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Protective Effects of *Basella rubra* Against Fluoridated Pyrethroid Insecticide Induced Reproductive Health Toxicity in Mice

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Accepted: 2023 Nov 2
Epub as e251: 2023 Nov 2

ABSTRACT

Purpose: The present research work was designed to explore the effects of Bifenthrin (BF) and Lambda-Cyhalothrin (Lct) (fluorinated type II pyrethroid) insecticides on testicular histopathologies and their amelioration on the post-treatment of *Basella rubra* extract (Br).

Methods: Sixty male albino mice were divided into six groups (n=10) as follow: 1: Control group (CO) received 0.1mL corn oil on day 1 & 2, no treatment (day 3) and drinking water (day 4-6) through gavage; 2: *Basella rubra* group (Br) were given Br extract on day 1 & 2, no treatment (day 3) and drinking water (day 4-6); 3,4:Bifenthrin (BF) and Lambda-Cyhalothrin (Lct) were treated with their respective 5mg/kg insecticides dissolved in CO on day 1 & 2, no treatment (day 3) and drinking water (day 4-6); 5,6: Lct+Br and BF+Br were given respective insecticides on day 1 & 2, no treatment (day 3) and Br (day 4-6). Both testes were excised on euthanasia and processed on day 7th.

Results: Both insecticide-exposed groups (BF and Lct) had extensive damage to seminiferous tubules and interstitial tissues. Empty spaces in the tubules and interstitial tissues, wavy borders of the tubules, decrease in spermatids with immature spermatozoa, and demaged spermatogonia was also observed in both insecticides treated groups. Simultaneously, the mean cross sectional area of seminiferous tubules also showed significant ($p<0.05$) increase in Lct and BF than CO and Br groups. Altogether, results demonstrated that BF and Lct causes detrimental effects on testes and may influence fertilization, decrease spermatids and spermatozoa and in Lct and Bf respectively as compared to CO, and damaged spermatogonia were observed.

Conclusions: Based upon these findings it is concluded that BF and Lct exposure cause alterations in testicular histology that can be rescued through Br treatment indicating the importance of *Basella rubra* fruit in male reproductive health.

Key-words: *Basella rubra; Testosterone; Pyrethroid; Semniferous tubules*

INTRODUCTION

Pesticides are biological or chemical substances that are used to eliminate, repel, prevent, attract or control pests[1]. Pesticides cover a broad spectrum of chemicals used as insecticides, herbicides, molluscicides, rodenticides, fungicides and nematicides[2]. Pyrethroids, organochlorines, organophosphates and carbamates are all important insecticides families. Pyrethroids act as endocrine disruptors which are responsible for impairment of male reproductive system and damage its physiology[3,4].

Basella rubra is an abundantly available leafy vegetable in almost all places of Asian countries. It is used in traditional medicine for various purposes[5]. The aqueous plant extracts of *B.rubra* have healthy effects on male reproductive function in male albino wistar rats. So *B.rubra* enhance weight of seminal vesicles, serum level of testosterone, in vitro testosterone production, the activity of prostatic acid phosphatase and concentration of spermatozoa in the lumen of the seminiferous tubules significantly increased[6].

Basella rubra extracts have a strong antioxidant potential (Table.1) and are rich in natural colourant qualities, suggesting that natural pigments from fruits could be used as a bioactive ingredient like β-cyanin, gomphrenin I, gomphrenin II, and gomphrenin III[7], in associated product development[8]. Therefore, aims of the present study were to provide information about the ameliorative potential of *Basella rubra* fruit pulp extract against pancreas histopathologies develop by pyrethroid insecticides exposure in albino mice.

MATERIALS AND METHODS

Dose Groups

The present study was conducted on 60 male albino mice (*Mus musculus*) reared in animal house of The Department of Zoology, University of Sargodha that were divided into 6 groups.

