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Haematological Changes in *Duttaphrynus melanostictus* in Response to Furadan

KEYWORDS: Haematological parameters, furadan, *Duttaphrynus melanostictus*

Pragati Priyambada Pattanayak
P. G. Department of Zoology, North Orissa University, Baripada, Odisha, 757003 INDIA.

Puspanjali Parida
P. G. Department of Zoology, North Orissa University, Baripada, Odisha, 757003 INDIA

ABSTRACT *Effect of furadan on haematological parameters were studied in Duttaphrynus melanostictus as function of exposure time. Toads exposed to furadan 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 treated with furadan showed an increase in weight and decrease in buccal movement.*

INTRODUCTION

Hematology is the most straightforward and less invasive technique to access the health status of natural population of vertebrates (Artacho *et al.*, 2007). These parameters allow the detection of changes in physiological, pathological, ecological, and environmental conditions in natural population (Sarasola *et al.*, 2004; Seaman *et al.*, 2005). Blood parameters of Amphibia species were particularly affected by the negative environmental conditions. Amphibians are potentially reliable and efficient bioindicators (Welsh and Ollivier, 1998; Garg and Hippargi, 2007). They are very sensitive to even the slightest fluctuations in the environmental settings (Cunnigham and Saigo, 1999). Keeping the above an attempt has been made to study the effect of furadan on haematological parameters in *D. melanostictus* at different time intervals.

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 µl 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 µl 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 DISCUSSION

The buccal movement of *Duttaphrynus melanostictus* in time duration 0 h, 1 h, 24 h, 48 h and 72 h exposed to furadan were 121 $\pm$ 6.55, 89.33 $\pm$ 7.23, 94.33 $\pm$ 8.96, 95.67 $\pm$ 2.081 and 95.33 $\pm$ 3.05 respectively. It is revealed that buccal movement differed significantly [F (3, 11) = 12.353; P = 0.001] from each other at different time intervals after furadan treatment (Fig.1).

The toads have a body weight of 121.79 $\pm$ 65.9g but after 24 h of furadan exposure the body weight was found to be 100.37g. The toads have a body weight of 109.01 $\pm$ 41.44g but after 48 h of furadan exposure the body weight was found to be 28g. The body weight of toads was 123.1 $\pm$ 26.36g but after 72 h exposure to furadan it was decreased to 111.52 $\pm$ 19.62g. There was a significant and positive correlation between weight and exposure period (24h = 0.996, 48h = 0.926 and 72h = 0.998 for furadan) at 0.05 level. The results showed that the toads exposed to furadan showed a decrease in body weight (Fig.2).

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

Parameters	0 hour	24 hour	48 hour	72 hour
Total Erythrocyte Count ($X10^6$)	0.71 $\pm$ 0.02	0.55 $\pm$ 0.026	0.483 $\pm$ 0.02	0.43 $\pm$ 0.017

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Total Leukocyte Count ($X10^3$)	22.63 ± 1.16	17.23 ± 1.41	20.13 ± 0.99	27.6 ± 2.35
Haemoglobin (gm %)	9.46 ± 0.30	6.13 ± 0.11	5.6 ± 0.2	5.2 ± 0.11
Lymphocyte count	36.667± 1.527	29.33 ± 1.527	30.06 ± 1.154	35.33± 0.577
Monocyte count	9 ± 1	35.33 ± 0.577	35.333± 0.577	35.33± 0.577
Neutrophil count	32.333 ± 2.081	37 ± 1	36 ± 1.732	26.66 ± 1.527
Eosinophil count	16 ± 2	15.66± 0.577	14.33± 1.527	13.66 ± 3.214
Basophil count	6 ± 1	6.666± 1.5275	5.333 ± 2.081	6.333 ± 3.055

The RBC count of *Duttaprynus melanostictus* in time duration 0h, 24h, 48h and 72h exposed to furadan were 0.71 ± 0.026, 0.44 ± 0.043, 0.33 ± 0.017, 0.263 ± 0.015 $X10^6 mm^3$ (Table 1 and Fig 3) Toads exposed to pesticides showed a time dependent decrease in RBC count during exposure period. It was found that there was a time dependent significant (ANOVA: $P<0.05$) decrease in total erythrocyte count at different time intervals. It is revealed that RBC count differed significantly [$F (3, 11) = 163.965$; $P = 0.000$] from each other at different time intervals after furadan treatment.

