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ORIGINAL RESEARCH ARTICLE

Effect of Systemic Pesticide Phosphamidon on Haematological Aspects of Common Frog *Rana tigrina*

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ABSTRACT

The impact of phosphamidon toxicity on biochemical aspects of blood cells and nucleus size of RBC of R*ana tigrina* resulted in a significant decreased in protein level were as increased in carbohydrate and lipid level. Cell and nucleus size of RBC also exhibited a decreasing trend

Key words: phosphamidon, haemocytes, carbohydrates, protein, lipids and toxicity.

1. INTRODUCTION

Water is one of the most important natural resources required essentially for the life and health of living organism. Such waster gets contaminated due to land reclamation, construction of huge building structures, urbanization intensive farm practices and large scale industrialization. According to estimates, about one million pollutants of different kinds are introduced into natural water. In developing countries extensive use of pesticides to meet with increased agriculture needs is inevitable and the indiscriminate use, misuse or mishandling of the pesticides always results in acute poisoning of the bio – system. Long term exposure to such pesticides is known to adversely affect a number of vital functions not only the human beings but also to the aquatic organisms[2,5]. Pesticides used for pest control in agriculture fields seem to produce many physiological and biochemical changes in fresh water organisms by influencing the activities of several enzymes. Toxicity of pesticides endosulfan, melathion and furdan to tadpole of *Rana hescadactyla* [1]. Studied the variation in the haematology of frogs in relation to toxicity of different pollutants[11]. Haematological changes in male frog *Rana hexcadactyla* due to effect of dimecron[8]. The reduction in the blood glucose, haemoglobin, haematocrit mean erythrocyte value of *R.hexcadactyca* due to the effect of Ekalure[10]. Ezemonye Lawrence and tango Isioma. 2011. Studied actue toxic effects of endosulfan and diazinon pesticide on adult amphibians *Bufo regularis*[3]. Amphibian declines and endocrine disruption due to pesticides mixture[13]. For a clear understanding and to fill the gap in research trends in frog toxicology, an attempt is made here to evaluate 96 hrs LD50 value and the effect of phosphamidon on biochemical changes in blood of *Rana tigrina*.

2. MATERIALS AND METHODS

2.1 Test Species:

In the present study the test species *Rana tigirna* (Daudin) the common Indian bull frog collected local paddy field. They were acclimatized to laboratory condition for a week and were regularly fed with insects.

2.2 Experimental Set Up:

During experimentation, the chosen frogs in the range of 55 to 65 gms were maintained in round plastic tubs, (10 frogs in each tubs) and they were covered with suitable wire mesh. During the period of study (nearly for 15 days) the temperature range between 28 to 30^0 C and control group also kept for the same period of time.

2.3 Test Chemical:

The present investigation is so designed in order to under stand the event of toxicity of an organophosphate insecticide namely "Phosphamidon 85%SL"

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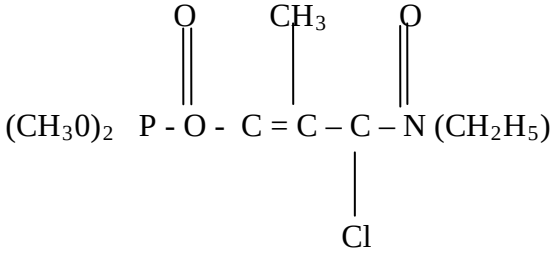
2.4 Structure:

Phosphamidon is Dimethy 1-Phosphate, Ester with 2-Chloro – N, N Diethyl1 -3 Hydroxy croton amide.



This compound is an oil which is soluble in water and most organic solvents[8].

2.5 Toxicity Evaluation:

The pesticide used for the present investigation was phosphamidon. The stock solution was prepared by adding 2.35ml of phosphamidon in 1 liter of distilled water. The media is equivalent to 2000ppm. From the stock solution different concentrations (240, 260, 280, 300, 320, 340, 360, 380 and 400) were prepared by suitably diluting with water, to determined LD50 value.

