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EFFECT OF A COMBINATION OF POLLUTANTS ON THE FISH RASBORA DANICONIUS (Ham.)

Mattu N. Madhyastha and Rajendra Nayak*
Department of Bio-Sciences
Mangalore University
Mangalore - 574 152, INDIA

*
Department of Biology
K.J. Somaiya College of Science
Bombay - 400 077, INDIA

ABSTRACT

In the present study laboratory experiments were carried out on Rasbora daniconius (Ham.) by exposing them to a mixture of sodium lauryl sulfate, an active part of the detergent and DDT a commonly used insecticide for time intervals of 7, 14, 21 and 28 days. The clinical diagnostic characters widely used in human medicine like haematological, histochemical and histological studies were employed to assess the health of the fish.

Haematological changes like decrease in the TRBC count accompanied by an increase in TWBC count was recorded throughout the 28 days of exposure. The fish exhibited anemia by a decrease in the THb content and changes in the morphology of the RBCs. Erythrocytic, leucocytic and thrombocytic responses were observed in the haematopoietic organs. Pathological changes in the morphology of the peripheral blood cells and their precursor cells were noted in the peripheral blood smears and in the imprints of the haematopoietic organs. The histochemical tests also exhibited a decrease in their intensity both in the peripheral blood cells and in their precursor cells. Degenerative changes were noted in the tissues like gills, liver and kidney. In all, the mixtures of SLS + DDT caused profound changes in the exposed animals much earlier to the time intervals taken by the individual pollutants separately.

1. INTRODUCTION

It is evident that different anthropogenic wastes ultimately find their way to the acquatic environment in varying amounts and disturb the ecological system. Their action on the communities may vary from severe lethality to minor disturbances based on their individual toxicity, biodegradability, half-life, strength and presence of other compounds and on the physico chemical quality of the ambient environment. Thus both intrinsic and extrinsic factors ultimately decide the condition of the reaction and fate of individuals in the aquatic ecological system.

The detergents due to their surface active property are believed to affect the aquatic animals living in the water bodies. Agricultural use of pesticides and domestic use of detergents have remained high in an agrarian country like India. This is of particular concern so far

as the ecological safety of smaller water bodies are concerned because the runoff from the farm land reach them. A few reports are available on the synergistic effect of the detergent in combination with the metals [13] and pesticides [8, 13, 35, 36]. Hence a study was undertaken with combination of an active compound of the detergent, sodium lauryl sulfate (SLS) and DDT, to evaluate the impact of this mixture at the sublethal level using a fish R. daniconius (Ham.) as a test animal in the laboratory. R. daniconius (Ham.) is a larvicidal fish inhabiting small waterbodies and streams of India. This fish is often exposed to a threat of the combination of surface active agent and pesticides.

Blood often shows immediate pathological changes before external signs of poisoning can be seen. As in human medicine, the assessment of the haematological parameters of the fish has been emphasized by many workers to diagnose the disease conditions of the fish [26, 18, 20, 6]. The behavioural and histological effects are often studied in routine to assess the effect of many pollutants. So the fish were exposed to a sublethal concentration of SLS + DDT mixture and the effects of this combination was studied on the behavioural, haematological and histological parameters of the fish.

2. MATERIALS AND METHODS

Laboratory acclimated fish of average size 9.2 cm and average weight 4.6 gm. were taken for the study. Healthy fish irrespective of sex were used for the experiment.

The detergent SLS was supplied by M/s Loba Chemie, Indoaustranal Co., P.B.NO. 6130, Colaba, Bombay - 5. A stock solution of SLS was prepared in distilled water. The insecticide DDT (technical grade para para isomer 72%) was supplied by M/s Hindustan Insecticides Ltd., New Delhi. A stock solution of DDT was prepared in AR grade acetone. Only one ml of the stock solutions of SLS and DDT were added to 42 liters of water to attain a experimental concentration of 0.127 mg/l of SLS and 0.00074 mg/l of DDT in each of the four experimental tanks. One ml of distilled water and one ml of AR grade acetone used for preparing the stock solutions were added to 42 liters of water in each of the four control tanks.

Since no reproducible results could be obtained by using different combinations of SLS + DDT to find out the LC50 values it was necessary to revert back to the LC50 values of individual toxicants used in our previous studies [23, 30]. The 96 hr. LC50 values of SLS and DDT were multiplied by the factor 0.02. All the standard methods as cited in [2] were taken into consideration. The hydrological parameters of the water used for the toxicity test as determined by the Standard methods [2] are given in Table 1.