- **Control (CO) group:** Received 0.1mL corn oil (CO) on day 1 & 2, no treatment (day 3) and drinking water (day 4-6) through gavage.
- ***Basella rubra* (Br):** This group given Br extract on day 1 & 2, no treatment (day 3) and drinking water (day 4-6).
- **Bifenthrin and Lambda cyhalothrin (BF and Lct**): Both groups were treated with their respective 5mg/kg insecticides dissolved in corn oil on day 1 & 2, no treatment (day 3) and drinking water (day 4-6).
- **Lct+Br and BF+Br**: Both of the groups were given respective insecticides on day 1 & 2, no treatment (day 3) and Br (day 4-6). All animals were dissected on day 7th.

Fruit extract and dose preparation

Frozen *Basella rubra* fruits were thawed at 5°C for 12 h then crushed in a mortar and pestle. Fruits were macerated and centrifuged at 15000rpm at 8°C for 10min at ≈ 15±1°C. Dose of 0.1mL given to each animal in Br group on day 1 and 2 while in Lct+Br & BF+Br group 0.1mL fruit extract was provided from day 4-6.

Sampling, Histology and Data Analysis

Each animal was dissected at 7th day. All the animals were weighed before dissections. Both testes from each animal were excised on euthanasia and processed for micrometry. Microtome slices section of 5µm placed on glass slides followed by staining process using E & H staining. Binocular microscope used to observed slides. One-Way ANOVA is used to analyze the data.

RESULTS

Histological Results

All of the cellular distribution landmarks in the testes were revealed by histology in the CO group. The section depicts a rounded cross section of

seminiferous tubules with concentric arrangements of spermatogonia, spermatocytes, spermatids, and maturing spermatozoa from the tubular margin to the center. Interstitial space between the tubules was densely populated by interstitial cells, which are endocrine in nature and produce male sex hormones known as androgens (Figure 1A).

The Br group showed typical histological markers of healthy testes. These distinguishing features include the rounded shape of the seminiferous tubules, as well as the normal shape and arrangement of spermatogonia, spermatocytes, spermatids, and maturing spermatozoa (Figure 1B).

The interstial tissue in Lct and Bf testicular sections was shrunken, with fluid-filled empty spaces present in place of interstitial cells. The seminiferous tubular section was greatly enlarged, revealing large empty spaces in the center with only a few spermatozoa. The spermatogonia along the basement membrane were also disrupted, with large gaps between them. The spermatocytes also have a lot of space around them (Figure 1C & 1E).

Various signs of pathology normalisation were seen in the Lct+Br and BF+Br treated groups. These include the partial regeneration of intestitial tissue and the regeneration of spermatogonia, which obviously increased the number of these cells along the basement membrane. The spermatocytes were discovered to be at various stages of rehabilitation in various sections, including almost complete normal arrangement of spermatocytes and spermatids on one hand, and enlarged vacuolated spermatocytes and number of spermatids in between two complete destruction forming a thick halo of cellular debris on the other (Figure 1D & 1F).

Micrometric Results

Mean body weight

According to statistical analysis, the mean body weight of mice treated with Lct and BF decreased by a very significant. Body weight increased in the Lct+Br and BF+Br groups compared to the insecticide-treated groups. On the other hand, there was a significant difference between the control and *Basella rubra* groups (Table 2).

Mean organ weight

The mean testicular weight of Lct and BF decreased significantly when compared to the control group. Weight gain was observed in the LCT+Br and BF+Br groups when compared to the insecticide-treated groups. Highest testes weight was observed in control group followed by *Basella rubra* group (Table2).