The total leukocyte count of *Duttaphrynus melanostictus* in time duration 0h, 24h, 48h and 72h exposed to furadan were 22.63 ± 1.16,17.08 ± 0.51, 18.067 ± 0.17 and 19.34 ± 0.8 $X10^3$ mm^3 respectively (Table 1 and Fig 4).. There was a time dependent significant (ANOVA: $P<0.05$) decrease in total leukocyte count at 24, 48 and 72 hours of exposure period. It is revealed that WBC count differed significantly [$F (3, 11) = 30.370$; $P = 0.000$] from each other at different time intervals after furadan treatment.

The haemoglobin content of *D. melanostictus* in 0h, 24h, 48h and 72h exposed to furadan were 9.46 ± 0.3, 7.6 ± 0.34, 6.6 ± 0.2 and 6.06 ± 0.11g respectively (Table 1 and Fig 5).It was found that there was a time dependent significant (ANOVA: $P<0.05$) decrease in haemoglobin content at different time intervals. It is revealed that haemoglobin content decreased significantly [$F (3, 11) = 286.639$; $P = 0.000$] from each other at different time intervals after furadan treatment.

The percentage of lymphocyte in time duration 0h, 24h, 48h and 72h exposed furadan were 36.667 ± 1.527, 29.333 ± 1.527, 30.066 ± 1.154 and 35.333 ± 0.577 respectively. The percentage of monocyte in time duration 0h, 24h, 48h and 72h exposed to furadan were 9 ± 1, 11.333 ± 1.154, 13.666 ± 1.152 and 18 ± 1 respectively (Table 1 and Fig 6). Lymphocyte count also differed significantly at 24 and 48 hours [$F (3, 11) = 23.860$; $P = 0.000$] but do not show significant variation at 72 hours after furadan treatment. It is revealed that monocyte count differed significantly at 72 hours [$F (3, 11) = 31.216$; $P = 0.000$] but do not show significant variation at 24 and 48 hours after furadan treatment.

The percentage of neutrophil in time duration 0h, 24h, 48h and 72h exposed to furadan were 32.333 ± 2.081, 37 ± 1, 36 ± 1.732 and 26.666 respectively. The percentage of eosinophil in time duration 0h, 24h, 48h and 72h exposed to furadan were 16 ± 2, 15.666 ± 0.577, 14.333 ± 1.527 and 13.666 ± 3.214 respectively (Table 1 and Fig 7). The percentage of basophil in time duration 0h, 24h, 48h and 72 h exposed to furadan were 6 ± 1, 6.666 ± 1.527, 5.333 ± 2.081 and 6.333 ± 3.055 respectively (Table 1 and Fig 7). Neutrophil count also differed significantly at 24 and 48 hours [$F (3, 11) = 24.583$; $P = 0.000$] but do not show significant variation at 72 hours after furadan treatment. Eosinophil count differed significantly at 72 hours [$F (3, 11) = 8.944$; $P = 0.006$] but do not show significant variation at 24 and 48 hours after furadan treatment. Basophil count do not show any significant [$F (3, 11) = 1.844$; $P = 0.217$] variation at different time intervals after furadan treatment.

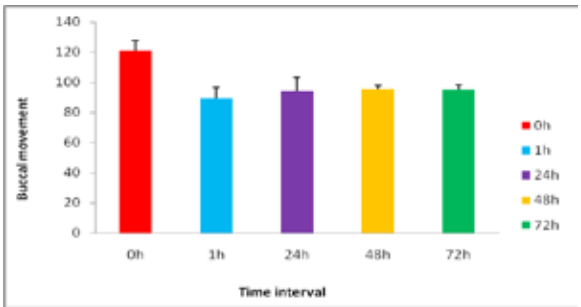

Fig-1: Buccal movement in furadan treated animals at different time intervals

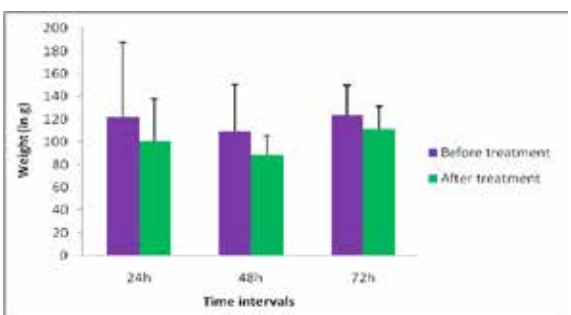

Fig-2: Change in weight of animal treated with furadan at different time intervals

