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Quarterly reports

Comparative Histopathology of Endocrine Pancreas on Halogenated Type II Pyrethroid Exposure in Male Albino Mice

Unique digital address (Digital object identifier [DOI] equivalent):
https://www.fluorideresearch.online/epub/files/256.pdf

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Accepted: 2023 Nov 24

Epub as e256: 2023 Nov 24

ABSTRACT

Purpose: This work aimed to define the histopathological severity of fluorinated, brominated and chlorinated pyrethroid insecticides on endocrine pancreas of mice.

Methods: Fourty male mice (*Mus musculus)* were categorized into four groups and each group containing ten animals. 0.1mL dose of corn oil was given to the vehicle contro (VC) group animals for three days. This group was kept as a reference group. The 0.1mL corn oil containing 5mg/kg dose of each insecticide such as Cypermethrin (CYP), Deltamethrin (DLT) and Lambda cyhalothrin (LC) was given to three group of mice for three days, followed by three days of none treatment. Dissection procedure was carried out at 7th day.

Results: All the micrometric results and histopathological alterations have shown that pyrethroid insecticides were highly toxic to endocrine pancreatic tissues. The various histopathological sign such as strip arrangements of endocrine cells in islets, endocrine cellular necrosis mainly in the central and pericentral areas of islets and increased vacuolation in surviving endocrine cells etc. In three insecticides treated groups, there were significantly decline in the relative abundance of endocrine cells per unit area (1230μm^2) such as CYP (8.8±0.25) DLT (7.5±0.23) and the LC (7.5±0.23) as compare to control group (9.7± 0.24). It indicates that type II pyrethroid insecticides are generally pancreo-toxic and they specifically target the islets of langerhans cells.

Conclusions: The severity of the mentioned pathological sign in LC and DLT groups indicated that these non-dietary halogenated (fluorinated and brominated respectively) pyrethroid inflict more severe histopathological impacts on the endocrine pancreas as compare to the simple chlorinated type II pyrethroid the CYP. The findings reflects that these otherwise consider comparatively safer or less toxic insecticides for the non-target organisms like mammals have come out to be highly toxic to the endocrine tissues particularly the pancreas.

Key-words: *Insecticides; Pyrethroids family; Histopathological changes in pancreas.*

INTRODUCTION

Pyrethroids are the most extensively used category of insecticides in the whole world. Deltamethrin (DLT), Cypermethrin (CYP) and Lambda cyhalothrin (LC) are insecticides which belong to the pyrethroid family.[1-3] DLT is assumed to be very powerful synthetic pyrethroids. It has very broad spectrum control.[4] Small concentration of DLT dose induce the apoptosis and decreased the cell viability.[5] Different studies have revealed that chronic exposure to DLT was linked with increase of blood glucose concentration and the decrease of plasma insulin levels. Similarly hyperglycemia in rabbits and in rats exposed to a single intraperitoneal injection of DLT.[6] Many reports have shown that the observed hyperglycemia due to toxic effect of insecticides is a result of pancreatic β cell dysfunction.[7]

The pancreas functions as a secretory organ, mainly composed of acinar tissue and the islets of Langerhans. The acinar tissue secretes pancreatic juice that contain variety of digestive enzymes (lipase, trypsinogen, phospholipase, cholesterol esterase, lysophospholipase and amylase) while mainly the islets consist of alpha and beta cells that are responsible to produce the hormones glucagon and insulin, to regulate the glucose in the blood plasma.[8-10] Delta cells in the islets of langerhans also produce somatostatin which decrease the secretion of insulin and glucagon. Toxicity of pancreas may result in diabetes mellitus acute pancreatitis with the loss of digestive capacity and an inability to regulate the blood glucose level.[11]

Cytotoxic effect of CYP on the cells of Pancreas has been reported, leading to insulin deficiency.[12] Pro-longed administration of LC, DLT and cismethrin to rats and rabbits respectively induced significant increase glucose level in brain and blood. In pancreas it can disturb structure of acinar cells, increase cellularity along with no definite boundaries of cells and absence of nuclei in some cells. .[13, 14] In the present study we investigated the histopathology of endocrine pancreas on halogenated type II pyrethroid insecticides exposure in male albino mice.

