EFFECT OF MALATHION ON THE BLOOD GLUCOSE LEVEL IN THE INDIAN LIZARD CHAMELEON ZELENICUS.

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ABSTRACT

The present study is aimed to investigate the effect of malathion on the blood of Indian lizard, Chameleon zelenicus. In the present study, the level of blood glucose was observed on the median lethal dose of malathion (42.7 mg/kg body weight) for 6,12,18,24,48,72,96 and 120 hours of exposure and lethal dose of (59.9 m malathion g/kg body weight) for 6,12 and 18 hours of exposure. The present study showed the level of blood glucose was decreased in the blood of indian lizard,. Chameleon zelenicus

KEYWORDS: Malathion, Blood Glucose, Chameleon zelenicus.

1. INTRODUCTION

A wide range of toxic chemicals called pesticides are used to control or eradicate the pests of agriculture, horticulture and other different crops throughout the world by people. The uses of pesticides have served to improve our crops from the ravages of insect pests. Control of pests have always been one of the major factors in enhancing the agricultural production (Hague and Freed[19], 1975).

The synthetic group of pesticides are broadly-classified into three major groups viz., Organochlorines, Organophosphorus and Carbamates (Corbett,[11] 1973). Among them organochlorine pesticides are commonly used in agriculture due to their high effectiveness in reducing the pests even at low concentrations. The organophosphorus compounds dichlorvos, monocrotophos, parathion, fenitrothion, malathion, phosalone, dimethoate, thiometon are degradable and are quickly broken down into harmless products in the milieu. Though they are highly toxic, they are not pollutants if used carefully, whereas the carbamate compounds isolan, carbaryl, carbofuran, aldicarb, carbathion are least in their toxicity. Among the organochlorine compounds DDT, aldrin, lindane, dieldrin, endrin, chlordane and heptachlor

are remarkably long lasting chemicals in the milieu. For instance, residues of DDT and some other pesticides have been reported to be found even after 12 years from their day of application and thereby causing biomagnification.

Blood plays an important role in the normal functioning of the body of any organism. Seasonal variations in the blood contents of reptiles have been reported by Hutton and Goodnight[11] (1957), Binyon and Twigg[5] (1965), Kekic[27] (1970). Banerjee and Banerjee[4](1969) and Choubey[9] (1975) have also reported the seasonal variations in the blood components of tropical reptiles. Jayaraman[25](1976) has studied on the modulation of humoral and cell mediated responses to sheep erythrocytes in the lizard, Chameleon zelenicus. The influence of post helminth infection on haematological indices of Chameleon zelenicus was reported by Kameswari and Narsimha Rao[26] (1979). Banerjee (1981) has reported the seasonal variations of some blood constituents of the Indian lizard Chameleon zelenicus.

Blood glucose levels have been reported to be a sensitive indicator of many stresses viz., handling, forced activity, salinity changes and few chemical pollutants (Ghosh[18] et al., 1969; Wedemayer,[51] 1972; Silbergeld,[45] 1974 and Watson and Mckeown,[49] 1976). A definite seasonal variation in the blood glucose level has been reported to occur in reptiles (Dessauer,[14,15] 1953). Pancreatectomy affects the blood glucose differently in different reptiles (Houssay and Penhos[21], I960; Madhavan and Rangnekar, 1985). The effect of Cad_2 on the cells of endocrine pancreas in the snake, *Natrlx natrlx* was worked out by Calugrenan[6] (1970). Light and electron microscopic studies on pancreatic islets of the lizard, *Lygosoma laterale* was observed by Rhoten and William[43] (1971). Hussaini[22] and Amani (1976) studied the effect of intramuscular injections of two different doses of alloxan on the pancreatic islet tissue and the blood sugar level of the toad, *Bufo regularis* and the lizard, *Agma stillio* at different time intervals.).

An interesting study was made by Chan[7] et al. (1970) on the water and electrolyte composition of the Iguanid lizard, *Dipsosaurus dorsalis* with reference to the control in the pituitary gland and the adrenal cortex. Lofts[29] et al. (1971) have observed the seasonal changes In the histology of the adrenal gland of the Cobra, Naja naja. Unsicker[47] (1974) has investigated the innervation of adrenal cells in the lizards, *Lacerta dugesi* and *Lacerta pityusensis*. Contiguity of the adrenaline -s-rorir.g chromaffin cells with the interrenal tissue in the adrenal gland of a lizard was worked out by Conte[10] (1977).

