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Impact of Pesticide Endosulfan on Haematological Parameters of Etroplus Suratensis

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Abstract:- Pesticides are used to control pests of food crops, due to their enormous usage, water bodies are continuously polluted.Endosulfan is an organochlorine pesticide, known to be highly toxic to aquatic life, causing behavioural to biochemical pathology in fish. Blood is a pathophysiological reflector of whole body and therefore, blood parameters are an asset in diagnosing the structural and functional status of body organs exposed to toxicants. The aim of this study was to investigate the effect of sublethal concentration of endosulfan on haematological parameters of *Etroplus suratensis.* Exposure resulted in microcytic-hypochromic anaemia in the fish. The extent of microcytosis was significant and progressive up to 96 h post-exposure and it persisted to a lesser extent during further exposure. Mean corpuscular haemoglobin was significantly and progressively lowered with period of exposure from 24 h post-exposure onward causing hypochromia. At 24 h post- exposure, even though microcytosis was significant, MCH, and therefore, CI was unaffected suggesting the occurrence of microcytic normochromic anemia at the onset of exposure. The changes during exposure were fast recouped once the fish was relieved of the presence of the toxicant.

Keywords:- *Toxints, Pesticides, Haematology, Fish.*

I. INTRODUCTION

Aquatic poikilotherms such as fish whose physiology is very sensitive even to minor changes in their aquatic milieu. A major part of the world's food is being supplied from fish source, so it is essential to secure the health of fishes [31]. Blood being the only tissue that has intimate contact with all organs and tissues of an animal, changes in the quality of the environment would reliably be indicated by changes in the blood [25]. Blood is a pathophysiological reflector of whole body and therefore, blood parameters are an asset in diagnosing the structural and functional status of body organs exposed to toxicants [42]. A wide array of factors affects the peripheral hematological make-up of fishes. Significant decrease in Hb level after exposure to a sub lethal dose of toxicants may impair oxygen supply to various tissues thus resulting in slow metabolic rate and low energy production [12]. Decrease in Hb content may be due to either its increased rate of destruction or decrease in its rate of synthesis [32]. Evaluation of the haematological make-up could provide insight into the nature and extent of physiological damages caused by various toxicants and contaminants. Haematological tests provide important information on the erythropoietic condition.Erythrocyte and leucocyte counts, haemoglobin content and packed cell volume are sensitive parameters suitable for measuring toxicity in fish [39]. Pesticides cause erythropenia and lowering of haemopoiesis, causing anemia in fish [30]. Hypochromasia in erythrocytes and shrinkage of cell membrane have been observed in fish exposed to pesticides [31]. Anaemia, polycythaemia or erythrocyte swelling may occur in certain situations. RBC count and haemoglobin content decrease due to toxicants that lead to (macrocytic) anemia in fish [19]. Decrease in RBC count and haemoglobin concentration occurs due to haemolysis, which is because of alterations in the selective permeability of the cell membrane [8]. Haemoglobin content of fish is known to be a useful index of health. Pesticides cause a catalyzing action on the incorporation of stored body iron into haemoglobin thereby increasing the haemoglobin concentration. Haemoconcentration resulting from water loss under the stress of pesticides is a major and perhaps the most important factor, which explains the per unit volume increase in haemoglobin at the initial phase of stress [36]. Decrease in Hb content and RBC count may result in hypochromic, microcytic anemia in fish.

Pesticides are used to control pests of food crops, livestock and human health. Due to their enormous usage, water bodies are continuously polluted. Pesticides are known to primarily affect the erythropoietic tissues causing failure in red blood cell production [44]. Organochlorines are generally cumulative poisons [28]. Once inside an animal body they are mobilized into the blood and transported to the brain, where they may cause serious sublethal to lethal effects [2]. Endosulfan is an organochlorine pesticide, known to be highly toxic to aquatic life, causing behavioural to biochemical pathology in fish [6]. The aim of this study was to investigate the effect of sublethal concentration of endosulfan on haematological parameters of *Etroplus suratensis.* It is an economically important food fish, much relished by the local people. It has a high content of proteins and essential amino acids, especially lysine, methionine and isolucine as per the FAO/WHO (1973) recommended pattern of essential aminoacidrequirements. 100 g protein from this fish can provide a balanced protein diet for human adults [27].

