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Haematological effects of sublethal concentration of formalin on *Sarotherodon mossambicus* (Peters)

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Abstract. Exposure of fish *Sarotherodon mossambicus* (Peters) for 24 h to 80 ppm formalin induced lowering of total erythrocyte count and increase in haemoglobin and haematocrit. Erythrocyte constants and indices of exposed fish were higher than of the control fishes. The sum total of the effect of sublethal concentration of formalin on the peripheral blood of *Sarotherodon mossambicus* was macrocytosis and hyperchromia.

Keywords. Formalin toxicity; haematology; *Sarotherodon mossambicus*.

1. Introduction

Despite the fact that the toxic effects of several chemicals employed in fish disease therapy have been analysed by various workers, there seems to be little attention given to formaldehyde—an effective chemotherapeutic agent successfully employed against several infectious conditions, particularly those affecting the body surface/skin of fishes (Schnick 1974; Roberts and Shepherd 1979). The only publications that have given attention to formaldehyde poisoning of fishes are those by Wedemeyer (1971), Smith and Piper (1972) and Williams and Wootten (1981). In this paper the results of the study on the effects of sublethal concentration of formalin on the peripheral blood of *Sarotherodon mossambicus* (Peters) are presented.

2. Materials and methods

Specimens of *S. mossambicus* collected from a local freshwater pond, were transported alive to the laboratory in large polythene buckets. Fishes were acclimatized for one day to laboratory conditions by keeping them in large aquarium tanks containing filtered well-water.

Apparently healthy fishes ranging from 9·5–11 cm in standard length were selected for the present study. Ten fishes were maintained as control and 10 others were used for experimental purpose.

For TLm determination and for experimental studies, glass troughs of size 30 cm diameter, 15 cm height and a capacity of 6 L were used. Filtered well-water (mean temp. 27 ± 1°C; pH 7·9) was used throughout the experiment. TLm concentration (24 h) was determined by adopting the procedure suggested by Doudoroff *et al* (1951) for sewage and industrial wastes.

Haematological analyses were carried out employing standard techniques (Hesser 1960; Blaxhall and Daisley 1973). Blood samples were collected by severing the caudal peduncle. A 3:2 mixture of ammonium oxalate and potassium oxalate at the rate of 0·5–1 mg/ml blood was used as anticoagulant.

Total erythrocyte count (TEC), haemoglobin content (Hb) and haematocrit (Ht) were determined and from these results, the erythrocyte constants [mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)] and the erythrocyte indices [volume index (VI), colour index (CI) and saturation index (SI)] were computed. The results were statistically tested by the Student's '*t*' test.

3. Results

In tables 1 and 2 the results of haematological analyses of the control and experimental fish respectively are presented. Table 3 shows the results of Student's '*t*' test comparing the haematological parameters of control and experimental fish.

3.1 *TEC* ($\times 10^6$/mm^3)

TEC of the control fishes ranged from 2·22–2·56 and of the experimental from 1·75–2·65. The mean counts for the two groups were 2·35 and 2·14 respectively. The results indicate that exposure to formalin reduced the erythrocyte count of the fish. From the results of the '*t*' test it may be seen that the observed difference in TEC between the control and experimental fish is statistically significant ($P < 0{\cdot}05$).

3.2 *Hb* (g%)

For the control fish Hb content recorded a mean value of 11·19 with a range from 9·2–11·8. For the experimental fish it recorded a significantly higher mean value of 15·08 ($P < 0{\cdot}01$); the range of Hb content here was from 13·8–16·2. The result shows that exposure to formalin had an advantageous effect over Hb content of the fish.

3.3 *Ht* (%)

In the control fish Ht ranged from 24·27–35·37 with a mean of 28·63. In the

Table 1. Haematological parameters of 10 control specimens of *S. mossambicus*.

