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Protective Role of *Basella rubra* Against Bifenthrin and Lambda-Cyhalothrin Induced Thyro-Pancreatic Histopathology in Mice

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ABSTRACT

This study was designed to explore the ameliorating effects of *Basella rubra* fruit extract against thyro-pancreatic histopathologies in lambda cyhalothrin and bifenthrin treated mice.

Method: Thirty mature male albino mice were divided into 6 groups as follows: (i) and (ii) Vehicle control group/VC and *Basella rubra/*Br group (0.1mL corn oil and 0.1mL *Basella rubra* extract, respectively, for days 1-2; (iii) and (iv) Lambda-cyhalothrin (Lct) and Bifenthrin (Bf) groups (5mg/kg Lct and Bf solution in 0.1mL corn oil, respectively, for days 1-2); (v) LctBr and BfBr groups (5mg/kg lambda-cyhalothrin and bifenthrin in 0.1mL corn oil, respectively, for days 1-2 and 0.1mL of *Basella rubra* solution on days 4-6); For histological study, pancreas and thyroid were excised from all animals on day 7.

Results: Lct and Bf exposure was found to cause acinus focal degeneration, islets hypertrophy and hyperplasia in pancreas, ruptured follicles, thyroglobulin depletion and abnormally enlarged follicles in thyroid gland. The micrometric outcomes revealed that relative area occupied by endocrine cells and their relative abundance was reduced in Lct and Bf (51.98±1.55 ; 61.46±1.32) groups compared to VC (88.87±2.86). Relative area occupied by endocrine cells per unit area was increased in Lct & Bf (51.98±1.55 ; 61.46±1.32) and increased in Br group (114.0±2.70) in than VC (88.87±2.86). In thyroid gland mean number follicular and C-cells were increased in Lct (229.64±9.82 ; 209.60±9.72) decreased (59.7±2.7 ; 80.4±4.2) in Bf group comparative to VC (97.80±3.70 ; 106.45±3.36).

Conclusions: Both of the glands were equally and highly affected by these pyrethroids. All these deleterious effects of Lct and Bf were markedly reversed in LctBr and BfBr groups. Use of Basella rubra showed a remarkable amelioration in Lct and Bf treated groups. Basella rubra fruit extract showed rehabilitative potential against the histopathological signs of Lct and Bf in male mice.Purpose: Insert text here.

Key-words: Lambda Cyhalothrin, Bifenthrin, *Basella rubra*, Thyro-pancreatic toxicity

INTRODUCTION

Lambda-Cyhalothrin (type-II) and Bifenthrin (type-I) are the synthetic pyrethroids which are commonly used in agriculture for pests control.[1]

