



Haematological Changes in *Duttaphrynus Melanostictus* as A Function of Exposure Period of Malathion

KEYWORDS

Haematological parameters, malathion, *D. melanostictus*

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ABSTRACT Effect of malathion on haematological parameters were studied in *Duttaphrynus melanostictus* as function of exposure time. Toads exposed to malathion produced a time dependent significant ($P < 0.05$) decrease in total erythrocyte count, haemoglobin content, lymphocyte count, monocyte count and increase in total leukocyte count, neutrophil count and eosinophil count. Toads exposed to malathion showed an increase in weight and decrease in buccal movement.

INTRODUCTION

According to Global Amphibian Assessment nearly one third (1,856 species) of the world's amphibian species are threatened and 165 species may have already gone extinct (IUCN, 2006). Habitat loss and fragmentation, climatic changes, diseases and chemical contaminants have been identified as some of the main causes of amphibian decline worldwide (Blaustein and Wake, 1995). Haematological indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999b). Erythrocytes are the major and reliable indicators of various sources of stress (O' Neal and Weirich, 2001). Blood is highly susceptible to internal and external environmental fluctuations because it is the vehicle for the transport of such toxic substances (Blaxhall, 1972). The haematological parameters have been considered as diagnostic indices of pathological conditions in animals. Hence, an attempt has been made to study the effect of malathion haematological parameters of *D. melanostictus*.

MATERIALS AND METHODS

Animal: Female *Duttaphrynus melanostictus* ($n = 20$) were collected by hand net from April to August, 2013 in and around Baripada, Mayurbhanj. They were fed with insects and earthworms and divided into four groups: control group ($n=5$) and experimental group ($n=15$). They were acclimatized for seven days in laboratory conditions prior to the experiment. The control group (untreated) were treated with 3 μ l water. The experimental group ($n=15$) further divided into three subgroup (E1, E2 and E3), each include 5 number of animals and were treated orally with 3 μ l of furadan dissolved in acetone (0.005g of furadan per 1ml of acetone).

Buccal movement: The weight of animals were measured in experimental group before and after the treatment, with a time intervals 24 h (E1), 48 h (E2) and 72 h (E3). Similarly, the number of buccal movement (beat) were counted for a minute, both in control and experimental group using a stopwatch.

Collection of Blood Sample: The animal were sacrificed. Blood samples were obtained by intra-cardiac puncture carried out with sterilized syringe and needle. Blood was kept with a 10% solution of ethylene diamine tetra acetic acid (EDTA).

Blood corpuscles and Haemoglobin

The erythrocyte or red blood corpuscle (RBC) and leucocyte or white blood corpuscle (WBC) counts were made by Neubauer's haemocytometer by using Hayem's solution and Turk's fluid respectively. Haemoglobin was estimated by acid haematin method. A drop of blood was placed on a clean microscope slide, and a second slide was used to smear the blood on the first slide. All slides were air-dried, fixed with methanol and then stained with Leishman's stain for differential count of leucocytes (granulocytes and agranulocytes). Counting procedures was followed by Davis and Maerz (2008 a, b).

Statistical analysis

The statistical analysis was done with the help of statistical package SPSS 16.0. Correlation analysis test was carried out to find out the level of significance between *Bufo* treated with furadan at different time intervals of 24 h, 48 h, and 72 h along with that of untreated or control (0 h). According to Steven's guidelines if the correlation data is more than 0.72 then the data is significant. A difference was taken as significant when P was less than 0.05.

RESULTS AND DISCUSSIONS

The buccal movement per minute in *Duttaphrynus melanostictus* at time duration of 0 h, 1 h, 24 h, 48 h and 72 h exposed to malathion were 121 ± 6.55 , 91.67 ± 2.51 , 95.67 ± 3.21 , 101 ± 6.24 , 101 ± 3.46 respectively. It is revealed that buccal movement differed significantly [$F(3, 11) = 17.28$; $P = 0.001$] from each other at different time intervals after malathion treatment (Fig.1).

The toads have a body weight of 125.48 ± 21.79 g but after 24 h of malathion exposure the body weight was found to be 149.19 ± 28.27 g. The toads have a body weight of 130.55 ± 17.95 g but after 48 h of furadan exposure the body weight was found to be 151.47 ± 23.75 g. The body weight of toads was 150.04 ± 44.79 g but after 72 h exposure to furadan it was decreased to 169.69 ± 53.28 g. There was a significant and positive correlation between weight and exposure period (r : 24h = 0.971, 48h = 0.990 and 72h = 0.792 for malathion) at 0.05 level. The results showed that the toads exposed to malathion showed an increased in body weight (Fig.2).