TEC ($\times 10^6$/mm^3)	Hb (g%)	Ht (%)	MCV (μ^3)	MCH (pg)	MCHC (%)
2·56	11·4	28·73	112·23	44·53	39·68
2·45	11·0	30·00	122·45	44·90	36·67
2·27	11·8	26·67	117·49	51·98	44·24
2·49	11·8	28·96	116·30	47·79	41·09
2·39	11·8	24·27	101·55	49·37	48·62
2·26	10·6	32·58	144·16	46·90	32·54
2·23	9·2	24·73	110·90	41·26	37·20
2·30	11·0	35·37	153·78	47·83	31·10
2·28	11·4	25·72	112·81	50·00	44·32
2·22	11·8	29·28	131·89	53·15	40·30
Mean ± SE.					
2·35 ± 0·04	11·18 ± 0·25	28·63 ± 1·05	122·36 ± 4·87	47·77 ± 1·08	39·58 ± 1·63

Table 2. Haematological parameters of 10 fish (*S. mossambicus*) exposed to sublethal concentration of formalin.

TEC ($\times 10^6$/mm^3)	Hb (g%)	Ht (%)	MCV (μ^3)	MCH (pg)	MCHC (%)	VI	CI	SI
2·65	15·2	31·0	116·98	57·36	49·03	0·96	1·21	1·24
2·44	14·8	29·0	118·85	60·66	51·03	0·97	1·27	1·29
2·34	14·8	31·0	133·48	63·25	47·74	1·08	1·33	1·21
1·83	13·8	28·0	153·01	75·41	43·28	1·25	1·58	1·10
1·80	14·0	26·0	144·44	77·78	53·85	1·18	1·63	1·36
1·75	16·0	29·0	165·71	91·43	55·17	1·35	1·92	1·39
1·90	16·2	34·0	178·95	85·26	47·65	1·46	1·79	1·20
2·20	15·8	32·0	145·45	71·82	49·38	1·19	1·51	1·25
2·12	15·4	28·0	132·08	72·64	55·00	1·08	1·53	1·39
2·34	14·8	32·0	136·75	63·24	46·25	1·12	1·33	1·17
SEM ± SE								
2·14 ± 0·09	15·08 ± 0·24	30·0 ± 0·72	142·57 ± 5·85	71·89 ± 3·31	49·84 ± 1·18	1·16 ± 0·10	1·51 ± 0·07	1·26 ± 0·03

Table 3. '*t*' values comparing haematological parameters of control and formalin exposed *S. mossambicus*.

Haematological parameters	'*t*' values
TEC	2·0087[a]
Hb	10·7137[b]
Ht	1·0205
MCV	2·2667[a]
MCH	6·5754[b]
MCHC	4·8503[b]
VI	2·7735[a]
CI	8·8230[b]
SI	8·4602[b]

[a] $P < 0·05$; [b] $P < 0·01$.

experimental fish mean Ht was slightly higher (30·0) than in the control. However, the difference in Ht between control and experimental fish was not statistically significant.

3.4 *Erythrocyte constants*

MCV of the experimental fish was 142·57 μ^3 compared to 122·36 μ^3 for the control fish. The difference in MCV between the control and experimental fish was statistically significant ($P < 0·05$).

MCH was also significantly altered by exposure to formalin. In the control fish MCH recorded an average of 47·77 pg whereas in the experimental fish it was much higher, with an average of 71·89 pg. The observed difference in MCH between the control and experimental fishes was significant at 1% level.

MCHC recorded very high value in formalin exposed fishes; the mean MCHC for this group was 49·84% in comparison with 39·58% for the control group. Here the difference between the control and experimental groups was statistically highly significant ($P < 0·01$).

3.5 *Erythrocyte indices*

In conformity with the increased MCV, VI of the experimental fish recorded an average value of 1·16. This clearly indicates that exposure to formalin induced swelling of the erythrocytes of the fish.

An increase in Hb content and MCH has been induced by formalin, consequently the CI of the experimental fish also recorded a considerably high mean value of 1·51 and this, along with the high SI (1·26), indicate that exposure to formalin induced hyperchromia.

4. Discussion

Wedemeyer (1971) observed pituitary activation in rainbow trout treated with 200 ppm formalin. Smith and Piper (1972) reported an increase in Ht and TEC in formalin treated rainbow trout. Williams and Wootten (1981) found that immediately after exposure to 200 ppm of formalin, Ht and Hb of rainbow trout significantly increased.