MATERIAL AND METHODS

Maintenance and Feeding of experimental animals

The present study was done on forty male Swiss Webster albino mice, age 3-4 months . These animals were kept in the 12''×16'' iron frame cages which covered with stainless steel guaze with appropriate pore size. Twenty albino mice 28-30g weight were placed randomly (10 individuals in each) into (VC), (CYP), (DLT), and (LC) groups. The VC group was maintained without any treatment for 06 days. Food and water kept in each cage separately in the access of all animals and these food and water were replaced on daily bases. Animal feed had all essential gradients like, sugar, vitamins, protein, minerals, calcium etc. In animal house normal condition of temperature (24±2°C) and humidity 35-40% were maintained.

Preparation of required dose of Insecticide

Desired dose of three different halogenated type II pyrethroid insecticides, which are DLT, CYP and LC were measured.The require solution of each insecticide was 5mg/kg.[15] The dose of 0.1mL of CYP, DLT and LC solution were given to groups of animals, except group VC. The Rafhan corn oil which was given to animals in pure form, bought from market (Sargodha).

Study Groups

Fourty animals were divided into four groups and each contain five animals. These groups were named as:

(i)DLT Group: 0.1mL gavage of prepared solution of DLT insecticide was given to each animal of this group for three days at a fixed time.

(ii)CYP Group: 0.1mL gavage of CYP insecticide solution was given forcefully to selected five animals for three days at a fixed time.

(iii)LC Group: In the same way 0.1mL gavage of LC insecticides was given to this group animals for three days at a fixed time.

(iv)Vehicle Control Group: No dose of insecticides was given to Vehicle control group. VC group was kept as a reference group. Just 0.1mL gavage of pure corn oil was given to the animals following the same protocol.

Daily observation

Four groups of animals were weighed on daily bases prior to dose treatment; any changed in the body weight of animals were noted. For this purpose digital balance were used which was available in animal house. This digital balance had 0.1g precision.

Excision of pancreas by dissection

At the seventh day of study, the dissection of animals was done. Prior to their dissection all animals were weighed. Cervical dislocation of mice was done to start the dissection procedure. Entire pancreas was excised very carefully and was put it into saline solution (0.9%). After a few minutes, pancreas was transfered into fixative solution for 48 hours.[15]

Histological Observation

After fixation pancreas was embeded into wax. So the tissues were dehydrated in different grades of ethanol (50%, 70%, 90%, 100%) afterthat clearance of tissues were done in xylene. With the help of rotary microtome (ERMA TOKYO 422) 2-3 microns thin sections were obtained of each pancreas. These sections were stained by using H & E stain according to standard protocol.

Digital photomicrograph

Stained sections were photographed at 400x ,100x and 40x magnification with the help of trinocular research microscope (Labomed CXR_2). It was attached with 7.2 mega pixel digital camera (Sony DSC-W35).

Computerized processing

To highlight the pathological signs, randomly selected photographs were improved in corelDRAW11 software for contrast, color and cropping and were presented in result section.

Micrometry

In micrometry, the data was generated from digital photomicrograph, with the help of computer assisted technique corelDRAW11. The selected images were proceed to get the micro-metric data on computer monitor by pre-calibrated scale of corelDRAW11. The measurement of mean CSA of islets of langerhans, mean cross sectional area of endocrine cells in islets of langerhans, relative abundance of endocrine cells/unit area of islets, and relative occupied area by endocrine cells of islets were obtained. The calibrated value were processed according to following formula.

$$CSA= (length \times width/4)\pi$$

Relative area occupied by endocrine cells in islets measured by following formula.

Relative area occupied by endocrine cells = (CSA of endocrine cells) X (mean number of endocrine cells /unit area)

Analysis of Data and Statistical Applications

The data which is obtained on the basis of micrometry and histology was analyzed by Tukey multiple Range Test (TMRT) and One way ANOVA .