Table 1. Changes of haematological parameters in response to malathion at different time intervals

Parameters	0 hour	24 hour	48 hour	72 hour
Total Erythrocyte Count (X10 ⁶)	0.71 ± 0.03	0.44 ± 0.04	0.33 ± 0.02	0.26±0.02
Total Leukocyte Count (X10 ³)	22.63 ± 1.16	17.08 ± 0.51	18.07± 0.17	19.34 ± 0.80
Haemoglobin (gm %)	9.46 ± 0.30	7.6 ± 0.34	6.6 ± 0.2	6.06 ± 0.11
Lymphocyte count	36.67±1.53	30.03±2.08	32.33±1.53	38.33± 0.58
Monocyte count	9 ± 10	10 ± 1	9.67 ± 2.52	15 ± 1
Neutrophil count	32.33 ± 2.08	37 ± 1	37 ± 1.73	29.33±1.527
Eosinophil count	16 ± 2	17.33±1.15	15 ± 3	11.33±0.58
Basophil count	6 ± 1	5.33±0.58	5.33 ± 0.5	6 ± 1.73

The erythrocyte or RBC count of *Duttaphynus meanoticus* treated with malathion at the time intervals of 0h, 24h, 48h and 72h malathion were 0.71 ± 0.03, 0.44 ± 0.04, 0.33 ± 0.02, 0.26 ± 0.02 X10⁶mm³ respectively (fig 3 and table 1). It is revealed that RBC count differ significantly [F (3, 11) = 96.582; P = 0.000] from each other after different time intervals.

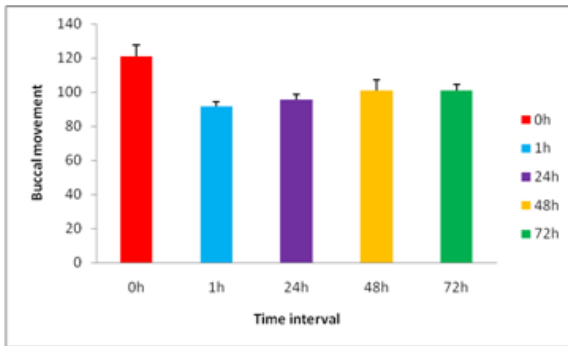


Fig-1: Buccal movement in Malathion treated animals

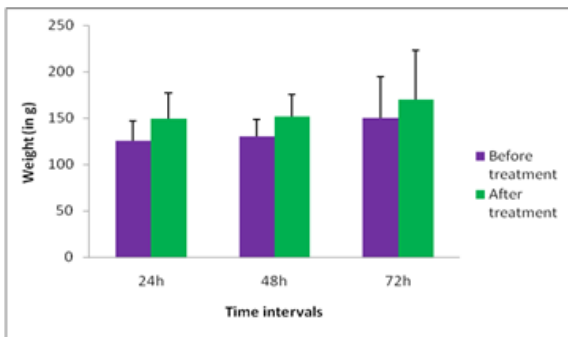


Fig-2: Weight in malathion treated animals

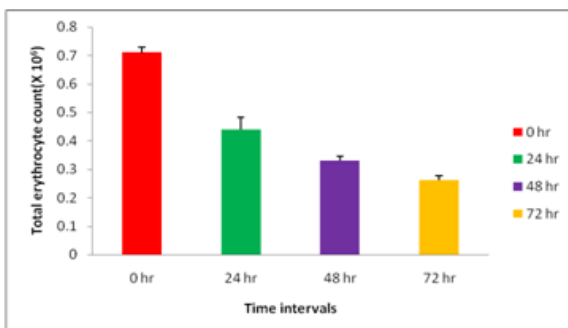


Fig 3: Total erythrocyte count in malathion treated animals at different time intervals

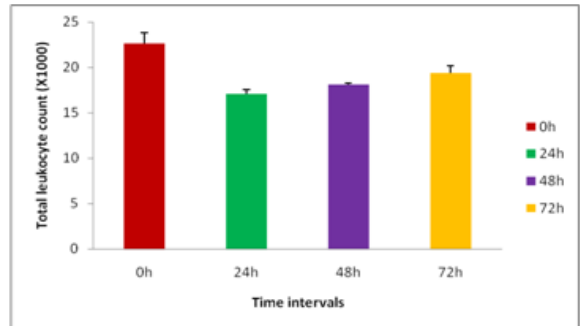


Fig-4: Total leukocyte count in malathion treated animals at different time intervals

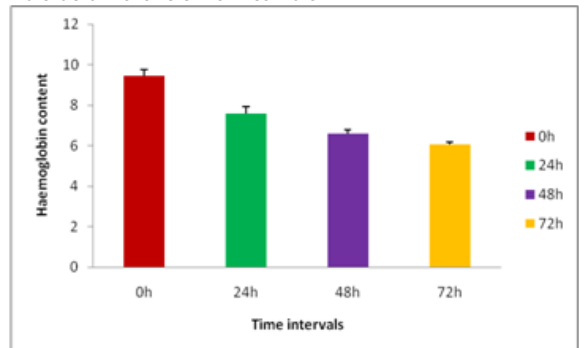


Fig-5: Haemoglobin content in malathion treated animals at different time intervals