2. MATERIALS AND METHODS

Procurement, acclimatization and selection of the experimental animals

The experimental animals were live trapped in and around Angul area. The collected experimental animals were lodged in cages of 18 x 18 x 45 cms size and were acclimated for a week. They were nourished with live cockroaches, grasshoppers and butterflies. Water was placed ad libitum. Only the female animals of Chameleon zelenicus were taken for the present study. Healthy individuals weighing about 30 - 37 gms were selected for the control and the experimental studies. Much care was taken to avoid injured, pregnant and infected animals.

Chemical and physical properties of the selected pesticide

Malathion is organophosphorous pesticide extensively used to control a wide range of sucking and chewing pests of field crops, fruits and vegetables. It has many structural similarities with naturally occurring compounds and their primary target of action in insects is the nervous system; it also inhibit the release of the acetylcholonesterase at the synaptic junction (Cabello et al 2001). Acetylcholinesterase plays a key role in the control of nerve excitability at post synaptic sites. Malathion is found to inhibit the acetylcholinesterase. Inhibition of liver acetyl cholinesterase (AChE) activity is generally regarded as an useful indicator of poisoning by Before this, lethal toxicity tests were carried out for four different concentrations of the malathion.

Toxicity studies

The toxicity tests have been classified into different categories based on the types of pollution in the environment as preliminary screening tests and tests to establish water quality criteria, etc. In the laboratory, determinations of toxicity may be divided into two types viz., short term or acute toxicity tests and long term or chronic biological responses (Negilski,[39] 1975).

Range finding tests

To avoid much time delay and effort the preliminary exploratory tests were conducted to get a range but not an accurate dose of the pesticide, which should be covered in the full scale tests. To conduct these tests, 1% stock solution of the pesticide, malathion was prepared by dissolving it in the coconut oil. Test solutions of required concentrations were prepared from the stock solution.

Lizards were orally administered with 1.0 ml of the pesticide solution, containing the required dose of pesticide by using hypodermic syringe without the needle. Much care was taken to avoid regurgitation of the pesticide. The exploratory tests showed the range of toxicity for this pesticide *ho* this experimental animal between 47.7 to 69.7 rag/kg body weight.

Full scale tests

Based on the exploratory tests, full scale tests were conducted to assess the acute toxicity of the selected pesticide, malathion. In these tests seven concentrations viz., 42.7, 44.7, 47.7, 50.7, 53.7, 56.7p.nd 59.9 mg/kg body weight were administered and for each concentration six lizards were used. Simultaneously the control animals were maintained and they were administered with 1.0 ml of coconut oil by using hypodermic syringe without the needle. The control and experimental lizards were observed for 120 hours. None of the control individuals attained mortality. In the pesticide treated lizards, the mortality rate of the lizards were observed at different hour's viz., 6, 12, 18, 24, 48, 72, 96 and 120. The dose at which 50% mortality attained for a period of 120 hours was taken as median lethal dose (120 hours, LD_{50}) and the dose at which cent percent mortality attained in the test animals for a period of 24 hours was taken as lethal dose (24 hours, LD_{100}) for the present study.

From the observed results, it was inferred that the pesticide concentration of 42.7 mg/kg body weight at which 50% mortality occurred for 120 hours was taken as median lethal value (120 hours, LD_{50}) and the pesticide concentration of 59.9 mg/kg body weight at which cent percent mortality occurred for 24 hours was taken as lethal dose value (24 hours, LD_{100}).

Calculation of LD_{50} value

The LD_{50} was estimated by the method of Litchfield and Wilcoxon (1949). The LD_{50} value was obtained by the straight line interpolation method based on observed percentages of test animals surviving at concentrations lethal to more than half and less than half of the test animals. The LDc_{0} value was derived by plotting the experimental data on a one scale log x probability sheet taking test concentration on the probability scale. A straight line was drawn between the points representing the mortality percentages Vs concentration (Standard methods published by American Public Health Association, 1960). From the point at which this line intersects 50 percent mortality, a perpendicular line was drawn to the concentration marked in ordinate and this indicate the LD_{50} of 120 hours exposure period.