II. MATERIALS AND METHODS

The cichlid fish, *Etroplus suratensis* of 50-80 mm size were collected from Vembanadu Lake in Kerala, India. The fish were acclimatized to lab conditions for four days. An organochlorine pesticide Endosulfan 35% EC was used for the study. Preliminary tests were conducted to determine the range of concentration of the toxicant to be used for a definitive toxicity test. This test was conducted by using

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standard methods [46]. The sub lethalconcentration of endosulfan used was 0.7 ppb. The control and experimental fishes were maintained for 30 days, to evaluate the long term effect of endosulfan. The medium was changed once in two days and no mortality of fishes was recorded during the period of investigation. Blood was collected by severing the caudal ends of fishes and the different blood parameters like total count of erythrocytes (TEC), hemoglobin content (Hb), haematocrit (Hct) and erythrocyte sedimentation rate (ESR) were determined. From the values of TEC, Hb and Hct, the erythrocyte constent, 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 calculated. Student t-test was applied to find out whether there is any significance difference between the control and treated groups for the various hematological parameters studied.

III. RESULT

A. Total Erythrocyte Count (TEC= x106 mm-3)

The mean TEC of control fish was 2.36. Exposure to endosulfan caused a progressive increase in TEC up to 96 h when it recorded 3.02. Further exposure resulted in the decrease of TEC to 2.62 at 10 days post-exposure and to 2.37 at 30 days post-exposure. At 30 days post-exposure the TEC was almost similar to that of the control fish. During recovery, TEC was higher than of the control fish. It progressively increased from 2.53 at 24 h recovery to 2.75 at 72 h recovery. At 96 h recovery however, TEC registered a decrease to 2.24, which was lower than that of the control (Table 1). It is noticeable that all through the exposure and recovery, except 96 h recovery, TEC registered higher values than of the control.

B. Haemoglobin Content (Hb= g%)

The mean Hb of control fish was 11.0. The variation in Hb during exposure followed a pattern similar to that of TEC: progressive increase up to 96 h post-exposure and thereafter a decrease at 30 days (9.9) through 10 days (10.8) post-exposure. At 30 days post-exposure, however, the Hb content was lower (9.9) than that of the control fish. The Hb content during recovery was lower than that of the control fish but higher than that at 30 days post exposure(Table 1).

C. Haematocrit (Hct= %)

The mean Ht of control fish was 29.4. Exposure to endosulfan initially caused a decrease in Ht to 28.4 at 24 h and then an increase to 32.2 at 96 h, through 30.3 at 72 h. At 10 days post-exposure it decreased to 30.2 and further to 28.3 at 30 days post-exposure. During recovery Ht was higher than of the control fish. It increased from 30.8 at 24 h recovery to 32.3 at 72 h recovery. But at 96 h recovery Ht decreased to 30.0, which was slightly higher than of the control fish (Table 1).

D. Erythrocyte Sedimentation Rate (ESR=mm h-1)

The mean ESR of control fish was 2.9. Exposure to endosulfan resulted in a progressive decrease in ESR to 1.1 at 96 h. Thereafter it increased to 1.9 at 10 days post-exposure and to 2.4 at 30 days post-exposure. During recovery at 24 h (1.9) and 72 h (1.9) ESR was lower than of the control fish. At 96 h recovery ESR recorded 2.4. In fish exposed to endosulfan ESR was significantly different from that of control fish throughout exposure (Table 1).

E. Mean Corpuscular Volume (MCV=µm3)

The mean MCV of control fish was 125.2. Exposure to endosulfan caused a progressive decrease in MCV to 107.0 at 96 h. Thereafter, it increased to 116.6 at 10 days post-exposure and to 120.9 at 30 days post-exposure.During recovery, MCV increased to 123.3 at 24 h recovery to 134.2at 96h recovery (Table 1).

F. Mean Corpuscular Haemoglobin (MCH = pg)

The MCH of control fish was 46.8. It progressively decreased to 40.4 at 96 h post-exposure. Thereafter, it got elevated to 42.0 at 30 days post-exposure through 41.5 at 10 days post-exposure. All through the exposure MCH was well below that of control fish.Up to 72 h recovery MCH dropped from 42.0 at 30 days post-exposure, to reach 39.3. But at 96 h recovery it increased to 46.7, which was equal to that of the control fish (Table 1).

G. Mean Corpuscular Haemoglobin Concentration (MCHC= %)

The mean MCHC of control fish was 37.3. After an initial increase to 40.1 at 24 h post-exposure, MCHC progressively decreased to reach 35.2 at 30 days post-exposure. The decrease in MCHC continued during recovery and it reached its lowest at 72 h recovery (33.4)(Table 1).

H. Volume Index (VI)

Volume index of exposed fish was well below unity at 24 h, 72 h and 96 h post-exposure (0.89, 0.88, 0.87, respectively). At 10 days post-exposure VI improved to 0.95 and again to 0.98 at 30 days post-exposure. At 24 h recovery VI registered unity. It dropped slightly to 0.96 at 72 h recovery but got elevated to 1.1 at 96 h recovery (Table 1).

