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ORIGINAL ARTICLE

EFFECT OF ENDOSULFAN ON HISTOPHATHOLOGY OF PANCREAS IN THE GARDEN LIZARD, CALOTES VERSICOLOR (DAUD.)

[1]*M.Gnanambigai and [2]G.Ramaswamy

[1*]Department of Zoology, Periyar Arts Collge, Cuddalore-607001, Tamilnadu

[2]Department of Zoology A.V.C.College, Mannampandal-609305, Tamilnadu

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ABSTRACT

An attempt was made to observe the effect of endosulfan on the pancreas of garden lizard, *Calotes versicolor*. In the present study, the histoarchitecture of pancreas was observed on the median lethal dose of endosulfan (41.7 mg/kg body weight) for 120 hours of exposure and lethal dose of endosulfan (59.7 mg/kg body weight) for 18 hours of exposure. The present study showed the size of α and β was decreased and α and β appeared red in colour due to endosulfan in the pancreas of garden lizard, *Calotes versicolor*.

Key words: Endosulfan, Pancreas ,*Calotes versicolor*

1.INTRODUCTION

The physico-chemical properties of the pesticides together with environmental transport processes translocate a portion of the applied chemicals elsewhere in the environment. The direct result of the applications of chemicals is relatively easy to observe while the indirect effects are difficult to detect (Ritakumari and Nair, 1978.About 12,500 chemicals are used as pesticides throughout the world. Pesticides are generally classified in relation to pest to be controlled such as insecticides, herbicides, fungicides, nematicides, ascaricides, rodenticides and molluscicides (Metelev et al., 1983).

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. These insecticides are insoluble in water and so they are stored in fatty tissues instead of being excreted. In this way they accumulate at each trophic level. Indiscriminate use of these pesticides resulted in the contamination of ecosystem, causing hazards to several non-target organisms (Anees, 1976; Matsumura et *al.*, 1972 and Natarajan, 1981).

**Corresponding author: M.Gnanambigai, Department of Zoology, Periyar Arts College, Cuddalore-607 001,Tamilnadu*

2.MATERIALS AND METHODS

Procurement, acclimatization and selection of the experimental animals

The experimental animals were live trapped in and around Mannampandal 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 Calotes versicolor 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

In the present study endosulfan (99% technical grade), α,β , - 1, 2, 3, 4, 7, 7 - Hexachloro bicyclo -(2, 2, 1) heptene - (2)-bisoxy methylene - (5, 6) sulphite supplied by Bharat Pulverising Mills (Private Limited), Bombay was used (Source:Mercier, 1981).

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, 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, endosulfan 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 41.7 to 59.7 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, endosulfan. In these tests seven concentrations viz., 41.7, 44.7, 47.7, 50.7, 53.7, 56.7p.nd 59.7 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 41.7 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 59.7 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.

Histophathological study

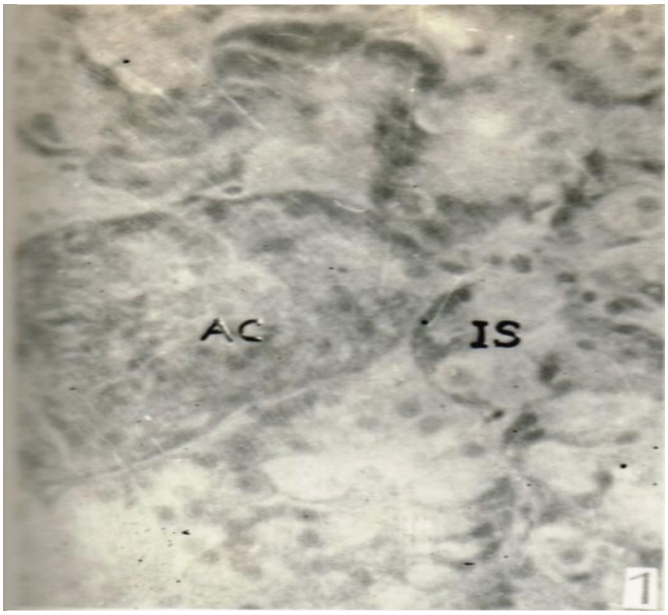
Histological studies were undertaken to comprehend the knowledge about the pathological lesions caused by the administration of endosulfan. To undertake this, two principal endocrine organs viz,, pancreas tissue was gently dissected out from the control and the treated animals and fixed in Bouin's fixative for 24 hours. After fixation, the tissues were washed overnight with running water, dehydrated with ascending grades of alcohol and embedded in paraffin wax, whose melting point was between 58ºC - 60 ºC. Sections were cut at 5µ thickness and were carefully deparaffinised and Stained with Iron-alum haematoxylin using alcoholic eosin as Counter stain by the usual method. The stained sagittal section of pancreas was examined under microscope and they were photomicrographed.