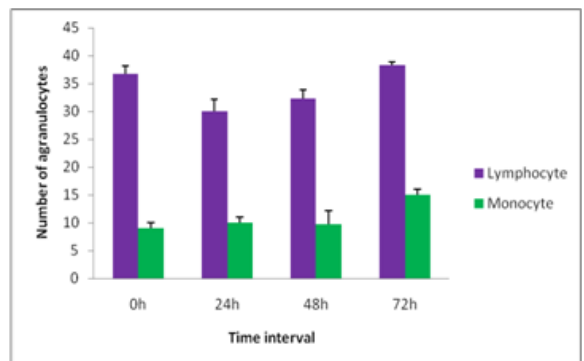


Fig 6: Number of agranulocytes in malathion treated animals at different time intervals

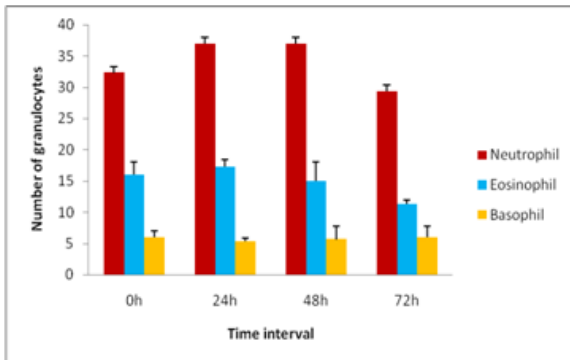


Fig 7. Number of granulocytes in malathion treated animals at different time intervals

The leucocyte or WBC count of *Duttaphynus meanostictus* treated with malathion at the time intervals of 0h, 24h, 48h and 72h malathion were 22.63 ± 1.16 , 17.08 ± 0.51 , 18.07 ± 0.17 , $19.34 \pm 0.80 \times 10^3 \text{mm}^3$ respectively (fig 4 and table 1). The results of the present investigation showed a significant [F (3, 11) = 23.476; P = 0.000] difference in WBC count at 0.05 level.

The haemoglobin content of *Duttaphynus meanostictus* treated with malathion at the time intervals of 0h, 24h, 48h and 72h malathion were 9.46 ± 0.30 , 9.46 ± 0.30 , 6.6 ± 0.2 , $6.06 \pm 0.11 \text{ gm } \%$ respectively (fig 5 and table 1). It is revealed that there is decrease in haemoglobin content at 24, 48 and 72 hours of exposure period. It is revealed that haemoglobin content decreased significantly [F (3, 11) = 100.867; P = 0.000] from each other at different time intervals after malathion treatment

The percentage of lymphocyte in time duration 0h, 24h, 48h and 72h exposed to malathion were 36.67 ± 1.53 , 30.03 ± 2.081 , 32.33 ± 1.53 and 38.33 ± 0.58 (fig 6 and table 1). It is revealed that lymphocyte count differed significantly in 48 hour [F (3, 11) = 4.661; P = 0.036] after malathion treatment but did not show significant variation in 24 and 72 hours.

The percentage of monocyte in time duration 0h, 24h, 48h and 72h exposed to malathion were 9 ± 1 , 10 ± 1 , 9.67 ± 2.52 and 15 ± 1 (fig 6 and table 1). It is revealed that monocyte count differed significantly [F (3, 11) = 9.750; P = 0.005] from each other at different time intervals after malathion treatment (Table- 11) and also differed significantly at 72 hours [F (3, 11) = 31.216; P = 0.000]

The percentage of neutrophil in time duration 0h, 24h, 48h and 72h exposed to malathion were 32.33 ± 2.08 , 37 ± 1 , 37 ± 1.73 and 29.33 ± 1.53 respectively (fig 7 and table 1). It is revealed that neutrophil count differed significantly in 48 hour [F (3, 11) = 15.948; P = 0.001] from each other at different time intervals after malathion treatment.

The percentage of eosinophil in time duration 0h, 24h, 48h and 72h exposed to malathion were 16 ± 2 , 17.33 ± 1.15 , 15 ± 3 and 11.33 ± 0.58 respectively. Eosinophil count differed significantly at 72 hours [F (3, 11) = 5.417; P = 0.025] but did not show significant variation at 24 and 48 hours (fig 7 and table 1.) and also differed significantly at 72 hours [F (3, 11) = 8.944; P = 0.006]

The percentage of basophil in time duration 0h, 24h, 48h and 72 h exposed to malathion were 6 ± 1 , 5.33 ± 0.58 , 5.67 ± 2.08 and 6 ± 1.73 respectively (fig 7 and table 1). Basophil count did not show significant [F (3, 11) = 0.141; P = 0.933] variation at different time intervals.

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