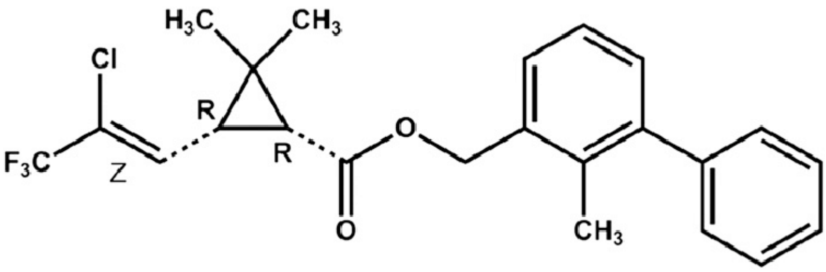

Figure. 1. Chemical structure of bifenthrin

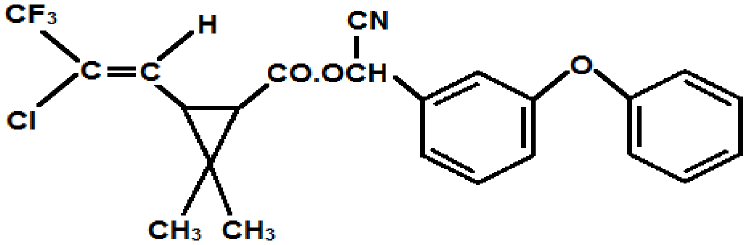

Figure 2. Chemical structure of Lambda-cyhalothrin

The toxic effects of Lambda Cyhalothrin and Bifenthrin exposure on different body organs and endocrine glands are well reported.[2,3] Although, very little is documented about Thyro-pancreatic toxicity of Lambda-cyhalothrin and bifenthrin. Pancreas is responsible for digestion by producing digestive enzymes in acinar cells and blood glucose regulation by producing glucagon, insulin and somatostatin in islets.[4] Exposure to pyrethroids toxicity disrupts the proper functioning of pancreas and thyroid.[5] Lct and Bf have injurious effects on insulin secretion and glucose digestion.[6] Lct and Bf exposure cause oxidative stress which may induce apoptosis in beta cells of pancreas and follicle cells of thyroid. These cell injuries ultimately results into diabetes and pancreatitis.[7,8] Thyroid is an endocrine gland responsible for regulating body's metabolism, development and growth.[9] Thus it was reported that λ-cyhalothrin and bifenthrin toxicity can disrupt thyroid functioning.[10]The oral LD50 of lambda cyhalothrin in mice is 19.9mg/kg.[11] While, LD50 of bifenthrin is 43mg/kg. [12] According to this study, λ-cyhalothrin and bifenthrin exposure caused various injuries e.g., disintegrated and reduced sized follicles with less colloid and overflowing blood vessels which may leads to hyperthyroidism.

Basella rubra (Nagan Bail) also known as Indian spinach, malabar spinach, red climbing spinach or vine spinach is a green leafy herb of the family Basellaceae has a significant therapeutic effect.[13] *Basella rubra* fruits consists of various phytochemicals such as β-cyanin, gomphrenin I, II and III, flavonoids, bioactive phenolics and betalains generic acid, ferulic acid, chlorogenic acid, sinapic acid and coumaric acid.[12] These flavonoid components of basella rubra have been reported to exhibit the ameliorative effects against different cytotoxicities.[5,14] Therefore, it has been considered advantageous to conduct a study to evaluate rehabilitative effects of Basella rubra against Lct and Bf inducted thyropancreatic toxicity. The objective of present study was to evaluate the rehabilitative properties of *Basella rubra* and its amelioration against thyro-pancreatic toxicity of male mice, induced by Lambda cyhalothrin and Bifenthrin. Pancreas and thyroid glands were choosen to know the toxicity of same toxicants in two different glands.

MATERIAL AND METHODS

Experimental animals and their maintenance

Thirty adult male albino mice (*Mus musculus*) were used in this study. All of these animals were housed under standard conditions, which included a temperature of 23°C, humidity (45%) and 12-12 hour dark/light cycle). Water and food were unrestrictedly available to them throughout the trial period.

Preparation of Lct and Bf solutions

Lct and Bf pyrethroid insecticides were selected for study because these are routinely used fluoridated pyrethroids and they were found to disturb the physiology and activities of endocrine glands in human.[14] Moreover they have great affinity for lipids so they can easily cross the cell membrane.

The following method was used to make 20mg/kg stock solutions: The dose needed for a 1000g animal is = 20mg

The dose necessary for each 1g of animal is = 20mg/1000g

The needed dose for a 30g animal is = 20mg/1000g×30

0.6mg/100uL = 0.6mg/0.1mL = 600mg/100mL = 0.6g/100mL

By dissolving 0.6g of both insecticides in 100mL of corn oil, 20mg/kg stock solution was obtained. From this stock solution, 10mL of 5mg/kg was made by using following formula:

$$C_1V_1 = C_2V_2$$

Preparation of Basella rubra extract

After collecting fresh *Basella rubra* fruit, pulp was separated from seeds. Juice was obtained by grinding pulp in electric grinder. To remove fibers, fruit exract was centrifuged for 35 minutes at 32g. Then extract was stored in freezer.

Basella rubra Fruits (Figure. 3)

Animals treatment groups

Thirty albino male mice were randomly divided into six groups (Table.1)

Organs recovery and their histological processing

Dissection made on 7th day to recover pancreas and thyroid and then both organs were fixed in formyl ethanol for 48hr and further processed for dehydration in various grades of alcohol, cleared in xylene, embedded in wax to cut 3-4 µm thick sections by rotatory microtome (ERMA TOKYO 422). These sections were mounted on albumin coated slides and then stained with Eosin and Hematoxylin and mounted in Canada balsam

Histological observations and micrometry

Pancreas and thyroid sections were examined and photographed (at 400×) in a mobile camera an then CorelDRAW11 was used to improve the images to present the photographic data. The micrometric measurements for relative abundance of alpha, beta and delta cells, relative area occupied by endocrine cells, mean CSA of pancreatic islets and endocrine cells, thyroid follicles, follicular cells and their nuclei, C-cells and their nuclei were obtained. Following formulas were used to collect data:

$$\textbf{CSA} = (\text{Length} \times \text{Width} / 4)\ \pi$$

Relative Area occupied by endocrine cells = CSA of endocrine cells × mean no. of endocrine cells per unit area

Relative Area occupied by follicular cells = CSA of follicular cells × mean no. of follicular cells per unit area

Statistical Data Analysis

Statistical data was analyzed through one way ANOVA & TMRT by using SPSS20 software. Results were presented as bar graphs in result section.