RESULTS

Histological Results

Histological slide of vehicle control group showed that the endocrine cells in the islets of langerhans were healthy, contain prominent nuclei and evenly distributed throughout the islets. Blood vessels were also present nearer to islets. The islets of langerhans were mostly rounded in shape. The presence of eosinophilic cytoplasmic granules in the endocrine cells of the islets indicate the stored cytoplasmic proteins, probably the pro-hormones (pro insulin). The acinar tissues (exocrine portion) which also contained prominent nuclei were present in the surrounding of islets. Fig: 1&2(A)

The histological section of pancreas of the CYP treated animal group showed peculiar sign of histopathology such as the presence of longitudinal fluid filled spaces mimicking the typical sinusoidal spaces of usual hepatolobuler section. This lead to the strip or cord like arrangement of endocrine cells, nevertheless a few islets cells have shown intracellular fluid accumulation. The strip and sinusoid like special arrangement of the endocrine pancreatic tissue mostly restrict to the central/core area of the islets while the marginal area of the islets remained more or less undisturbed. Fig:1&2(B)

The pancreatic sections of DLT treated group have shown much of the typical sign of general endocrine cells necrosis. As indicated by the intracellular vacuolation and edema of the endocrine pancreas indicating intracellular toxicological improvisation of insecticides leading to general intracellular fluid accumulation that proceed further events of cellular necrosis. The strip formation of endocrine cells have observed in this group at scattered places in the sub marginal zone of the section of islets of Langerhans. Fig: 1&2(C)

The Histological sections of LC treated group animals have shown most severe damage to the islets. Where only very few healthy functional endocrine cells were observed while most of the islets structure showed macrophages infestation. The core area of islets was almost completely devoid of endocrine cells and was replaced by the fibrotic mass. Nevertheless a few typical strip of functional endocrine cells were observed in the sub marginal area of the islets of the langerhans in this group. Fig: 1&2(D)

Micrometric findings

The micrometric results have shown all micrometric parameters have highly significant difference between groups. For example One way ANOVA based analysis of mean CSA of endocrine cells of islets have shown highly significant variation among groups ($p \leq 0.0001$). TMRT based post hoc analysis has shown significant ($p \leq 0.05$) increase in mean value for LC and DLT (due to vacuolation) as compared to vehicle control. However

there was less significant difference between VC and CYP group (Table. 1).

Table 1. Statistical results of endocrine pancreas

Micrometric Parameters	Mean±SEM			
	VC	**CYP**	**DLT**	**LC**
*Mean CSA of Islet of Langerhans (μm^2)	168.5±14.9[a]	208.5±19.3[b]	208.5±19.3[b]	247.5±19.9[c]
Relative area occupied by endocrine cells of islets (μm^2) ¥	191.82±23.17[b]	136.92± 19.82[a]	125.3±18.27[a]	130.79±21.39[a]
*Mean No.of cells of islets of Langerhans per unit area (1230μm^2) ¥	9.7± 0.24[b]	8.8±0.25[a]	7.5±0.23[a]	7.5±0.23[a]
*Mean CSA of endocrine cells of islets of Langerhans(μm^2) ¥	32.9 ± .7[a]	35.7±1.1[a]	48.6±2.01[b]	43.6±2.4[b]

*:anlysed by One Way ANOVA, CSA (cross sectional area); ¥ :p≤0.0001; The mean values in a row not sharing a common superscript differ significantly (p≤0.05) with each other.