So the LD50 for 96 hours was calculated from the toxicity of different concentration of phosphamidon was given through intraperitonial injection to each animal as per experimental flow chart. The causalities were counted at intervals of 24, 48, 72 and 96 hours, the percentage was calculated. For further experiments animals were divided into two groups namely lethal (320ppm) and sub lethal (32ppm), and they were exposed to toxicant continuously the period of 7 days (lethal group) and 15 days (sub lethal group) using the same procedure.

2.6 Buccal Movement:

After a particular period of experiment (7 and 15 days) both the control and experimental animals were kept in glass jars, separately. The number of buccal beat was enumerated for a minute, using a stop watch. Enumeration was repeated for 10 times, by using the same method 10 animals from each tub was enumerated and mean value was calculated to get the buccal movement for a particular animal.

2.7 Micrometry:

Blood was collected from the heart region by cardiac puncture and a smear was made in the conventional manner fixed with Methanol and stained with Leishmen stains. Size (Length & Width) of the cells and nucleus of maximum 10 RBC cells were measured by using calibrated ocular micrometer.

2.8 Biochemical Analysis:

At the end of experiment the control and treated animals were weight and killed lightly anesthetizing by chloroform, blood samples were collected from ventricle with 2.5ml syringe and 21 gauge needle, previously rinsed with 10% Sodium heparin.

2.9 Serum:

Blood transfer into a clean dry tube without anticoagulant and it was allowed to clot, the clot was loosen with a clean glass rod and centrifuged. The serum was transferred into a clean dry tube.

2.10 Estimation of Glucose: (Carbohydrate)

Glucose in presence of hot acetic reacts with O-Toludine and gives a blue coloured complex which is measured calorimetrically at 620-660 nm red filter within 30 minutes against blank.

2.11 Cholesterol:

Cholesterol reacts with ferric Perchlorate in presence of Ethyl acetate & Sulphuric acid when heated in a boiling water bath to produce a lavender coloured complex. The intensity of the colour produced is proportional to the Cholesterol concentration, the intensity of the colour measured in spectrophotometer at 560nm within 15 minutes.

2.12 Protein:

Peptide bonds of proteins react with Cupric ions in alkaline solutions to form a coloured chelate, the absorbance of which is measured at 550nm. The Biuret Reagent contains sodium-potassium tartrate to complex cupric ions and maintains their solubility at alkaline Ph, absorbance data are proportional to protein concentrations.

2.13 Statistically analysis

Data's were expressed as means ± SE and were analyzed using ANOVA. A "p" value < 0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

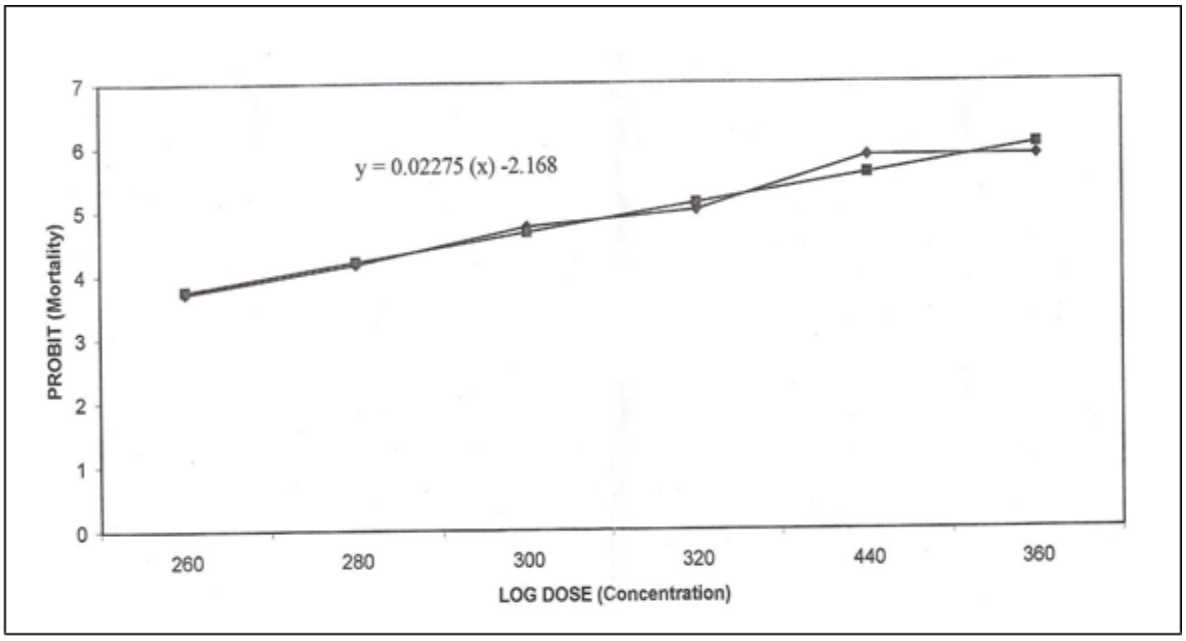
Toxicity test:

The 96 hr LD 50 value of phosphamidon for the frog *Rana tigrina* was 320 ppm. It was determined by probit analysis[4] (**Table 1**) shown in the present work as the concentration increases, the percentage mortality also increases. There is positive correlation between the concentration and morality (r=0.98). Regression equation has also been worked to estimate the mortality for a given concentration. Concentration as variable x and mortality as variable y are taken for consideration. The regression equation is y on y x is. Y = 0.02275 (x) – 2.168. (**Fig 1**) shows the dose response relationship of phosphamidon at 96 hrs. The logarithms of the dose are plotted against the probit mortality. The dotted line represents the regression line.

Table 1: Mortality rate of *Rana tigrina* exposed to different concentration of phosphamidon

Concentration (ppm)	Total Mortality				Percentage Mortality at 96 hrs	Probit values
	24 hr	48 hr	72 hr	96 hr		
Control	0	0	0	0	-	
240	0	0	0	0	-	
260	0	0	0	1	10	03.718
280	0	0	1	2	20	4.158
300	0	1	2	4	40	4.747
320	0	2	3	5	50	5.000
340	0	2	6	8	80	5.842
360	1	3	6	8	80	5.842
380	2	4	8	10	100	
400	3	5	8	10	100	

Fig 1: Dose response relationship of Phosphamidon at 96 hrs in *Rana tigrina*



Respiratory movement:

Pesticides and Heavy metal pollutions is known to affect a number of physiological system of fish. Respiration is one of the important parameter on which depend many of the vital functions like growth and reproduction. The Buccal movements in terms of number of beats per minute. Percentages from control level are given in (**Table 2**). From the table is calculated that there is decline in buccal movement of frog exposed to higher concentration 320ppm is more when compared with control and lower concentration 32 ppm, F values shows statistically significant, $p<0.01$ in both concentration.

Table 2: Test of significance difference of the (F-test) of the buccal movements between the control and experimental groups of *Rana tigrina* treated with different concentrations of phosphamidon (320 ppm &32 ppm).

Groups	Overall Mean ±SE	I	II	II	IV	V	F-Value
Control	112.00	118.5	112.4	113.0	109.3	112.9	
	2.29	6.9	4.6	5.3	4.5	5.4	
Experimental Groups 320 ppm	103.72	105.1	109.2	105.3	101.4	101.8	9.420**
	1.5	3.1	3.6	4.2	3.2	3.0	
32 ppm	103.20	101.3	111.0	101.8	103.8	104.7	7.984**
	2.6	4.2	11.6	4.4	1.4	1.5	

**p < 0.01

Values are expressed in Mean ± SE for 10 animals

Micrometry of RBC:

In micrometry studies, the size (length and width) of RBC cell and nucleus due to effect of phosphamidon are presented in (**Table 3**) along with control. The present observation reveals, significant decreased level in length and width of both cell and nucleus is $P<0.001$. Present study is in agreement with the earlier findings.