Eighty fish were divided into eight groups of ten each, of which four groups served as experimental and four groups as controls for the specific time intervals of 7, 14, 21 and 28 days of exposure to a mixture of the sublethal concentration of SLS + DDT. Each group of ten fish were reared in 42 liters of water during the experiment. Ten fish were removed from each experimental and controlled group at the end of specific time

Table 1. Hydrological parameters of the water used for the toxicity studies

	Parameter	Range
1.	Temperature	21-25°C.
2.	Total EDTA hardness mg CaCO3 /litre	122-156 mg CaCO3/litre
3.	Total Alkalinity mg $CaCO_3$ /litre	86-134 mg $CaCO_3$/litre
4.	p^H	6.8-7.3
5.	Conductivity	12.5-26.5 μ mho/cm
6.	Dissolved Oxygen	6.2-8.2 ppm.

interval of time and their length and weight were determined. They were then sacrificed for assessing their haematological parameters as per the standard methods [7]. Tests were carried out for the presence of myeloperoxidase enzyme, for the presence of sudanophil and PAS positive granules [7] on the peripheral blood cells. The red blood cells (RBCs) were measured to find out their C:N and N:C ratio. In case where the blood volume was not sufficient to conduct all the haematological tests, the pooled blood sample was used.

Tissues like gill, liver and kidney were fixed in Bouin's fluid and sections cut at 8μ were stained in haematoxylin and eosin.

Head kidney tissue imprints were prepared to study the blood precursor cells. The imprints were stained in 5% Giemsa stain as adopted for the differential count of peripheral blood cells. The above histochemical tests were also conducted on the blood precursor cells.

3. OBSERVATIONS

3.1 General Behaviour

The fish exposed to the mixture were highly irritable when compared to the controls. After 7 days of exposure, the feeding rate was reduced as evidenced by the unconsumed feed lying at the bottom of the aquaria containing the exposed animals. Shedding of the scales was also observed. Opercular movements were around 88 to 102 per minute in the experimental animals whereas it ranged from 68 to 80 per minute in the controls. The fish were slippery to touch indicating the hypersecretion of the mucous.

3.2 Haematological Parameters

Many changes were noted in the blood parameters of the exposed fish as given in the Tables 2 and 3.

Table 2. Effect of exposure to sublethal conc. of SLS (0.127 mg/l) + DDT (0.00074 mg/l) mixture exposed for different time intervals on the haematological parameters of R. daniconius (Ham.).

Parameters	Control	Time Intervals - Experimental 7 days	14 days	21 days	28 days
Average length cm.	9.20	9.20	9.10	9.20	9.20
Average weight gm	4.60	4.55	4.55	4.50	4.54
TRBC count[a] x 10^6 = No. of cells/mm^3	3.29 ± 0.23[c]	3.03 ± 0.21[2*]	2.97 ± 0.17[1]	2.90 ± 0.08[1]	2.73 ± 0.19[1]
TWBC count[a] x 10^3 = No. of cells /mm^3	49.70 ± 6.91	57.50 ± 0.21[2]	58.50 ± 7.97[1]	57.13 ± 5.32[1]	56.60 ± 6.54[1]
THb gm/100 ml	10.81 ± 0.39	10.20 ± 0.27[2]	10.10 ± 0.22[2]	9.80 ± 0.45[1]	9.60 ± 0.44[1]
PCV %	50.47 ± 2.94	53.16 ± 2.37[4]	49.37 ± 2.48	44.86 ± 3.34[1]	42.81 ± 4.34[1]
MCV μ^3	153.30 ± 14.3	175.40 ±12.5[1]	165.80 ± 16.20	154.60 ±11.40	156.20 ± 12.40
MCH pg	32.81 ± 3.5	33.66 ± 2.14	33.99 ± 2.14	33.84 ± 1.45[2]	35.04 ± 2.81[2]
MCHC %	21.41 ± 1.55	19.18 ± 1.27[1]	20.47 ± 1.48[5]	21.84 ± 0.90	22.42 ± 1.28[5]
RBC Dimensions[b]					
CYTOPLASM					
Average length μ	10.80 ± 0.45	11.20 ± 0.48[2]	10.60 ± 0.55	10.30 ± 0.46[1]	10.10 ± 0.36[1]
Average length μ	5.70 ± 0.48	5.90 ± 0.40	5.50 ± 0.26	5.45 ± 0.45[2]	5.30 ± 0.24[1]
NUCLEUS					
Average length μ	4.80 ± 0.48	4.80 ± 0.55	4.20 ± 0.45[1]	4.30 ± 0.38[1]	4.20 ± 0.41[1]
Average width μ	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00	2.50 ± 0.00
N:C ration	0.194	0.181	0.180	0.196	0.191
C:N ratio	5.13	5.50	5.55	5.34	5.09