Comparing the results of the present study on *S. mossambicus* with the foregoing, a much lower concentration of formalin (85 ppm) itself was found to be lethal to the fishes. As for the effects, the haematological parameters of exposed fish were characterised by a decrease in TEC and an increase in Hb and Ht. Smith and Piper (1972) and Williams and Wootten (1981) also recorded increase in Hb and Ht in rainbow trout exposed to formalin. However, unlike the results obtained by the former workers for TEC, during the present study TEC was significantly lowered by formalin treatment. It may be noted that the mean corpuscular volume of formalin treated *S. mossambicus* was much higher than that of the control fish. This naturally would result in the reduction of the number of erythrocytes in a unit volume of blood. From the results, it would seem that it is the excessive increase in erythrocyte volume that apparently produced a picture of reduced TEC. It is, therefore, reasonable to conclude that the direct effect of formalin has been on the erythrocyte volume, not on its numerical abundance.

Williams and Wootten (1981) found that, even though immediately after exposure to formalin Hb and Ht of rainbow trout increased significantly, after 24 h exposure both parameters tended to return to normalcy. The present results on *S. mossambicus* are in disagreement with those of Williams and Wootten (1981) as the significant increase in Hb and Ht persisted even after 24 h exposure to formalin.

Increased Hb may be looked upon as a sort of compensatory reaction. Wedemeyer (1971) explained the increased pituitary activity in formalin treated rainbow trout on the basis of the chemical adversely affecting gill function. Such an interference with gill function can be expected to reduce its respiratory efficiency. It may be to compensate for this impaired respiratory efficiency that the Hb was increased in the treated fish. This sort of compensatory reaction is known to occur in fishes infected by certain parasites (Kabata 1970).

Exposure to formalin was found to result in an increase, though not statistically significant, in the Ht of *S. mossambicus*. Since the TEC of the experimental fish recorded a lower value than that of the control fish, presumably the increased Ht is the result of the increased MCV. Smith and Piper (1972) and Williams and Wootten (1981) also found an increase in the Ht of rainbow trout following exposure to

formalin. In the former study, since TEC also was higher, increase in Ht might have been due to increased number of blood cells; but in the latter study the cause of increase in Ht is not deducible.

Direct comparison of the results on erythrocyte constants and indices obtained during the present study with other like observations is not possible owing to lack of similar data from elsewhere. The results indicate that the erythrocytes of formalin exposed fish became hyperchromic (high CI) and macrocytic (high VI). The observed high CI is obviously not because of supersaturation of erythrocytes with Hb but because of the increased cell volume; the larger cells have accumulated more Hb (compared with the normal erythrocytes) resulting both in increased Hb and MCH. The overall toxic effect of sublethal concentration of formalin on *S. mossambicus* as indicated by the present study may be summarised as macrocytosis and hyperchromia.

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References

Blaxhall P C and Daisley K W 1973 Routine haematological methods for use with fish blood; *J. Fish Biol.* **5** 771–781

Doudoroff P, Anderson B G, Burdick G E, Galtsoff P S, Hart W B, Patrick R, Strong E R, Surber E W and van Hora W M 1951 Bio-assay methods for the evaluation of acute toxicity of industrial wastes on fish; *Sewage Ind. Wastes* **23** 1380–1397

Hesser E F 1960 Methods for routine fish haematology; *Prog. Fish-Cult.* **22** 164–170

Kabata Z 1970 *Diseases of fishes. Book I. Crustacea as enemies of fishes* (New Jersey: TFH Publ.)

Roberts R J and Shepherd C J 1979 *Handbook of trout and salmon diseases* (West Byfleet: Fishing News (Books) Ltd.)

Schnick R A 1974 Formalin as a therapeutant in fish culture; *US Fish Wildl. Serv. Lit. Rev.* 74–09 NTIS No. PB 235 448/AB p 145

Smith C E and Piper R G 1972 Pathological effects of formalin treated rainbow trout (*Salmo gairdneri*); *J. Fish Res. Board Can.* **29** 328–329

Wedemeyer G A 1971 The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*); *J. Fish Res. Board Can.* **28** 1899–1904

Williams H A and Wootten R 1981 Some effects of therapeutic levels of formalin and copper sulphate on blood parameters in rainbow trout; *Aquaculture* **24** 341–353