Estimation of blood glucose

Blood glucose was determined by the method of Murrel and Nace (1958). The Blood samples (0.01m) were collected by caudal puncture using heparinized hypodermic syringe and were immediately deproteinized in 10 ml of 10 per cent tungstic acid. The solutions were filtered and the filterate was used for glucose estimation. 0.5ml of the filtrate and 0.5ml of dilute tungstic aoid were taken in a clean test tube and then 1.0ml of potassium ferricyanide solution was added. The test tube was placed in boiling water bath for 15 seconds and cooled in running tap water, when the contents were sufficiently cooled, 1.0ml of cyanide carbonate solution (buffer solution) was added and the tubes were again placed on boiling water bath for 15 minutes and then quickly cooled to 25-30°C. Then into each tube, 2.0ml of ferric dupanol reagent was added followed by the addition of 6.0ml of distilled water and the solution was mixed well and shaken well after 10 minutes the intensity of blue colour formed was read at 630 nm in a spectrophotometer against the reagent blank. A standard curve was constructed from the absorbance of the standard glucose solution. The unknown glucose values of blood samples were directly read from standard curve. The glucose values are expressed as mg/ml of blood.

Statistical analysis

The data obtained from the control and experimental parameters were analyses to determine the level of significance at various exposure periods by student't' test.

3. RESULTS

Biochemical studies

The mean blood glucose values of the controls and the median lethal dose treated lizards are presented in the Table 3 and illustrated in the Table 1. The median lethal dose treated lizards had decreasing trend in their blood of lizards, after administrating with the pesticide, at malathion different hours viz., 6,12,18, 24,48,72,96 and 120 were 102.25 ± 7.53 mg/100 ml, 99.98 ±8.51 mg/100 ml,96.86 ± 7.41 mg/100 ml,95.26±4.60mg/100ml,94.19±5.91mg/100ml, 91.22±5.91mg/100 ml, 78.89± 6.54 mg/100 ml and 79.17 ± 4.81 mg/100 ml of blood, while the control values were 115.27±5.63 mg/100 ml, 114.40±5.26 mg/100 ml, 114.83 ±5.73 mg/100 ml,114.87±6.75mg/100ml,115.10±6.51 mg/100 ml,115.50±7.35 mg/100 ml, 114.50 ± 6.92 mg/100 ml and114.64±5.45mg/100 ml of blood during the respective hours. The difference between the control and the treated lizards were appeared to be significant during

all the experimental hours and the 't' value of different hours viz, 6, 12, 18, 24, 48, 96 and 120 as 3.39, 2.76,3.29, 4.70, 5.89, 5.83, 8.65 and 12.94 (P<0.05) respectively.

Table: 1 Level of blood glucose (mg/100ml) of Chameleon zelenicus in the control and Malathion exposed animals at median lethal dose

Hours of administration	Control	Malathion exposure
6	115.28±5.63	102.25±7.53*
12	114.45±6.26	99.98±8.51*
18	114.83±6.73	96.86±7.41*
24	114.88±7.75	95.26±4.60*
48	115.10±7.51	94.19±5.91*
72	115.50±7.35	91.22±5.91*
96	114.50±6.92	78.89±6.54*
120	114.65±5.45	79.17±4.81*

Mean+S.D of six individual observations

*Indicates statistically significant at 5% level

Table:-2 Level of blood glucose (mg/100ml) of Chameleon zelenicus in the Control and Malathion exposed.

Hours of administration	Control	Malathion exposure
6	115.28±5.63	98.43±8.32*
12	114.40±6.26	93.71±8.36*
18	114.84±6.73	88.85±7.86*

Mean+S.D of six individual observations

*Indicates statistically significant at 5% level

The mean blood glucose values of the control and the lethal dose treated lizards are presented in Table 4 and illustrated in the Table 2. The lethal dose treated lizards had decreasing trend in thei r blood glucose levels when compared to the control values upto 18 hours and later they died. The blood glucose level of lizards, after administrating with the pesticide malathion, at different hours viz 6, 12 and 18 were 98.43.+8.32 mg/100ml and 83.85 +7.86 ml/ 100ml of blood, while the control values were 115.28 ± 5.63 mg/100 ml, 114.40 ± 5.26 mg/100 ml and 114.83 ± 5.73 mg/100 ml of blood during the respective the hours. The difference between control and treated lizards were statistically significant during all the experimental hours and their 't' values were 5.09, 6.12 and 8.65 (P<0.05) at 6, 12 and 18 hours respectively.

