Changes in haematocrit value in fish exposed to different environmental stressors or chemicals have been reported by several workers [16,17,18, 19, 20, 27]. Aldrin indudced erythropenia was reflected by reduced haemoglobin content and haemotocrit as well as rapid sedimentation of erythrocytes. These changes have also been reported in *Channa punctatus* and *Gopy melanostomus* post exposure to some insecticides of OC group. Other worker[17,16] also reported decreased level of haematocrit in fish exposed to different concentratons of detergent and GOAW effluent. Grizzle[28] attribute the decrease in haematocrit and haemoglobin content in fish to impairment of gas exchange by the gills. The changes in haematocrit value in this study might have occurred due to a slight hypoxia, during exposure to both the toxicants.

Clotting Time

The fish exposed to acute, subacute and sublethal concentrations of carbaryl and 2,4-D sodium salt for acute (96h) and short (10-20 days) term intervals exhibited significant delay in blood coagulation. Similar response was also observed by several workers after exposure of catfish to pesticides, dyes, detergent and metals [16-19,27]. Several workers have reported an inverse relationship between thrombocyte count and blood clotting time in several fish species [29,30]. In the present study, it seems that the catfish developed thrombocytopenia which led to a concomitant increase in the clotting time of the blood. Perhaps toxicosis of these toxicants triggers a rapid mobilization of the haemostatic system and the fish normally appears to deal with it by adjusting blood clotting time and thrombocyte concentations. The fish not showing any significant changes in clotting time at long (30-60 days) term exposure to subacute and sublethal concentration of carbaryl and 2,4-D sodium salt.

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