RESULTS

Histological Results

Histological Results of Pancreas

Histological sections of the VC group displayed normal, rounded and evenly distributed islets having aggregates of endocrine cells with prominent nuclei. The endocrine cells in islets were supplied with rich blood vessels. Fig: 4(A). In the Br group, histological sections displayed more pronounced features than those from the control group. Islets grew bigger and healthier so hypertrophy and hyperplasia were more clearly observed. Fig: 4(B). The histological section of Lct group showed severe damage to islets. Endocrine cells shrank as a result of lipid accumulation inside islets. Fig: 4(C). In Bf group, endocrine cells became enlarged so their nuclei were present more distantly as compared to the control group. There were also some apoptotic signs, such as nuclear dissolutions and distortions. Fig: 4(D)

Histological sections of LctBr group showed both degenerative as well as rehabilitative features in terms of lipid deposition inside the islets and islets emergence in endocrine area respectively. Regenerative signs in endocrine depicting mitigating potential of *Basella rubra* against lambda cyhalothrin intoxication. Fig 4(E). The histological sections of the BfBr group revealed the regenerative signs of endocrine pancreas. The size of the islets increased, and additional islets may have emerged as a result of the migration of endocrine cells from the existing islets. Fig: 4(F)

Histological Results of Thyroid

The histological sections of VC group showed that follicles were rounded and well-positioned, filled with thyroglobulin, and lined by one or two whirls of cuboidal follicular cells. C-cells were present in vicinity of thyroid follicles and surrounding the follicular cells. Fig: 5(A). The histological sections in Br group displayed the pattern similar to that was observed in the control group. Fig5(B)

In the Bf group, ruptured follicles, thyroglobulin depletion and water-filled thyroid follicles were clearly observed. Fig 5(C). The BfBr group showed remarkable rehabilitative capacity of *Basella rubra* against bifenthrin toxicity. Follicles partially filled with thyroglobulin, regeneration of follicular cells and newly emerging follicles (micro-follicles) were clearly observed. Fig 5(D). In Lct group the follicles ruptured by shrinking, converted into micro-follicles along with interstitial tissue lesions. While most of the follicles exhibit depletion of eosinophilic thyroglobulin and accumulation of colloid fluids, some fluid-filled enlarged follicles have also been seen. Fig 5(E). In the LctBr group there were mostly microfollicles of extremely small size. Few medium sized follicles were also observed. Most of the interstitial tissue lesions were found healed although few remnants of these tissue lesions were also observed. Fig 5(F)

Micrometric Results

For all micrometric parameters, the results showed a highly significant ($p \leq 0.0001$) difference among all the groups.

Micrometry of Pancreas

Mean CSA of Islets and endocrine cells were significantly increased in Lct while decreased in Bf group. In LctBr group, mean CSA of islets and endocrine cells were significantly ($p \leq 0.05$) lowered than Lct group and significantly ($p \geq 0.05$) increased in BfBr group than Bf. The relative area occupied by endocrine cells and relative abundance was significantly ($p \leq 0.05$) lowered in Lct and Bf groups comparative to VC and Br groups. In LctBr and BfBr groups, occupied area and abundance of endocrine cells were significantly ($p \geq 0.05$) lowered than LCT and Bf groups (Table 2).

Micrometry of Thyroid

Mean CSA of follicles, follicular cells and their nuclei were significantly ($p \geq 0.05$) increased in Lct group while significantly ($p \leq 0.05$) decreased in Bf group comparative to VC and Br group. Mean CSA of follicles, follicular cells and their nuclei were significantly ($p \leq 0.05$) decreased in LctBr group and significantly increased ($p \geq 0.05$) in BfBr group comparative to Lct and Bf groups. Mean CSA of C-cells and their nuclei were significantly ($p \geq 0.05$) increased in Lct and decreased ($p \leq 0.05$) in Bf group comparative to VC and Br groups. Mean CSA of C-cells and their nuclei were significantly ($p \leq 0.05$) lowered in LctBr group comparative to Lct group while significantly ($p \geq 0.05$) increased in BfBr group than Bf group (Table 3).