3.RESULTS

Histomorphology of the pancreas

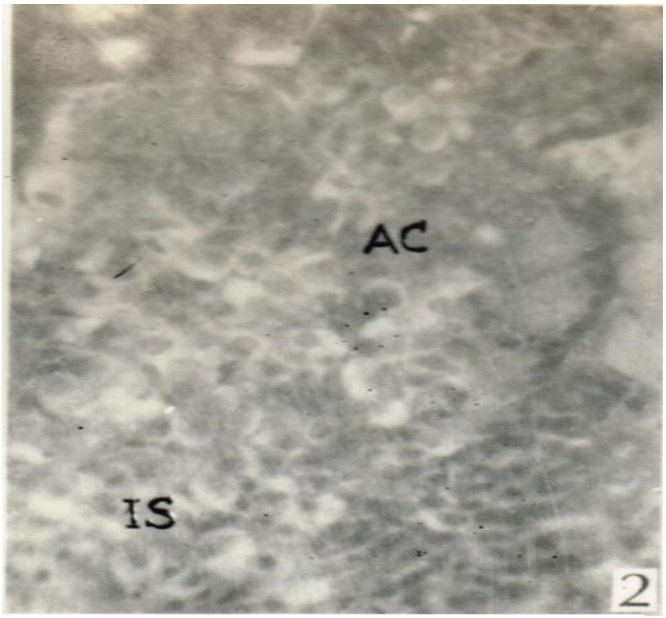
The pancreas in *C. versicolor* is a diffused whitish yellow leaf like structure extending from the gall-bladder to the duodenum. The margin is lobulated and attached to the stomach and duodenum by mesentries which are interspersed with blood capillaries. The common bile duct also opens into the duodenum. Distally the pancreas ends blindly at the duodenal 'U'bend and it is referred to as the pyloric lobe. A lobe of pancreas is attached to the spleen which is referred to as spleenic lobe while the lobe attached to the upper region of the stomach is referred to as cardiac lobe.

Fig. 1 shows histology of normal pancreas in *Calotes versicolor*



AC- acinar cels; IS-islet cells

Fig. 2 shows histopathology of median letal dose of endosulfan exposed pancreas in *Calotes versicolor*



AC- acinar cels; IS-islet cells

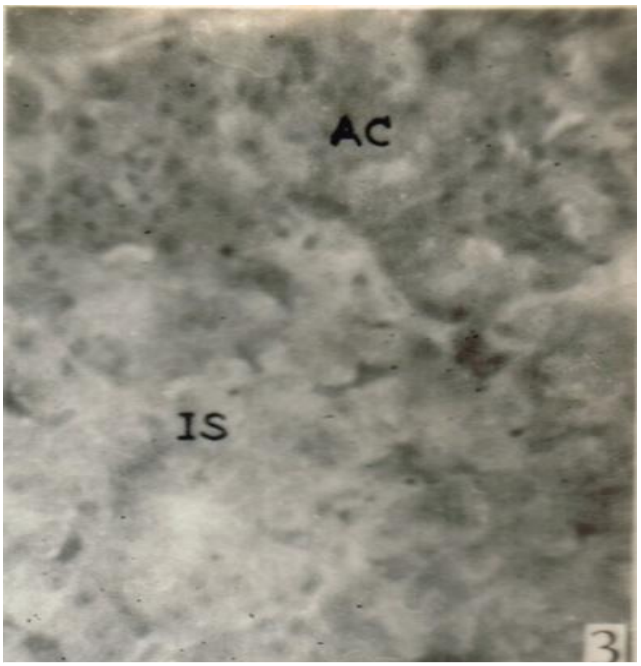
Histoarchltecture of the pancreas in control lizards

The pancreas is a complex structure consisting of exocrine and endocrine tissues. The exocrine constituent secretes pancreatic juice which is poured into the duodenum through the pancreatic ducts. The islets of Langerhans are aggregations of endodermal cells which liberate their hormones directly into the blood circulation. The islet cells are not easily distinguished from the acinar tissue. The islet cells are arranged in irregular cords which are separated by a system of very rich capillary vessels or sinusoids. There were 75% islets in the cardiac lobe, 12.5% in both pyloric and spleenic lobe. The endocrine pancreas was stained lightly with Iron-alum haematoxyl in-alcoholic eosin while the exocrine pancreas or acinar tissue stained darkly (Fig.1). Two types of cells, the α and β cells were observed. The α cells are more in number than the β cells. In one islet of cardiac, pyloric and spleenic lobe, both the α and β cells were enumerated to 57.4% cells, 24.5% cells and 18.03% cells respectively. The α cells are elongated and their average size measured to 7.5μ while the β B cells are ovoid in shape and their average size was 4.75 μ and their nuclei were centrally situated. The cytoplasm of β cells contained secretory granules.