Table 3: Test of significance difference of the RBC Cell / Nucleus size of the control and experimental groups of *Rana tigrina* treated with different concentration of phosphamidon (320 ppm & 32 ppm)

Red Blood Corpuscles	Control Group			Experimental Group (n=10)							
				320 ppm				32 ppm			
	n	X	SD	n	x	SD	t-value	n	x	SD	t-value
CELL SIZE											
Length	10	22.69	2.678	10	14.34	1.264	8.72***	10	17.04	1.807	5.32***
Width	10	14.79	1.520	10	11.01	0.329	9.41***	10	11.22	1.437	6.2***
NUCLEUS SIZE											
Length	10	8.20	1.541	10	4.83	1.287	5.40***	10	5.7	1.011	4.39***
Width	10	5.06	1.231	10	3.39	0.448	5.52***	10	4.06	0.568	4.19***

***p<0.001

Glucose:

The results obtained due to phosphamidon toxicity on glucose, protein and cholesterol are presented in the (**Table 4**) along with control.

In the present investigation it is found that a level of blood glucose increased significantly in treated animals those are intoxicated to lethal and sub lethal concentration of (320ppm and 32ppm) phosphamidon, (**Table 4**) shows the mean different between control and treated is (P<0.001) the percentage change over control of glucose level of treated animals of both concentrations (320ppm and 32ppm) are (+14.02, +9.25) respectively. Similar results were observed in some fishes like *Barbus conchonius* exposed to endosulfan for 4 weeks Gill *et al* (1991), is C*larias batrachus, Saccobram chaes fossilis* and M*ystis vittatus* exposed to sub lethal concentration of a thorax for 30 days. *Verma et al,(* 1983).

Bakthavathasalam and srinivasa reddy, (1981) have reported that the higher blood glucose level in the treated fish might be due to an impairment in the carbohydrate metabolism. Impairment of glucose level in the blood of *Tilapia mossambica* exposed to phosphamidon clearly indicates the occurrence of diabetes mellitus Jayantha rao et al, (1984). The foregoing observation show that the occurrence of hyperglycemia is an important phenomenon in animals subjected to pesticide stress.

Protein

In the present study the total soluble protein level in blood of treated decreased. The decrease in total protein level after treatment is statistically significant (p<0.001) and percentage change over control in treated animals of both concentrations were (-33.56 & -42.65) respectively. The significant decrease in Albumin and Globulin level in treated animals both concentration is (p<0.001). The percentage change over control in treated animals both concentration are Albumin (-52.88 & 59.61) (Globulin -20 & -22.35).

The decrease in total and soluble protein of level in blood after treatment enhancement of proteolysis to meet high energy demand under pollutant stress condition. In the present study also a perceptible decrease in the protein content total and soluble blood suggests its mobilization to the ready uses to adjustment the energy crisis.

Cholesterol:

In the present investigation, the plasma cholesterol levels are increased in treated animals when compared with control. The difference in Mean ± SD is statistically significant (P<0.001 in 320ppm and P-<0.01 in 32ppm) and percentage change over its both experimental groups (320ppm & 32ppm) are (+46.07 and +40.04) respectively significant difference in cholesterol level between control and treated animals are given in (**Table 4**). Plasma cholesterol levels are considered as valuable indication of chemical induced disruption of lipid metabolism. Hypercholesterolemia in the frog, treated with pesticides might be the result as a decreased rate of conversion of cholesterol to bile acids constriction of biliary duct of impaired liver functions.

CONCLUSION

The observations documented in the present investigation are summarized as follows. Toxicity test was carried out to measure that effect of phosphamidon on *Rana tigrina*. The 96 hrs LD 50 Value of Phosphamidon for the *Rana tigrina* is 320 PPM. It was determined by probit analysis. Pesticides and heavy metal pollutions are known to effect a number of physiological system of aquatic animals. Respiration is one of the important parameter on which depend many of the vital roles. In view of this, the present study has been under taken to evaluate the buccal movement of *Rana tigrina* after exposed to higher concentration is more when compared with control and lower concentration. Toxicity impact of carbohydrate (glucose) shows that there is significant increase in blood glucose level in treated animals due to intoxication of

phosphamidon. Toxicity effect on cholesterol level increase in treated animals, result as decrease rates of conversion of cholesterol to bile acids. Results are expressed in decreased rates of protein levels in treated animal.

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