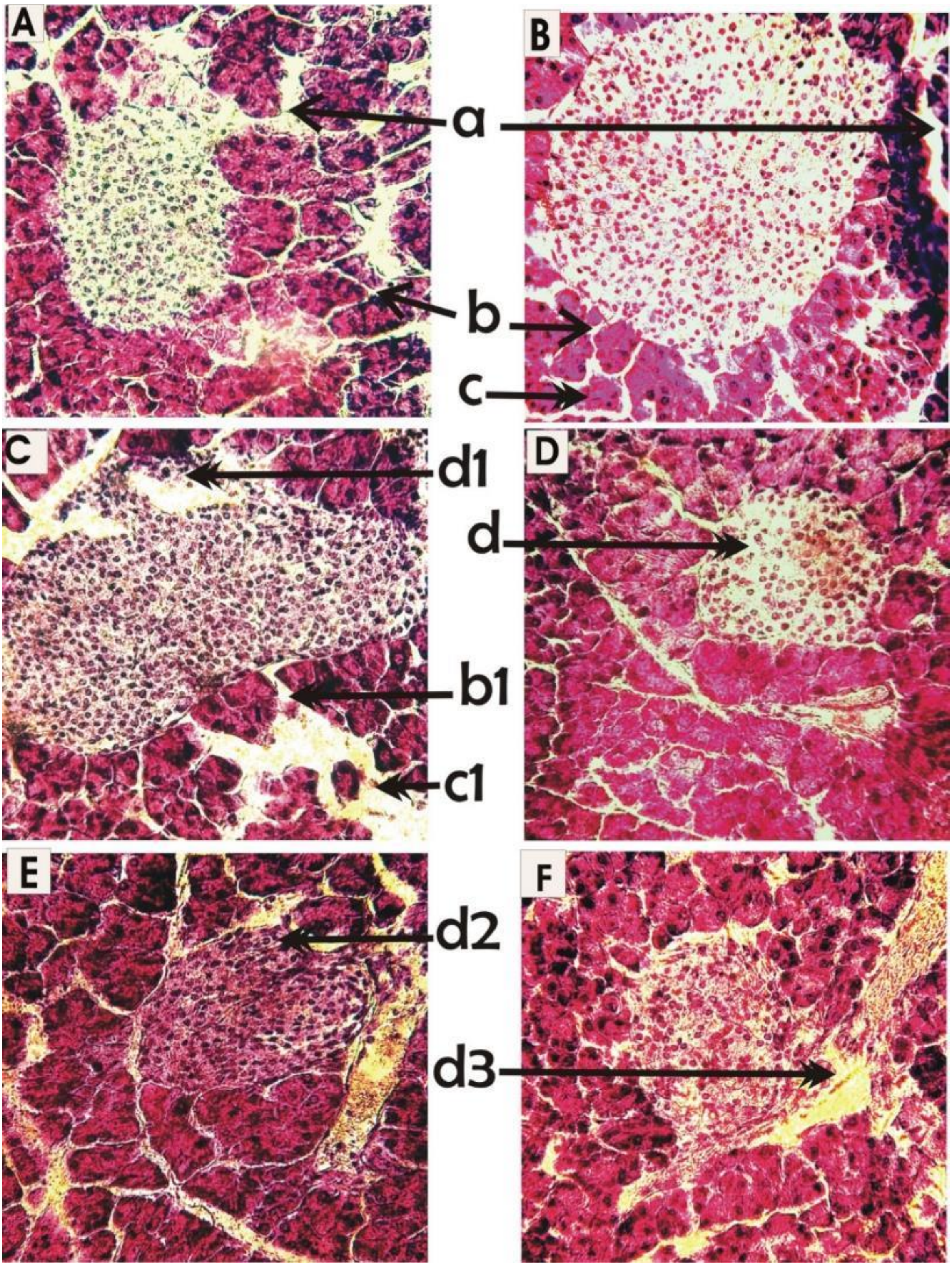

Figure 4A-4F : H & E stained Histopathological slides of the Panrease gland (400X);

A: Vehicle control; **B:** *Basella rubra*; **C:** Lambda cyhalothrin; **D:** Bifenthrin; **E:** Lambda cyhalothrin+*B.rubra*, **F:** Bifentrin+*B.rubra* [a: intralobular duct, b: intercalated duct, b1: intercalated enlarged duct, c: single acinus, c1: acinus focal degenerations d: islet of Langerhans; d1: islet of Langerhans hypertrophy; d2: islet of Langerhans probably with secondary hypertrophy, d3: islet of Langerhans with hyperplasia