Histoarchitecture of the pancreas in treated lizards

In the median lethal dose treated lizards which received 41.7 mg of endosulfan/kg of body weight showed remarkable changes in their pancreatic islets. The number of α and β cells were reduced. The size of α cells were decreased to 5.25 μ and the β cells were decreased to 2.5 μ. The α and β cells appeared red in colour and on the verge of disintegration (Fig. 2). On the other hand, the lethal dose treated lizards which received 59.7 mg of endosulfan/kg of body weight showed more pronounced remarkable reduction in the number of both type of cells (Fig. 3). In the lethal dose treated animals, the size of the α cells were decreased to 3.75 μ, and the β cells decreased to 2μ. The whole islet appeared shrunken because of the clear space occurring at the peripheral region of the islet.

Fig. 3 shows histopathology of median letal dose of endosulfan exposed pancreas in *Calotes versicolor*



AC- acinar cels;IS-islet cells

4.DISCUSSION

The pancreas is a complex structure consisting of exocrine and endocrine tissues. The exocrine constituent secretes pancreatic juice which is poured into the duodenum through the pancreatic ducts. The islets of Langerhans are aggregations of endodermal cells which liberate their hormones directly into the blood circulation. The islet cells are arranged in irregular cords which are separated by a system of very rich capillary vessels or sinusoids.

The islet tissue of all vertebrates except cyclostomes contains three functionally different types of cells viz., α cells producing glucagon, β cells producing insulin and D cells, the source of gastrin. The pancreatic islets of the lizard, *Calotes versicolor* is rich in alpha cells (Madhavan and Rangnekar, 1985). This has also been reported for other reptiles by Turner (1966) and Khanna and Kumar (1974). Several authors have studied the his to architecture and cellular composition of the pancreatic islets of Langerhans in different reptilian species (Miller, 1962; Miller and Wurster, 1956, 1958; Burton and Vensel,1966; Rhoten, 1971). Miller (1960) has studied the histology and carbohydrate metabolism of the pancreatic islets in amphibians and reptiles.

In the present study it was reported earlier that the pancreatic islet has two types of cells viz., α or A cells and β or B cells. The present observations under high power of a compound microscope are in concurrence with the observations of Miller and Wurster (1958), Miller (1962), Burton and Vensel (1966) and Titlbach (1967). However, three cell typesjviz.. A, B and D cells have been reported for different reptilian species in the pancreatic islets by Titlbach (1967, 1969), Gabe (1970) and Rhoten and William (1971). In the present study, the endocrine cells stained lightly while the exocrine cells stained darkly. The staining reaction revealed that the oC cells are believed to be the source of glucagon, the hyperglycemic factor and the P, cells are considered to be the source of insulin, the hypoglycemic factor (Foa et al., 1957).

The pesticide has instituted histological lesions in the islet tissues. Since the islets of Langerhans in amphibians and other poikilotherms answer differently to similar pesticides, the study becomes indispensable under experimental conditions to ascertain the functional significance of the different cell types in the islet tissues (Sheriff, 1987). In the present study the organochlorine pesticide, endosulfan caused deleterious changes in the histoarchitecture of the islets of Langerhans of *C. versicolor* and it has been reflected in its cell types.

The changes observed in the cell types of islets of Langerhans were as reduction in the number of α and β cells, shrinkage in their cell size and shape, damaged and degranulated nature of these cells. Falkmer and Hellman (1961) have stated that due to alloxan treatment, the B cells were destroyed in Cottus scorpius. Similar destruction and degranulation of β cells have been observed by Rangnekar and Sabnis (1967) for *R. tigrina* on administration of alloxan. The changes such as degranulation and vacuolization of the cytoplasm and pycnosis of the nucleus of the B cells have been reported for *R. cyanophlyctis* on administration with rogor by Sheriff (1987).

Besides this compatible histopathological lesions on β cells have been documented for different species of amphibians and other vertebrates (Lingeswari, 1985 and Paul Ravindran, 1989). In the present observations, the β cells exhibited destruction and damage in different stages. This might be due to the increased secretion of insulin. Thus, the treated lizards had a steep fall in their blood glucose level. This observation is in agreement with the earlier reports on other species of anurans by Arnold (1958). He has further reported that the decreased number of S cells in the islets of Langerhans could answer the presence

of toxic substances in the body which are having the ability to destroy the β cells and degeneration and exhaustion of over-worked β cells due to the high demand for insulin.

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