a. Calculated from haemocytometer. b. Calculated from the stained peripheral blood smears. c. ± Standard deviation. * Different levels of significance calculated from the student's t-test representing the difference between the control and the experimental groups.

1 < 0.001 2 < 0.002 3 < 0.005 4 < 0.01 5 < 0.02 6 < 0.05

Table 3. Effect of exposure to sublethal conc. of SLS (0.127 mg/l) + DDT (0.00074 mg/l) mixture for different time intervals on the peripheral blood cells of R. daniconius (Ham.).

Type of cell No./3000 cells	Control	Time Intervals - Experimental 7 days	14 days	21 days	28 days
		Differential count of 3000 blood cells[a]			
MRBCs	2686.30 ± 22.30[b]	2624.40 ± 42.79[1]	2596.10 ± 35.41[1]	2584.10 ± 11.38[1]	2554.40 ± 44.87[1*]
IRBCs	112.50 ± 21.50	141.30 ± 13.13[1]	153.00 ± 33.19[1]	156.60 ± 27.38[1]	165.90 ± 23.43[1]
Smudge Cells	104.00 ± 26.87	135.70 ± 17.87[1]	138.40 ± 12.53[1]	144.30 ± 30.91[2]	159.60 ± 12.87[1]
Thrombocytes	67.50 ± 8.01	65.40 ± 12.93	76.30 ± 10.83[5]	81.10 ± 15.57[2]	86.40 ± 13.72[1]
Small Lymphocytes	22.40 ± 2.94	23.20 ± 2.53	26.50 ± 3.87	22.40 ± 4.08	20.20 ± 4.74[1]
Large Lymphocytes	5.60 ± 1.92	6.70 ± 1.26[3]	6.20 ± 1.60[4]	4.80 ± 0.98	6.20 ± 1.61[4]
Neutrophils	0.90 ± 0.35	1.80 ± 0.92[2]	2.00 ± 0.84[1]	4.00 ± 2.07[1]	4.10 ± 1.90[1]
Monocytes	0.80 ± 0.55	1.10 ± 0.57	1.10 ± 0.72	2.00 ± 0.76[1]	1.80 ± 1.14[2]
Plasmacytes	-	0.40 ± 0.37[3]	0.40 ± 0.31[3]	0.70 ± 0.46[1]	1.40 ± 0.89[1]
No./100 cells		Differential count of leucocytes[a]			
Small Lymphocytes	87.60 ± 2.58	83.40 ± 2.76[1]	82.20 ± 4.30[1]	81.40 ± 3.59[1]	81.20 ± 2.19[1]
Large Lymphocytes	7.30 ± 1.94	9.20 ± 1.83[4]	9.20 ± 1.96[4]	10.00 ± 2.17[2]	10.10 ± 2.80[2]
Neutrophils	4.30 ± 1.25	6.50 ± 1.28[3]	5.90 ± 0.45[3]	6.00 ± 0.69[3]	6.10 ± 1.20[3]
Monocytes	0.80 ± 0.55	0.60 ± 0.65	2.30 ± 0.59[1]	2.00 ± 0.63	1.90 ± 0.66[3]
Plasmacytes	-	0.30 ± 0.38[4]	0.40 ± 0.37[1]	0.60 ± 0.39[1]	0.70 ± 0.60[1]

a Calculated from stained peripheral blood smears. b ± Standard deviation.

* Different levels of significance calculated from the student's t-test representing the difference between the control and the experimental groups.

1 < 0.001 2 < 0.002 3 < 0.005 4 < 0.01 5 < 0.02 6 < 0.05

Table 4. Effect of exposure to sublethal conc. of SLS (0.127 mg/l) + DDT (0.00074 mg/l) mixture for different time intervals on the blood precursor cells of the head kidney.