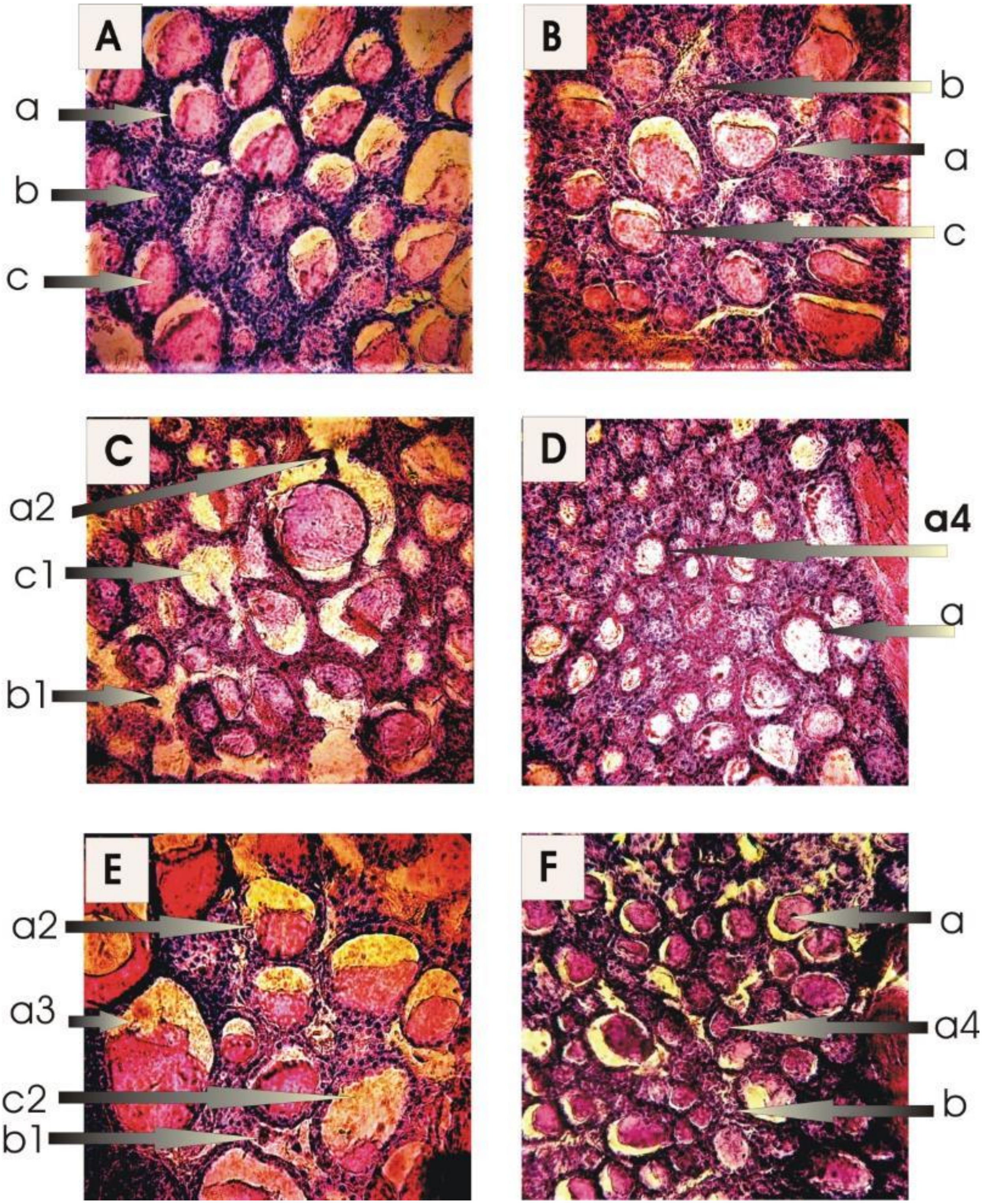

Figure 5A-5F: H & E stained Histopathological slides of the Thyroid Gland (400X)

A: Vehicle control **B**: *B.rubra* **C:** Bifenthrin **D**: Bifenthrin + *B.rubra* **E:** Lambda Cyhalothrin,**F**: Lambda Cyhalothrin + *B.rubra* Groups [a: Normal follicle, a2 Ruptured follicle, a3: abnormally enlarged follicle, a4: micro-follicles, b: C-cells , b1: tissue rupture, c: Thyroglobulin, c1: empty follicle without thyroglobulin, c2: little thyroglobulin]