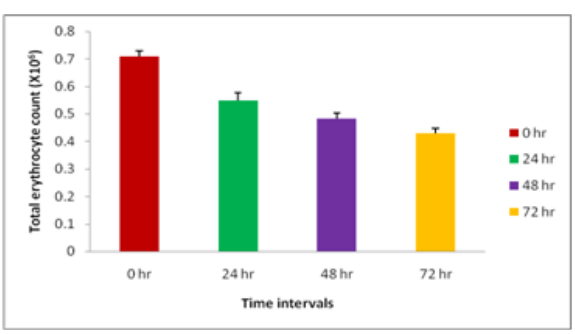

Fig-3: Total erythrocyte count in furadan treated animals at different time intervals

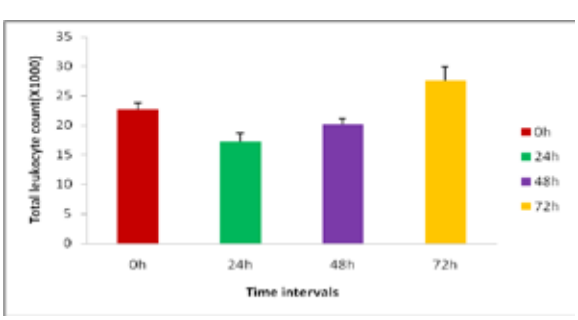

Fig-4: Total leukocyte count in furadan treated animals at different time intervals

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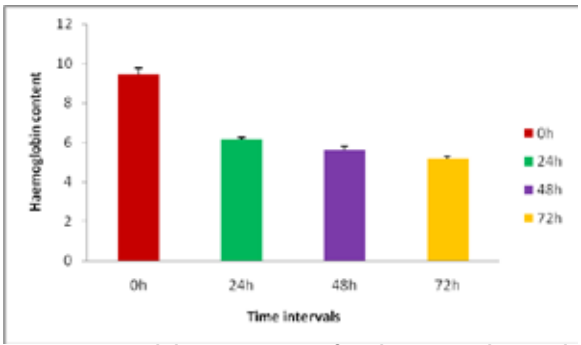

Fig-5: Haemoglobin content in furadan treated animals at different time intervals

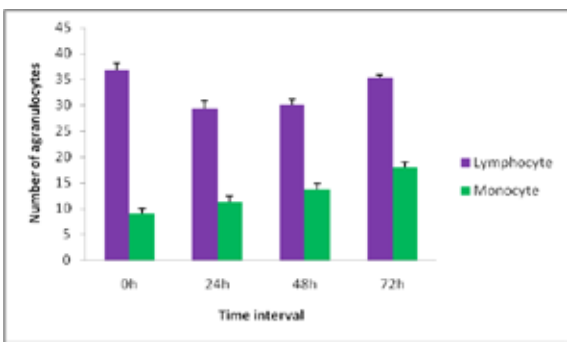

Fig-6: Number of agranulocytes in furadan treated animals at different time intervals

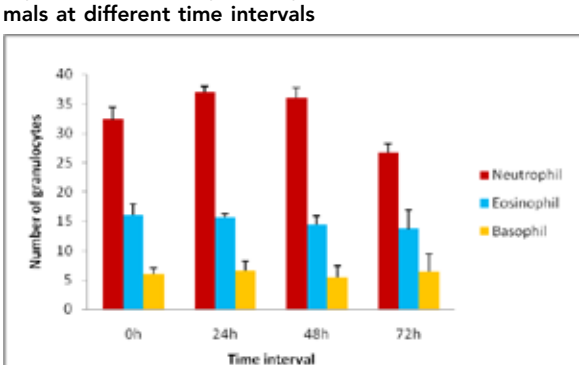

Fig-7: Number of granulocytes in furadan treated animals at different time intervals

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