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HEMATOLOGICAL RESPONSES TO ENDOSULFAN TOXICITY IN THE INDIAN GARDEN LIZARD, *CALOTES VERSICOLOR*

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ABSTRACT

The organochlorine insecticide, endosulfan is widely used in crop field as well as for garden plants to control insects. The Calotes, common habitant of garden, is unknowingly affected by such insecticides. Hematological parameters vary in response to different toxicants in different animals. Considering this the present study was undertaken to evaluate the effect of endosulfan on some hematological parameters of Indian garden lizard, Calotes versicolor. Endosulfan (2.5 µl) was introduced orally to it at 24, 48, 72 and 96 hrs. It was observed that hemoglobin declined gradually throughout the experimental period than that of control. The lymphocytes decreased suddenly at early phase of 24 hours and then increased after 48 hrs. in experimental lizards, but could not increase beyond the lymphocyte count of control throughout the experimental period. Eosinophils, red blood cells and total leucocytes decreased gradually upto 96 hours than that of control after exposure to endosulfan.

KEYWORDS: *Hematological indices, Endosulfan, Calotes versicolor.*

INTRODUCTION:

The pesticides developed for human welfare are being considered as the most disastrous factors in producing harmful effect in environment and have become the serious sources of pollutant (Rameshwarsing, 2004). The regular utilization of large quantities of these pesticides into environment creates a pesticide pollution which constitutes the most dangerous health hazards on non-target organism. Reptiles have been reported to be more sensitive to the effects of these pesticides than are birds and mammals (Davidson *et al.,* 2002). Some studies established that the reptiles face many impact from human activities (Khan and Law, 2005). Hall (1980) reviewed several studies showing deaths of lizards and other reptiles following use of organochlorine pesticides, especially DDT, Dieldrin, Endrin, and Heptachlor and reported widespread mortality in two lizard populations following application of Heptachlor. Alterations in hematological parameters due to water pollutants are well established (Radha *et al.,* 2005; Sah *et al.,* 2006; Gupta *et al.,* 2007). Reduction in erythrocytes is reported in *Labeo rohita* exposed to sub lethal concentration of Nuvan and it became anemic (Wedemeyer *et al.,* 1984). The hematology of fingerlings of *L. rohita* exposed to two sublethal concentrations i.e. 10.3 ppm and 2.06 ppm of Nuvan was studied by Das and Mukhaerjee (2001), and it was found that there was reduction in total erythrocytes count and hemoglobin percentage. Total counts of erythrocytes and hemoglobin were decreased and leucocytes were increased in endosulfan treated group of rats and differential counts of leucocytes showed significant increase in basophils and monocytes (Das *et al.,* 2010). Possible impacts on lizards include direct poisoning from eating (or licking) poisoned bait and secondary poisoning from eating dead, dying, or disorientated insects contaminated with insecticides. The impact of pesticides on reptiles exposed to it is by many routes but perhaps the most common route is agricultural practices which affect natural habitat in several ways. As endosulfan is a worldwide used synthetic insecticide that has an important role on management of pests in agriculture, the

present work was undertaken to determine the effect of endosulfan on the hematological parameters of *Calotes versicolor* which can be a promising procedure for use in biomonitoring programmes to diagnose pesticide exposure of wild populations.

MATERIALS AND METHODS

Calotes were collected from the nearest agricultural field without regard of sex. They were kept in cages, acclimatized for two weeks in laboratory conditions before the experiment and fed with insects during and before the experiment. The Endosulfan (2.5µl) was introduced orally to the *Calotes*. A batch of untreated lizard was also kept for comparison as control without administration of endosulfan. At 24, 48, 72, and 96 hrs. the blood was drawn from both control and experimental groups from the orbital sinus with the help of glass capillary tube for analysis of eosinophils, lymphocytes, monocytes, neutrophils, RBC and hemoglobin percentage.