Table 1. Shows brief detail of animal treatment groups

Groups	Duration of Treatment						
	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII
Vehicle control /VC	0.1mL corn oil	0.1mL corn oil	Rest	drinking water *ad labitum*	drinking water *ad labitum*	drinking water *ad labitum*	Recovery
Basella rubra /Br	0.1mL in Br	0.1 mL in Br	Rest	drinking water *ad labitum*	drinking water *ad labitum*	drinking water *ad labitum*	Recovery
Lambda-cyhalothrin / Lct	0.1mL of 5mg/kg sol of Lct in corn oil	0.1mL of 5mg/kg sol of Lct in corn oil	Rest	drinking water *ad labitum*	drinking water *ad labitum*	drinking water *ad labitum*	Recovery
Bifenthrin /Bf	0.1mL of 5mg/kg sol of Bf in corn oil	0.1mL of 5mg/kg sol of Bf in corn oil	Rest	drinking water *ad labitum*	drinking water *ad labitum*	drinking water *ad labitum*	Recovery
lambda-cyhalothrin and *Basella rubra* /LctBr	0.1mL of 5mg/kg sol of Lct in corn oil	0.1mL of 5mg/kg sol of Lct in corn oil	Rest	0.1mL Br extract and drinking water *ad labitum*	0.1mL Br extract and drinking water *ad labitum*	0.1mL Br extract and drinking water *ad*	Recovery
bifenthrin and *Basella rubra*/ BfBr	0.1mL of 5mg/kg sol of Bf in corn oil	0.1mL of 5mg/kg sol of Bf in corn oil	Rest	0.1mL Br extract and water *ad labitum*	0.1mL Br extract and water *ad labitum*	0.1mL Br extract and water *ad labitum*	Recovery

Table 2. Micrometric variations in the CSA of endocrine cells and islets, relative area occupied by endocrine cells and relative abundance of endocrine cells among different groups of pancreas (Values are mean ±SEM)

Micrometric parameters		Experimental Groups					
		VC	Br	Lct	Bf	LctBr	BfBr
CSA of endocrine cells***		1.83 ± 0.03^{b}	1.85 ± 0.04^{c}	2.53 ± 0.06^{d}	1.60 ± 0.04^{ab}	2.30 ± 0.07^{a}	1.79 ± 0.04^{b}
CSA of islets of Langerhans***		212.33 ± 11.68^{a}	228.74± 15.16^{a}	430.90 ± 20.13^{b}	182.14 ± 19.82^{a}	263.34 ± 21.40^{a}	2321.98 ± 235.97^{a}
Relative area occupied by Endocrine cells per unit area (µm$^{2)***}$		88.87± 2.86^{b}	114.0± 2.70^{d}	51.98 ± 1.55^{a}	61.46 ±1.32^{b}	67.89 ± 1.71^{b}	68.20 ± 1.78^{c}
Relative abundance of cells	Relative abundance of Alpha Cells***	11.32± 0.15^{c}	14.32± 0.15^{d}	7.32 ± 0.15^{a}	8.90 ± 0.13^{ab}	9.14 ± 0.14^{b}	10.26 ± 0.15^{b}
	Relative abundance of Beta Cells***	22.60± 0.30^{c}	25.66± 0.31^{d}	14.60 ± 0.30^{a}	16.30 ± 0.30^{ab}	19.58 ± 0.31^{ab}	21.60 ± 0.30^{bc}
	Relative abundance of Delta Cells ***	3.70 ±0.09^{c}	6.70 ± 0.09^{d}	2.06 ± 0.09^{a}	2.10 ± 0.08^{a}	3.28 ± 0.09^{b}	3.46 ± 0.07^{bc}

Mean values are significantly ($p \leq 0.05$) different among all the groups and not sharing a same lower case letter [abc].

Table 3. Micrometric variations in the CSA of follicles, follicular cells and their nuclei, C-cells and their nuclei among different groups of Thyroid (Values are mean ±SEM)

Micrometric parameters	Experimental Groups					
	VC	Br	Lct	Bf	LctBr	BfBr
CSA of Follicles***	540.65 ± 34.7^{a}	792.38± 41.4^{b}	1120.99 ± 114.8^{c}	463.06 ± 19.6^{a}	928 ± 70.9^{bc}	529.75 ± 33.8^{c}
CSA of Follicular cells ***	97.80 ± 3.70^{b}	106.45 ± 3.36^{c}	229.64 ± 9.82^{c}	59.77 ± 2.70^{a}	106.45 ± 3.36^{b}	69.15 ± 2.41^{a}
CSA of Follicular cells nucleus ***	69.15 ± 2.41^{b}	97.80 ± 3.70^{c}	229.6 ± 9.82^{d}	47.11 ± 2.17^{a}	106.45 ± 3.36^{c}	59.77 ± 2.70^{ab}
CSA of C cells**	106.45 ± 3.36^{ab}	118.74 ± 4.65^{bc}	229.64 ± 9.82^{d}	80.41 ± 4.28^{a}	132.98 ± 3.93^{c}	97.80 ± 3.70^{c}
CSA of C cells nucleus***	59.77 ± 2.70^{ab}	71.42 ± 2.43^{b}	229.64 ± 9.82^{d}	41.90 ± 1.95^{a}	97.80 ± 3.70^{c}	56.62 ± 2.37^{ab}

Mean values are significantly ($p \leq 0.05$) different among all the groups and not sharing a same lower case letter [abc].