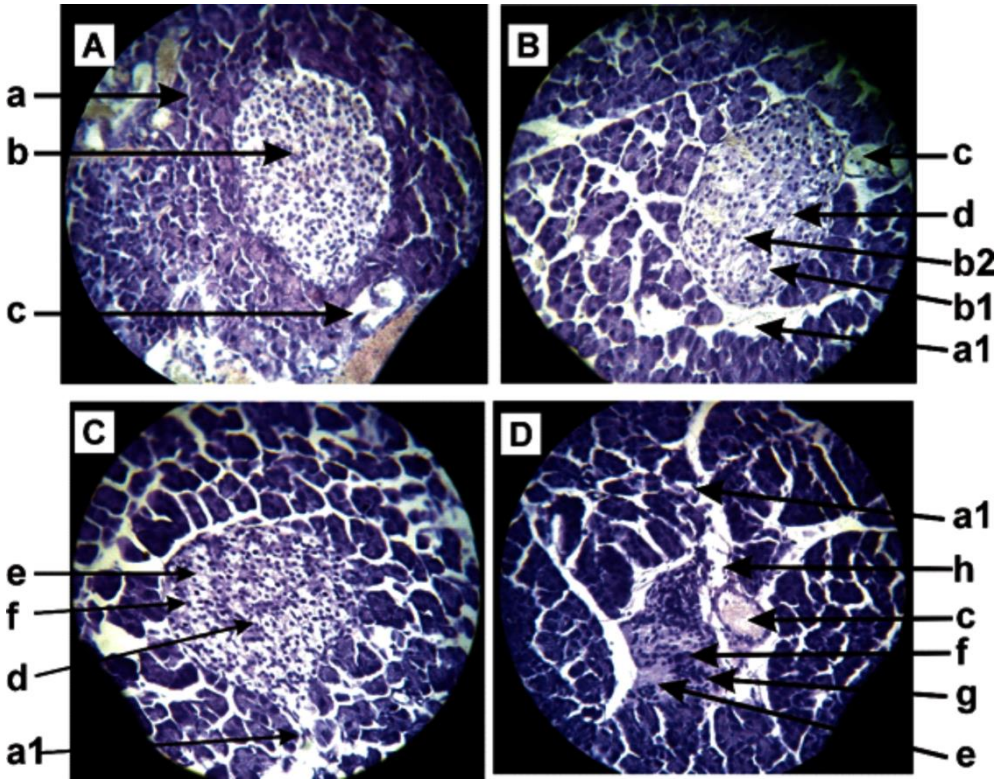

Figure 1. Histological sections of albino mice pancreas at 400x: VC(A), CYP (B), DLT (C) and LC (D).

a: Healthy exocrine tissue, (a1) Damage exocine acinar tissue, (b) Healthy endocrine cells in islets, (b1) Fibrotic mass in islets, (b2) Strip formation of endocrine cells, (c) Blood vessels, (d) cells vacuolation in islets, (e) empty spaces in islets (show degeneration of endocrine cells), (f) Megakaryocytes, (g) Macrophages infestation (h) apoptosis.

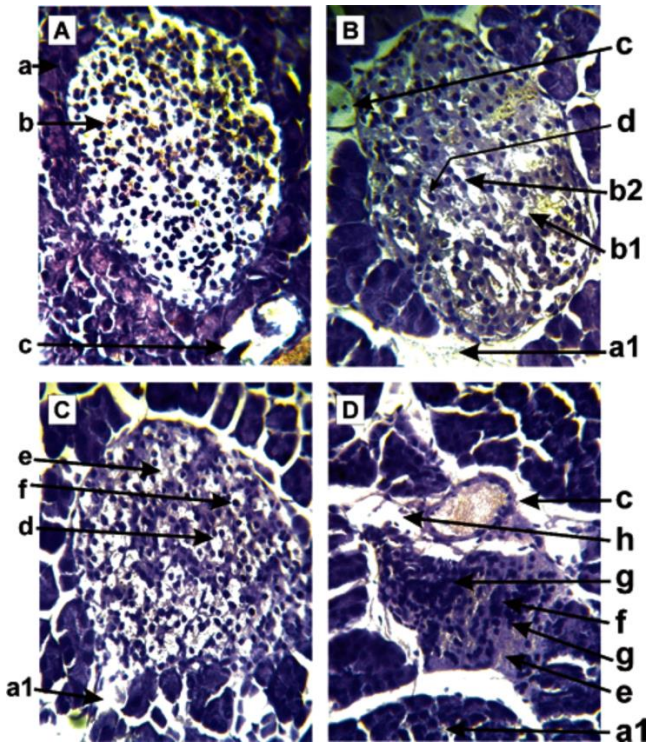

Figure 2. Histological sections of albino mice pancreas at 400x: VC (A), CYP (B), DLT (C) and LC (D).

a: Healthy exocrine tissue, (a1) Damage exocrine acinar tissue, (b) Healthy endocrine cells in islets, (b1) Fibrotic mass in islets, (b2) Strip formation of endocrine cells, (c) Blood vessels, (d) cells vacuolation in islets, (e) empty spaces in islets (show degeneration of endocrine cells), (f) Megakaryocytes, (g) Macrophages infestation (h) apoptosis.