I. Colour Index (CI)

Colour index also registered a pattern similar to that of VI during exposure. All through exposure CI was considerably lower than unity, the lowest of 0.89 being recorded at 96 h post-exposure. During recovery also CI was well below unity. But it got elevated to 0.93 at 96 h recovery (Table 1).

J. Saturation Index (SI)

At 24 h post-exposure, SI registered an increase (1.06), which dropped progressively during further exposure and reached the lowest at 30 days post-exposure (0.92). As with CI, SI also was well below unity during recovery and as in the former case it also slightly improved at 96 h recovery (0.92) (Table 1).

The sum total of the haematological changes induced by endosulfan suggests that exposure resulted in microcytic-hypochromic anaemia in the fish. The extent of microcytosis was significant and progressive up to 96 h post-exposure and it persisted to a lesser extent during further exposure. Mean corpuscular haemoglobin was significantly and progressively lowered with period of exposure from 24 h

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post-exposure onward causing hypochromia. At 24 h post-exposure, even though microcytosis was significant, MCH, and therefore, CI was unaffected suggesting the occurrence of microcytic normochromic anaemia at the onset of exposure. The changes during exposure were fast recouped once the fish was relieved of the presence of the toxicant.

Table:1. Haematology *of E. suratensis*: Peripheral haematological parameters of control, fish exposed to endosulfan and of recovering fish (Values are mean of 10 observations ± S.E.)

Haematological Parameters											
Group		TEC	Hb	Ht	ESR	MCV	MCH	MCHC	VI	CI	SI
Control		2.36 ± 0.15 [a]	11.00 ±0.12 [a]	29.40 ±0.18 [a]	2.9 ±0.11 [a]	125.2 ±2.17 [a]	46.8 ±1.18 [a]	37.3 ±0.47 [a]	1.00 ±0.02 [a]	1.00 ±0.02 [a]	1.00 ±0.01 [a]
Exposure	24 h	2.60 ±0.06 [b]	11.40 ±0.08 [c]	28.43 ±0.28 [b]	2.1 ±0.05 [b]	109.8 ±2.17 [b]	44.1 ±1.18 [a]	40.1 ±0.58 [a]	0.89 ±0.02 [b]	0.97 ±0.03 [a]	1.06 ±0.02 [a]
	72 h	2.80 ±0.03 [b]	11.90 ±0.13 [d]	30.29 ±0.19 [a]	1.8 ±0.08 [c]	108.5 ±1.26 [b]	42.5 ±0.55 [b]	39.2 ±0.57 [a]	0.88 ±0.01 [b]	0.94 ±0.01 [b]	1.05 ±0.02 [a]
	96 h	3.02 ±0.06 [c]	12.20 ±0.13 [b]	32.24 ±0.24 [c]	1.1 ±0.07 [d]	107.0 ±1.66 [b]	40.4 ±0.61 [b]	37.8 ±0.35 [a]	0.87 ±0.01 [b]	0.89 ±0.01 [b]	1.00 ±0.01 [a]
	10 d	2.62 ±0.10 [b]	10.80 ±0.20 [a]	30.10 ±0.44 [a]	1.9 ±0.08 [b]	116.6 ±5.38 [a]	41.5 ±1.14 [b]	36.0 ±1.18 [a]	0.95 ±0.04 [b]	0.91 ±0.03 [b]	0.95 ±0.03 [a]
	30 d	2.37 ±0.08 [a]	9.90 ±0.21 [e]	28.30 ±0.51 [a]	2.4 0.11 [e]	120.9 ±5.40 [a]	42.0 ±1.13 [b]	35.2 ±1.35 [a]	0.98 ±0.04 [b]	0.92 ±0.03 [b]	0.92 ±0.03 [b]
Recovery	24 h	2.53 ±0.08 [a]	10.40 ±0.17 [b]	30.82 ±0.48 [a]	1.9 ±0.08 [b]	123.3 ±5.43 [a]	41.1 ±0.98 [b]	33.7 ±0.91 [b]	1.00 ±0.04 [a]	0.90 ±0.02 [a]	0.89 ±0.02 [b]
	72 h	2.75 ±0.05 [a]	10.80 ±0.19 [a]	32.33 ±0.58 [b]	1.9 ±0.15 [a]	106.2 ±12.30 [a]	39.3 ±1.11 [a]	33.4 ±0.90 [a]	0.96 ±0.03 [a]	0.87 ±0.03 [a]	0.88 ±0.02 [a]
	96 h	2.24 ±0.05 [a]	10.50 ±0.10 [a]	29.97 ±0.37 [a]	2.4 ±0.13 [b]	134.2 ±3.27 [a]	36.7 ±0.78 [a]	34.9 ±0.68 [a]	1.10 ±0.03 [a]	0.93 ±0.10 [a]	0.92 ±0.02 [a]