DISCUSSION

The insecticides toxicity is a hot issue of research now a days.[15] The organo-flouride character of Lct and Bf and the highly sensitive nature of hormone producing cells in thyroid and pancreas were the main points of concern that motivated our focus for the current work. Lct and Bf have long-lasting toxic effects on thyroid and pancreas of non-target animals, including human. These two glands have high metabolic rates because of their responsibility for producing and releasing particular hormones that control body physiology.[5] Lambda cyhalothrin and bifenthrin being fluoridated pyrethroids release fluoride ions which can accumulate in pancreas and thyroid and affect their specific hormone production.[16]

Pancreas is a crucial organ for producing enzymatic and hormonal secretions for regulating digestion and blood sugar level in body. Normally, the acinars of exocrine portion releases digestive enzymes into pancreatic ducts. Duct system integrity may disturb by pyrethroids exposure. As a result, enzymatic secretions enter in interstitial spaces and this condition leads to severe tissue damage.[17]Exposure to pyrethroids results into lipid accumulation in endocrine cells and fatty acinar cells. Bifenthrin exposure can cause destruction of β-cells which leads to hyperglycemia. In this way, bifenthrin can also be responsible for diabetic conditions.[18,19]

Thyroid gland with its T3 and T4 (thyroxin) hormones is responsible for regulating the body metabolism, growth and differentiation. Exposure to lambda cyhalothrin and bifenthrin may cause thyrotoxicosis in the form of loss of membrane integrity, thyroglobulin depletion, water-filled thyroid follicles and cellular necrosis in the thyroid follicles.[20] Severe type of thyroid pathology may result into hyperthyroidism, exothalamic goiter and grave's disease.[21]

In present study, it was reported that lambda cyhalothrin and bifenthrin exposure showed a severe damage to islets of Langerhans in pancreas. Acinar cell degeneration, intercalated enlarged ducts, islets hypertrophy and hyperplasia were clearly observed in both the Lct and Bf exposed groups. Micrometric outcomes revealed that CSA of islets and endocrine cells increased in Lct and decreased in Bf group. But when we used Basella rubra along with Lct and Bf, it showed rehabilitative effects. The histological sections and micrometric outcomes revealed that LctBr group displayed a clear rehabilitation in the form of new islets emergence in endocrine area. CSA in LctBr was lowered comparative to Lct and increased in BfBr comparative to Bf group.

In BfBr group, rehabilitative signs of *Basella rubra* were also observed as the islets size increased and additional islets have also emerged as a result of the migration of endocrine cells from the existing islets. Lct and Bf exposure in thyroid gland resulted into ruptured or fluid filled enlarged follicles, thyroglobulin depletion, cellular necrosis and fibrosis. Micrometric results revealed that CSA of follicles and follicular cells increased in Lct and decreased in Bf group. CSA of follicles and follicular cells decreased in LctBr and increased in BfBr group comparative to Lct and Bf group. These outcomes were a clear evidence that *Basella rubra* post treatment has revealed marvelous signs of recovery in the pancreas and thyroid tissues. It is due to phytochemicals of *basella rubra* such as beta cyanin, gomphrenin I, II and III, betanin and isobetanin which possess rehabilitative potential against insecticides toxicities.

CONCLUSIONS

Results of this study reveal that exposure of laboratory mice with Lct and Bf at 5mg/kg/day for 7 days can result to various histopathological variations in pancreas and thyroid glands. However these pathologies can be ameliorated by using *Basella rubra*.

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FUNDING

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CONFLICT OF INTERESTS

None

REFERENCES

[1] Oyugi J, Maina N, & Rop K. Morphological Characterization of Vine Spinach (Basella Alba l. and

Rubra l.) in Western Kenya. Afri J Tech 2022;7(1):169-176.

[2] Ham J, You S, Lim W, & Song G. Bifenthrin impairs the functions of Leydig and Sertoli cells in mice via mitochondrion-endoplasmic reticulum dysregulation. Env Pol 2020;266, 115174. DOI: 10.1016/j.envpol.2020.115174.

[3] Miao J, Wang D, Yan J, Wang Y, Teng M, Zhou Z, & Zhu W. Comparison of subacute effects of two types of pyrethroid insecticides using metabolomics methods. Pest bio & Physio 2017;143, 161-167. DOI.10.1016/j.pestbp.2017.08.002.