Type of cell No. / 300 cells	Time Intervals - Experimental				
	Control	7 days	14 days	21 days	28 days
MRBCs	212.00 ± 6.36[b]	186.30 ± 5.68[1]	188.80 ± 4.26[1]	182.60 ± 6.00[1]	174.20 ± 10.42[1*]
IRBCs	18.10 ± 2.25	23.20 ± 3.76[1]	23.50 ± 5.25[4]	26.00 ± 3.70[1]	24.90 ± 5.39[1]
Normoblasts	5.10 ± 1.08	6.30 ± 2.50	6.90 ± 0.94[2]	5.80 ± 0.87	7.50 ± 2.06[1]
Large Lymphoid Hemoblasts	4.10 ± 0.59	5.80 ± 0.74[1]	4.90 ± 0.85[6]	5.60 ± 0.81[1]	6.80 ± 2.44[1]
Small Lymphoid Hemoblasts	3.50 ± 0.63	4.20 ± 0.60[1]	4.20 ± 0.96	4.80 ± 0.63[1]	4.40 ± 0.93[1]
Myeloblasts	2.60 ± 0.99	2.50 ± 0.47	2.80 ± 0.74	3.40 ± 0.85[1]	4.20 ± 0.87[1]
Myelocytes	2.40 ± 0.76	3.80 ± 0.82[1]	2.60 ± 0.42	3.30 ± 1.00[4]	3.60 ± 0.75[1]
Neutrophilic Meylocytes	3.50 ± 0.77	4.80 ± 1.60[2]	4.60 ± 0.80[1]	5.10 ± 1.10[1]	4.80 ± 0.64[3]
Neutrophils	4.20 ± 0.68	6.40 ± 0.60[1]	5.90 ± 0.80[1]	6.70 ± 0.90[1]	6.50 ± 2.32[1]
Small Lymphocytes	16.00 ± 2.53	20.10 ± 3.81[1]	20.90 ± 3.50[1]	20.00 ± 4.23[1]	20.10 ± 2.85[1]
Large Lymphocytes	4.40 ± 1.80	4.50 ± 1.62	6.20 ± 1.20[1]	4.40 ± 1.00	6.40 ± 1.45[1]
Promonocytes	2.50 ± 0.68	2.90 ± 0.67	2.40 ± 0.45	2.60 ± 0.49	2.90 ± 0.57
Monocytes	2.50 ± 0.66	2.90 ± 0.70	2.20 ± 0.52	3.00 ± 0.82	2.80 ± 0.67
Reticuloplasmacytes	1.20 ± 0.25	3.00 ± 0.90[1]	1.60 ± 0.57[5]	2.70 ± 0.83[1]	3.10 ± 0.72[1]
Plasmacytes	0.90 ± 0.48	1.90 ± 0.42[1]	1.40 ± 0.48[2]	1.30 ± 0.40[6]	2.40 ± 0.81[1]
Thrombocytes	17.00 ± 3.70	20.40 ± 2.41[1]	20.20 ± 3.73[1]	21.70 ± 4.42[1]	23.00 ± 3.25[1]
Megaloblasts	----	1.00 ± 0.51[1]	0.90 ± 0.35[1]	1.00 ± 0.54[1]	2.40 ± 1.12[1]

a Calculated from stained peripheral blood smears. b ± Standard deviation.

* Different levels of significance calculated from the student's t-test representing the difference between the control and the experimental groups.

1 < 0.001 2 < 0.002 3 < 0.005 4 < 0.01 5 < 0.02 6 < 0.05

From Table 2 it is evident that total red blood cell count (TRBC) and Total haemoglobin (THb) showed significant decrease starting from the seventh day of exposure onwards; whereas the total white blood cell count (TWBC) increased throughout the 28 days of exposure period. The packed cell volume (PCV) and mean cellular volume (MCV) values exhibited an increase after 7 days of exposure but PCV value decreased significantly after 21 and 28 days of exposure. MCV did not change after 7 days of exposure. The increase in the PCV and MCV values at the end of 7 days of exposure is accompanied by an increase in the average length of the RBCs. The significant decrease in the PCV values at the end of 21 and 28 days were concomittant with a decrease in the average length and width of the RBCs. Mean corpuscular haemoglobin content value (MCHC) decreased after 7 and 14 days of exposure but recorded an increase after 28 days, whereas the mean corpuscular haemoglobin value (MCH) exhibited a significant increase after 21 and 28 days of exposure.