IV. DISCUSSION

It is a well-established fact that as in higher vertebrates and human beings, in fishes also environmental contaminants cause dramatic changes in the circulating blood [29] [17]. Thus, measurable changes are induced in the haematological parameters such as cell counts, haemoglobin content, haematocrit, leucocrit and erythrocyte sedimentation rate by different types of stressors [21] [14]. Exposure of *E. suratensis* to endosulfan resulted in an initial increase in total erythrocyte count and haemoglobin content, both of which decreased subsequently with increase in exposure time. Haematocrit on the other hand increased throughout the exposure period. Reduction in TEC has been reported in *G. giuris* exposed to malathion [15].

Haemoglobin content of blood is a useful index of the health of fish[24]. According to [36], pesticides might have a catalyzing effect on the incorporation of ions in to haemoglobin and therefore, in increasing the haemoglobin content. Increase in Hb and Ht following stress is regarded to be the result of impairment of gas exchange by the gill [1]. [23]and [41] have attributed the increase in Hb and Ht in toxicant exposed fish to the increase in the size of RBCs. In the present study in endosulfan exposed *E. suratensis*, increase in Hb and Ht was noted during the initial phase of exposure. Abidi and Srivastava [36] reported on significant increase in Hb content of *C. punctatus* exposed to endosulfan. Another study have also noted marked increase in Hb content in *A. testudineus* exposed to different concentration of endosulfan[40]. In the present study, long term exposure of *E. suratensis* to endosulfan caused decrease in Hb content of the fish. Similar results have been reported in many fishes such as *C. punctatus* exposed to sublethal concentration of quinalphos for 45 days [20] and to malathion for 15 days [9]. *Clariusbatrachus* exposed to BHC for 60 days [7] and *L. rohita* exposed to nuvan for 45 days [8]. Haematocrit denotes the volume by percentage of red blood cells in blood and it is the most accurate measure for the detection of anaemia and polycythemia [10]. Mount and Putnick [11] reported that in fish, endrin poisoning causes tremendous decrease in Hct to about half that of the control fish. Similar decrease in Hct was noted in *S. mossambicus* exposed to sumithion and sevein [33] and [34], in *C. punctatus* ,*C. batrachus* and *H. fossilis* following malathion treatment [35] and in *C. idella* exposed to fenvelerate [5]. Decrease in haematocrit in danitol exposed *C. idella* remarked that it is an indication of the extent of the shrinking cell size due to insecticide intoxication[12]. Increase in Hct in fish exposed to low concentration of endosulfan; however at high concentration it registered decrease[18]. In the present study, in *E. suratensis* exposed to endosulfan initially caused decrease in Hct and then a gradual increase up to 10 days post-exposure. But at 30 days post exposure it again decreased.

Etythrocyte sedimentation rate is a complex biological process, governed by a diverse array of physicochemical factors. This parameter is useful in detecting fish diseases. Erythrocyte sedimentation rate is reported to be negatively correlated with TEC [4]. Thus, another study noted increase in ESR corresponding to decrease in RBC count in *H. fossilis* subjected to metal stress [37]. This was somewhat true in the present study also. In the endosulfan exposed fish, ESR initially decreased progressively up to 96h post

exposure during which period TEC progressively increased. The ESR increases in fish under pathological and stress conditions [38] andsStress causes haemodilution in turn augments ESR [13]. Increase in ESR is reported in *H. fossilis* exposed to fenthion [45]. Metal stress is also known to hasten the ESR of this fish [37]. In the present study in endosulfan exposed fish, ESR after an initial decrease up to 96 h post exposure, was found to increase up to 30 days post exposure. Increase in ESR in fish under long term exposure (>10 days) to pesticide probably is suggestive of tissue damage [45]. Based on MCV and MCHC, differential diagnosis may be made of anaemia in fish [16] and [26]. In the present study exposure of *E. suratensis* to endosulfan caused decrease in MCV throughout the exposure period. MCHC also essentially showed decreasing trend during exposure. Thus, exposure to sublethal concentration of endosulfan caused hypochromic microcytic anemia in *E. suratensis.* At the initial stage of exposure (24 h post exposure) the amemia was of normochromic micrcytic type.

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