RESULTS

Alterations in hematological parameters of *Calotes versicolor* after exposure to Endosulfan

Parameters	Groups	Periods of Exposure			
		42 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.
Hemoglobin (gm/dl)	Control	7.7±0.374	7.6±0.316	7.5±0.489	7.4±0.435
	Experimental	5.4±0.352** (-29.8)	5.1±0.556** (-32.8)	4.3±0.624** (-42.6)	4.2±0.331** (-76.1)
Eosinophil %	Control	10.6±1.213	9.5±1.048	10.3±1.633	10.8±0.983
	Experimental	7.8±1.189* (-26.41)	5.6±0.819** (-41.05)	3.5±1.048** (-66.01)	2.3±0.9** (-78.70)
Lymphocytes%	Control	6.0±2.0	5.1±1.69	5.5±2.345	5.6±1.833
	Experimental	2.6±2.18** (-56.66)	3.8±1.685 ^{NS} (-25.49)	4.1±1.691 ^{NS} (-25.45)	3.6±1.833** (-35.71)
Monocytes %	Control	1.6±0.521	1.8±0.409	1.5±0.547	1.5±0.707
	Experimental	0.83±0.752* (-48.1)	0.66±0.515** (-63.3)	0.5±0.836** (-66.6)	0.33±0.505** (-78)
Neutrophils %	Control	3.8±1.685	3.3±1.319	3.6±1.166	3.5±1.870
	Experimental	2.1±1.691** (-44.73)	2.8±1.68^{NS} (-15.15)	2.6±1.833 ^{NS} (-27.77)	2.5±1.224 ^{NS} (-28.57)
Red Blood Cells (Million/mm3)	Control	1.20±0.044	1.21±0.051	1.21±0.037	1.19±0.037
	Experimental	1.37±0.052* (14.16)	1.17±0.037 ^{NS} (-3.30)	1.13±0.046^{NS} (-6.61)	1.10±0.04 ^{NS} (-7.56)
Total Leucocytes count(cu.mm. in thousands)	Control	5.69±0.043	5.68±0.151	5.67±0.309	5.68±0.122
	Experimental	5.63±0.130^{NS} (-1.05)	5.59±0.026 ^{NS} (-1.58)	5.53±0.137^{NS} (-2.46)	5.50±0.167** (-316)

Values are mean ± SD of six observation NS- Not Significant Values are significant at *P<0.05, ** P<0.01 Values in parenthesis expresses percentage difference.

DISCUSSION

The toxicity of endosulfan varies depending upon the route of administration, vehicle, species and sex of animal. (Dikshith *et al.,* 1988). The experiments conducted on sexually matured male rats to determine the effect of endosulfan on the hematological and haemochemical parameters of albino rats fed with 5 mg/kg body weight endosulfan in mixed food stuff for 42 days revealed decreased total counts of erythrocytes and hemoglobin, whereas leucocytes were increased in treated group (Das *et al.,* 2010). In our experiment significant decrease was found in hemoglobin percent in *Calotes* in all the exposure periods treated with endosulfan. The percent changes in hemoglobin over the control were 29.8, 32.8, 42.6 and 76%. The decrease in hemoglobin percent may be due to the disruptive action of the pesticide on the erythropoetic tissue. Solanke and Singh (2000) also reported declination in hemoglobin percent in Rat, *Rattus rattus* when exposed to thidon 53EC and postulated that this decrease may be due to haemolysis, hemorrhage and reduced erythropoiesis resulted due to the stress of endosulfan. In our investigation a reduction in R.B.Cs. number was observed but statistically it was nonsignificant. Compared to control, decrease was noted in R.B.Cs. count at 48, 72 and 96 hrs., but a slight increase was observed at 24 hrs. Thus it can be concluded that the decreased hemoglobin percent may be due to the reduced number of R.B.Cs. Sayim *et al.,* (2005), also reported decrease in mean corpuscle volume, hemoglobin, RBCs count and mean corpuscular hemoglobin, whereas no statistically significant increase in WBCs count, but significant increase in number of lymphocytes and monocytes in hematological analysis of rats when orally administered with synthetic pyrethroid insecticide, cypermethrine (150 and 300 mg/kg for 28 consecutive days). This decrease in R.B.Cs. count was suggested due to disrupted erythropoiesis due to pesticidal stress. Johal and Garwal (2004) studied toxicity of Carbaryl on *Channa punctatus* and recorded a general decrease in R.B.Cs count suggesting metabolic stress. Thus it may concluded that decrease in R.B.Cs due to disrupted erythropoiesis caused by stress of Endosulfan and slight increase in initial exposure periods may be justified for increased demand of energy during the experimental period.