Table 3 depicts that in the differential count of 3000 peripheral blood cells, a significant decrease was exhibited by mature red blood cells (MRBCs), whereas the blood cells like immature red blood cells (IRBCs) smudge cells, neutrophils and plasmacytes exhibited an increase throughout the 28 days of exposure. However, the thrombocytes increased in their number after 14 to 28 days of exposure to the SLS + DDT mixture. The monocytes increased significantly after 21 days of exposure which may be due to large scale destruction of the blood cells. There was comparative elevation of large lymphocytes in the experimental animals after 28 days of exposure period whereas, the small lymphocytes exhibited a decrease. The large lymphocytes also exhibited a similar trend during 7 and 14 days of exposure to the above mixture.

In the differential count of the leucocytes, the number of leucocytes exhibited similar trend especially after 14 and 28 days of exposure.

Throughout the 28 days of exposure period, many interesting changes were noted in the morphology of different peripheral blood cells. After 7 days of exposure, RBC with double nuclei and with eccentric nuclei were very common. Some of the RBCs were hypochromic and had clumped chromatin material. A few IRBCs had ragged appearance and a few were anucleate. Orthochromatic normoblasts were many in number; whereas they were rarely seen in the controls.

In the 14 days of exposure period, the poikilocytes were very common and a few anucleate granular bodies were also observed. RBCs with dumbbell shaped nuclei were also noted. Some neutrophils had crescent shaped nucleus and a few had 2 nuclei connected by a thin thread of chromatin material.

21 days of exposure caused the following type of pathological changes in the peripheral blood cells compared to the controls. Many RBCs and large lymphocytes exhibited the presence of vacuoles. Some RBCs attained tear drop shape and a few thrombocytes had 2 nuclei with a chromatin connection in between. Many blood cells had nuclear bits in them. Unidentified blast cells were many in number.

Many degenerative changes were observed in the peripheral blood cells after 28 days of exposure to SLS + DDT mixture. A few neutrophils exhibited a reduction in their size and had pinkish red patches in their cytoplasm. Neutrophils with punctate nuclei were commonly seen. Monocytes had vacuoles both in the cytoplasm and the nucleus. Some large lymphocytes had lobed nuclei and intense basophilic cytoplasm.

From the Table 4, it is evident that in the differential count of 300 cells of the head kidney imprints, a general trend was observed throughout the 28 days of exposure. The MRBCs number decreased whereas the IRBCs, normoblasts, large lymphoid and small lymphoid haemoblasts, myeloblasts, myelocytes, neutrophilic myelocytes, neutrophils, small and large lymphocytes, promonocytes, monocytes, reticuloplasmacytes, plasmacytes, thrombocytes and megaloblasts increased in their number especially after 28 days of exposure to SLS + DDT mixture.

The myeloperoxidase enzyme, PAS positive granules and sudanophil granules exhibited a decrease both in the respective cells of the peripheral blood and those of the head kidney imprints in all the exposed animals throughout the experimental duration when compared to the controls.

In the differential count of 300 cells of the head kidney imprints, a general trend was observed throughout the 28 days of exposure. The erythropoietic response was exhibited by an increase in the number of IRBCs and normoblasts. The IRBCs increased in their number throughout the 28 days of exposure period, whereas the normablasts exhibited an increase only after 14 days and 28 days but not for 7 and 21 days of exposure periods. The neutrophilic response was also observed by an increase in the number of neutrophilic myelocytes and neutrophils throughout the 28 days of exposure period. The increase in the number of reticuloplasmacytes and plasmacytes may be due to an allergic response.

3.3 Histological Changes

Many histological changes were evident in the animals treated with SLS + DDT mixture.

The gill filaments started bending and exhibited degenerative changes like sloughing off of the epithelial layer, presence of cell mass and mucous at many places.

In the liver, the hepatocytes became distinct by getting separated from the neighbouring cells. The chromatin material exhibited pyknosis. The cytoplasm exhibited granules. The nucleus was pyknotic in some of the cells and was eccentric. The sinusoidal spaces were infiltrated by RBCs.