4. DISCUSSION

Blood glucose has been a sensitive biochemical indicator of environmental stress persuaded by handling, forced activity, thermal shock, contact with pesticides and other chemical pollutants (Chavin and Kavacevic[8], 1961; Nakano and Tomlinson[37], 1967; Hill and Fromm[20], 1968; Watson and Mckeown, 1976; Wedemayer and Yasutaka, 1977 and Meielev et al_., 1983). The blood glucose level which shows fluctuations represent a dynamic balance between the rate at which it enters the blood from the liver and the rate at which it is being removed by the body tissues from the blood for utilization (Saskin, 1941). In the present investigation, the normal blood glucose level was estimated in the laboratory acclimated lizards and its mean value was 115.5 + 7.35 mg/100 ml of blood. Khanna and Kumar[28] (1975) have stated that the lizards generally maintain a much higher level of blood glucose than do other reptiles. The present findings gain support from the findings of Dessauer[16] (1952) for Gekko gecko, Dessauer[14](1953) for Anolis carolinensis, Miller and Wurster (1958) for Eumeces obsoletus, Dawson[12] (1960) for Eumeces obsoletus, Dawson and Poulson[13] (1962) for Phrynosoma cornutum, Motelica and Constanta (1965) for Lacerta vlridis viridis, Sabnis and Rangnekar[44] (1967) for Varanus monitor, Calotes versicolor and Nabuya carinata, Khanna and Kumar[28] (1974) for Uromastix hardwlcki and Madhavan and Rangnekar[30] (1985) for Calotes versicolor.

In the present study, it was documented earlier that the lizards administered with median lethal and lethal doses of endosulfan exhibited gradual decrease in their blood glucose level, when compared to control level. Similar results were reported for different species. Paul Ravindran[40] (1989) also recorded a decreasing trend in the blood glucose level of R. tigrina. Balakrishnan[3] (1989) has been reported in the rat, Mlllardla meltada administered with lindane showed the decreased blood glucose level. A decline in the blood glucose level was also found in the bird, Lonchura malacca administered with malathion (Usha,[48] 1990). Neelanarayanan[38] (1990) observed a similar decreasing trend in the blood glucose level in Mlllardia mel tada when they poisoned with zinc phosphide. The present findings also gains support from the works of Mazeaud[31,32] et al., (1971) for different fishes and aquatic organisms. Jawale[24] (1985) has observed for the freshwater fish, Rasbora daniconius on exposure to lethal concentration of the pesticides like DDT, endosulfan, rogor and dimecron, the fish showed a sudden fall in its blood glucose level which resulted in its death. Similar results were observed for Anabas testudineus administered with lethal concentration of malathion has been reported by Amudha[2] (1986). Mazeaud[31,32] et al. (1971) have stated that when an animal was exposed to pesticides, which gives heavy stress on the animal and

such stressful condition elicits neuro-endocrine responses in the organisms, which in turn affect the carbohydrate metabolism.

The rapid fall in the blood glucose level found in the present study might be due to the severe stress which occured on the animal during the initial phase of anoxia. Wilhelmi[53] (1948) was of the opinion that under fully developed shock, the blood glucose level falls and then there is striking rise in the ratio of lactate to pyruvate and that the combination of an increased rate of utilization of carbohydrate. Engle[17] (1949) has also stated that stress regularly results in hypoglycemia and death in cases where the adrenal cortex fails to function. It may be concluded that hypoglycemia in treated animals may be either due to failure of the adrenal to secrete the adrenocorticoidal secretion or due to the damage caused in the adrenal due to the administration of the pesticide. The fall in the blood glucose level much below its normal threshold value resulted in hypoglycemic convulsions which might have caused the death of the treated animals while hyperglycemia has been reported for *C. versicolor* when administered with lindane (Thiyagesan[46], 1981) and when administered with malathion (Rajeswari[42], 1988). They have accounted hyperglycem as due to the increased secretion of glucocorticoids.

Blood glucose has been a sensitive biochemical indicator of environmental stress persuaded by handling, forced activity, thermal shock, contact with pesticides and other chemical pollutants (Chavin and Kavacevic,[8] 1961; Nakano and Tomlinson[37], 1967; Hill and Fr.

Chameleon zelenicus (Fe male)

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