Statistically no significant changes were noted in total leukocytes count in all the exposure periods but slight decrease in all the exposure periods may be due to the leucocytopenia exerted by endosulfan stress. A significant decrease was noted in neutrophil percent of treated *Calotes* in our study. Dikshith *et al.,* (1982) noted a reduction in neutrophil percent after repeated oral administration of Quinalphos to male goat. Similar result was also recorded by Rajini *et al.,* (1987) in Albino rat after the treatment of Primiphos which causes an adverse effect on normal functioning of the bone marrow or changes in the varied factors responsible for normal leucocyte balance. Thus, it can be concluded that the endosulfan interfere in the normal physiology of neutrophils. Mandal and Lahiri (1985) noted a significant decrease in lymphocyte number in blue rock pigeon after the treatment of Sumithion.

In our studies, also a significant decrease was noted in Lymphocyte percent in *Calotes*. Lymphocyte percent was decreased sharply at early period of 24 hours and later increased steadily in endosulfan administered lizards but could not increase than that of control group. Thus the resulted lymphocyte count may be justified due to immunosupression caused by endosulfan toxicity. The assessment of the acute toxicity (96-h LC50 median value of 2.6 µgl.) and physiological parameters after exposure to 0; 0.25; 1; 2; 3; 4 and 16 µgl. endosulfan for 96 h under semi-static conditions in a freshwater perciform fish, *Cichlasoma dimerus* was done by Da Cuña *et al.,* (2011) and found decrease in erythrocyte mean corpuscular volume and mean corpuscular hemoglobin concentration in exposed fish suggesting a state of anemia. Rehaman (2006) observed decrease in lymphocyte count in Endosulfan treated fish *Cyprinus carpio* where the red blood cell population was observed to be declined by 20% at 120 hr. and by 45.2% at 240 hr. of endosulfan exposure. The hemoglobin levels were also depressed by 32.2 % at 120 hr. and 64.3 % at 240 hr exposure. The decrease of 24.6 % of counts of small lymphocytes was noted at120 hr. which further declined 52.6%, at 240 hr. The counts of large lymphocytes were found to be lowered in endosulfan-affected fish by 19.5 at 120 hr, and 65.9% at 240 hr. Suppression of the basophil numbers was observed at 120 hr and by 37.9% at 240 hr of endosulfan treatment. Attenuation of neutrophils was 9.4%; not significant at 120 hr. and 12.5% at 240 hr. of the pesticide exposure. The eosinophils were diminished by 30.4% at 240 hr. The

thrombocyte was the only blood parameter studied showing elevated counts (9.5%) a 120 hr. The monocytes were depressed by 11.7% at 120hr and by 29.5% at 240 hr in endosulfan-exposed fish.

In our findings a moderate decrease was noted in eosinophil percent in *Calotes* and notable changes were recorded in monocyte count after the treatment of endosulfan in all the exposure period. Dikshith, *et al.,* (1982) observed a decreased monocyte count after oral treatment of Quinalphos to male goat. Nath and Banarjee (1999) also recorded a low count of monocyte in *Heteropneustes fossilis* due to toxicity of Rogor. Omoyakhi *et al.,* (2008) observed a progressive decrease in monocyte percent in growing rabbit due to the treatment of Acetellic dust. Mandal and Lahiri (1985) also observed a gradual decrease monocyte percent in pigeon *Columbia livia* exposed to Sumithion. According to them this decreased eosinophilic count may be due to splenic immunosupression caused by pesticidal stress. The above findings support our investigation and can be concluded that Endosulfan exert stress on eosinophils and moncytes count via suppressed leucocytes.

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