Many degenerative changes were noted in the epithelial cells of the kidney tubules. Many of the cells had pyknotic nuclei. Destruction of the epithelial cells lining the tubules and the glomerulus was evident from the open spaces present in the tissues. After 28 days of exposure, incursion of the intertubular spaces by the RBCs in very large numbers was also observed.

4. DISCUSSION

From the haematological and histological observations on R. daniconius (Ham.) exposed to SLS + DDT mixture at the sublethal level, it can be concluded that the fish exhibited a combined effect of SLS and DDT. The effect of DDT is more pronounced than that of SLS.

The decrease in the feeding rate as evidenced from the presence of unconsumed feed at the bottom of the aquaria has also been observed by

[9] in zebra fish danios Brachydanio rerio after exposure to sublethal concentration of zinc, pottassium dichromate and ABS mixture. The decrease in the feeding rate of the fish exposed to SLS + DDT mixture may be due to the effect on taste receptors of the fish. This view has been put forward by [17] who observed a decrease in the feeding rate of Jordanella floridae exposed to ABS. They suggested that lack of sensory information from the taste receptors prevented the fish from recognising the food as such and hence they spat out the food even after they ingested it. Hypersensitivity of the SLS + DDT mixture exposed fish to mechanical disturbance has been already noted in the fish exposed to sublethal concentration of DDT only [30]. Similar response has also been noted in Salvelinus fontinalis exposed to DDT [3]. The slippery body surface has been noted in fish exposed to SLS [23] and the commercial detergent (point) [31], whereas the shedding of the scales has been observed in fish exposed to DDT only [30].

The decrease in the TRBC count in fish exposed to SLS + DDT mixture were also observed in the fish exposed sublethal concentration of commercial detergent (Point) [31], DDT (unpublished) and SLS [23] after 14 days in the former two cases and after 21 days in the latter case. Decrease in the number of RBCs has been reported in stress conditions [28] and also during exposure to various pollutants [22, 4, 21, 11]. To compensate this decrease the erythropoietic response in the head kidney was evident as revealed by an increase in the number of the precursor cells of the RBCs.

The MCHC value decreased after 7 and 14 days of exposure. A decrease in MCHC value was observed in the blood of carp exposed to organophosphorous insecticides [37]. However it exhibited an increase after 28 days of exposure which is in conformity with the observations recorded for sublethal exposure of R. daniconius (Ham.) to DDT (unpublished). This might be due to a decrease in the dimensions of the RBCs.

The significant decrease in the dimensions of the RBCs after 28 days of exposure might be due to stress. A decrease in the RBC size has been reported in goldfish [28] and Ictalurus punctatus [27] maintained at different loading denisities which caused fish density syndrome for RBC morphology.

The presence of double nucleated RBCs, RBCs with fragmented nuclei and anucleate erythrocytes observed in this case have been observed in folic acid anemia in fish [38] and in Heteropneustes fossilis exposed to pollutants [29]. The mixture of SLS + DDT might have either caused an impairment in the uptake of vitamins due to reduced feeding or might have caused abnormalities during the haemopoiesis.

The thrombocytic response observed after 14 days may be due to stress. Stress has been found to cause an increase in the number of thrombocytes [10] in rainbow trout.

In performing the histochemical tests on the peripheral blood cells the decrease in the intensity of the reactions encountered may be due to the presence of immature cells in the circulation. The present finding is in agreement with the observations of [6] who have opined that immature cells exhibit lesser intensity to the different histochemical tests. This view is strengthened by an increase in the number of precursor cells of the myelocytic series in the head kidney.

The histological changes in the gill filaments of R. daniconius exposed to SLS + DDT mixture also conform with the reports in fish exposed to detergents [32, 1], to detergent + zinc mixture [8] and other pollutants [14, 34, 12].

Apart from the histopathological degradation of the hepatocytes and epithelial cells of the liver and kidney respectively which were also observed by many authors in fish exposed to different pollutants [24, 25, 14, 19, 15, 16], it is interesting to note that incursion of the RBCs in the sinusoidal spaces of the liver and the intertubular spaces of the kidney in large numbers is an observation recorded only in the case of fish exposed to SLS + DDT mixture and not in the individual exposures to SLS and DDT separately.

Thus from the present experiment it is evident that the SLS + DDT mixture at sublethal level concentration is more toxic to R. daniconius than the effects that were observed in the fish exposed to SLS and DDT separately in the laboratory findings.

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