[4] Moede T, Leibiger B, & Berggren O. Alpha cell regulation of beta cell function. Diabet 2020;63(10), 2064-2075. DOI.10.1007/s00125-020-05196-3.

[5] Ajiboye O, Diayi A, Agunbiade O, Akinyemi J, Adewale B & Ojo A. Ameliorating activity of polyphenolic-rich extracts of Basella rubra L. leaves on pancreatic β-cell dysfunction in streptozotocin-induced diabetic rats. J Comp Integ Med 2021;19(2), 335-344. DOI. 10.1515/jcim-2020-0304.

[6] Xiao X, Clark M & Park Y. Potential contribution of insecticide exposure and development of obesity and type 2 diabetes. Food Chem Tox 2017;105, 456-474. DOI. 10.1016/j.fct.2017.05.003.

[7] RojasJ, Bermudez V, Palmar J, Martínez S, Olivar C, Nava M & Velasco M. Pancreatic beta cell death: novel potential mechanisms in diabetes therapy. J Diabet Res 2018; DOI. 10.1155/2018/9601801.

[8] Mancino G, Miro E, & Dentice M. Thyroid hormone action in epidermal development and homeostasis and its implications in the pathophysiology of the skin. J Endoc Invest 2021;44(8), 1571-1579. doi.10.1007/s40618-020-01492-2.

[9] Guo D, Liu W, Qiu J, Li Y, Chen L, Wu S & Qian Y. Changes in thyroid hormone levels and related gene expressions in embryo–larval zebrafish exposed to binary combinations of bifenthrin and acetochlor. Ecotox 2020; 29(5), 584-593. doi.10.1007/s10646-020-02206-3.

[10] Pirahanchi Y, Tariq MA, Jialal I. Physiology, Thyroid. 2023 Feb 13. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 30137850.

[11] Yang Y, Wu N & Wang C. Toxicity of the pyrethroid bifenthrin insecticide. Env Chem L 2018; 16, 1377-1391.

[12] Kumar S, Manoj P, Giridhar P, Shrivastava R & Bharadwaj M. Fruit extracts of *Basella rubra* that are rich in bioactives and betalains exhibit antioxidant activity and cytotoxicity against human cervical carcinoma cells. J Func Food 2015; 15, 509-515. DOI. org/10.1016/j.jff.2015.03.052.

[13] Thambirajah A, Wade G, Verreault J, Buisine N, Alves A, Langlois S & Helbing C. Disruption by Stealth-Interference of endocrine disrupting chemicals on hormonal crosstalk with thyroid axis function in humans and other animals. Env resea 2022;111906. DOI.org/10.1016/j.envres.2021.111906.

[14] Gerunova K, Bardina G, & Sechkina V. Prenatal low-dose effects of pesticides and their long-term effects. Ear Env Sc-I 2020;421(2), 022072. DOI.10.1088/1755-1315/421/2/022072.

[15] Foda S & Shams G. A trial for improving thyroid gland dysfunction in rats by using a marine organism extract. Braz J Bio 2020; 81, 592-600. DOI.org/10.1590/1519-6984.226829.

[16] Cattley C, Popp A, & Vonderfecht L. Liver, gallbladder, and exocrine pancreas. In Tox Path 2018;(pp. 451-514). CRC press.

[17] Atkinson N, Mao Y, Chan X & McCormick J. Condensation of Rubisco into a proto-pyrenoid in higher plant chloroplasts. Nature Com 2020;11(1), 1-9. DOI.org/10.1038/s41467-020-20132-0.

[18] Guglielmi V & Sbraccia P. Type 2 diabetes: Does pancreatic fat really matter?. Diabet Metab Res 2018; 34(2), e2955. doi.org/10.1002/dmrr.2955.

[19] Alengebawy A, Abdelkhalek S, Qureshi R & Wang Q. Heavy metals and pesticides toxicity in agricultural soil and plants: Ecological risks and human health implications. Toxics 2021; 9(3), doi: 42.10.3390/toxics9030042

[20] Noyes D, Friedman P, Browne P, Haselman T, Gilbert E, Hornung W & Degitz J. Evaluating chemicals for thyroid disruption: opportunities and challenges with in vitro testing and adverse outcome pathway approaches. Env health persp 2019;127(9), 095001. DOI. 10.1289/EHP5297.

[21] Tomer M, Porter A, Boomer B, James E, Kostel A, Helmers J & McLellan E. Agricultural conservation planning framework: Developing multipractice watershed planning scenarios and assessing nutrient reduction potential. J Env Quali 2015;44(3), 754-767. DOI. 10.2134/jeq2014.09.03