DISCUSSION

It has already shown in previous study that the insecticides of pyrethroid type II group like cypermethrin, deltamethrin, lambdacyhalothrin etc can cause exposure related damage to pancreatic tissues like various damage to the exocrine acinar tissue and in particular the insulin secreting beta cells the islets of langerhans. This situation mainly to various structural (anatomical and physiological) alteration in the exposed animals for example it has been shown that exposure to deltamethrin, cypermethrin and cismethrin.[13-15]

Pyrethroid has caused acute hypoinsulinemia that have led to different physiological alteration such as In pancreas tissues, different result of insecticides toxicity were recorded, for rats and rabbits respectively induced significant increase glucose level in brain and blood. The B cells of pancreas secrete insulin in response to high level of glucose and also respond to other substances such as acetylcholine and glucagon carbohydrates are unavailable to body cells because insulin is not available for the transportation of glucose to the cells. Due to insulin deficiency, carbohydrates are not available for the energy demands of body and most of the energy is obtained from fats. So, the stored fats in adipose tissue is then hydrolysed and the level of free fatty acids in blood is increased, as a result the total lipid concentration in serum is increased, which lead the body in different complications.[16]

Similarly sub-chronic exposure to LC the fluorinated type II insecticides has caused characteristic damages to the pancreas including various micro anatomical arrangement such as disturbed structure of acinar cells, no definite boundaries of cells and increase of cellularity and absence of nuclei in some cells were recorded in pancreas tissues.[13] The current study have shown certain characteristics type of histopathological alteration in endocrine and exocrine pancreas related to the exposure of cypermethrin a type II pyrethroid insecticides, deltamethrin a brominated type II insecticides and lambdacyhalothrin a fluorinated type II pyrethroid insecticides.

Aim of the present study was to compare and to discover the patterns of pancreatic damages or histopathology damages to pancreas and micrometric alterations thereof in exposed mice. Interestingly the pattern and extent of damage inflicted by the three insecticides that primarily differ on the bases of the halogen radical's they contain were unique and specific to the particular insecticide, for example the

characteristic pattern of pancreatic pathology related to CYP have shown the cells of Pancreas cause deficiency of insulin,[12] whereas DLT have shown sever hypoinsulinemia and hyperglycemia conditions might be associated to alteration of pancreatic cells which was confirmed by the 323 histopathological results[7] and most interesting the LC have caused the histopathological features which related to LC features were unique and highly specific to these insecticides showing alteration in the pancreatic tissues Oxidative stress and decreased content of free radical are the consequences of lambdacyhalothrin toxicity.[17,18]

These pathological features which seem to be insecticides related may be caused by there on specific mode of toxicity, that may be overlapping with each other but also have certain toxicological effects of their own which may merely be align upon the specific halogen contain in them as mention above. The overlapping features must be various damages to the membrane and membranous organelles, cytoplasmic enzymatic system and the oxidative stress related changes. As DLT and LC respectively contain bromine and fluorine in their chemical structures, the extent of tissue damage and the toxicity of these two insecticides was more severe (like degeneration of endocrine cells, megakaryocytes formation and macrophages infestation) as compare to extent of damage inflicted by the CYP exposure in which marginal area of islets remained more or less undisturbed.

As indicated by the micrometric result and the sign of the pancreatic histopathology.On the bases of these findings it was concluded that although three insecticides harboring pancreatic toxicity, however DLT and LC were more toxic and inflicted more severe damage to the endocrine and exocrine pancreas as compare to the CYP indicating that the no dietary halogens (fluorine and bromine) contain in these insecticides have led to increase the toxicological potential of these two insecticides as compare to type II insecticides the chlorinated type II insecticides the CYP.

CONCLUSION

The severity of the mentioned pathological sign in LC and DLT groups indicated that these non-dietary halogenated pyrethroid inflict more severe histopathological impacts on the endocrine pancreas as compare to the simple chlorinated type II pyrethroid the CYP. In this study, the toxicological effects of just three pyrethroid insecticides. Furthermore, harmful impacts of numerous other pyrethroids can be investigated as well.

ACKNOWLEDGEMENTS

The authors express their gratitude to the university of Sargodha and University of Chakwal, Pakistan for allowing and supporting this study by granting permission, providing laboratory facilities, and financial assistance.

FUNDING

Not applicable

CONFLICT OF INTERESTS

None

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