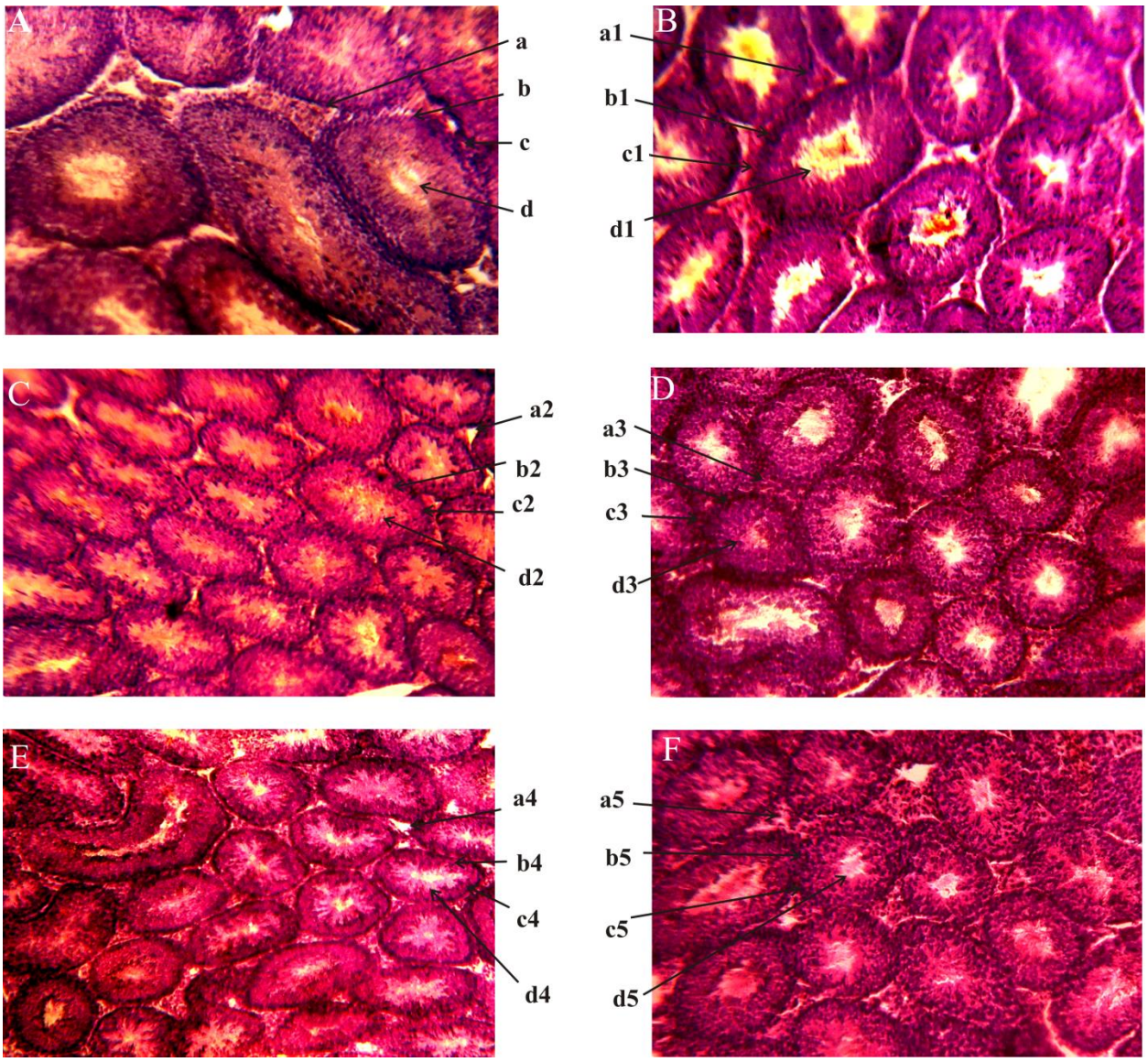

Figure 1: Hematoxylin and Eosin stained histological sections (100x) of mice testes.**A:** (CO); **B:** (Br); **C:** (Lct); **D:** (Lct+Br); **E:** (BF); **F:** (BF+Br) (a,a1: Healthy interstitial tissue) (a2,a4: damaged interstitial tissue) (a3,a5: recovering interstitial tissue); (b,b1: Healthy seminiferous tubule) (b2,b4: damaged seminiferous tubule) (b3,b5: recovering seminiferous tubule) (c,c1:Normal spermatogonia)(c2,c4: damaged spermatogonia); (c3,c5:recovering spermatogonia); (d,d1: Normal lumen with spermatozoa) (d2,d4: wide lumen with dead spermatozoa) (d3,d5: moderate lumen with spermatozoa)

Mean number of spermatogonia per unit circumference of seminiferous tubule

Data analysis using one-way ANOVA for the mean number of spermatogonia per unit circumference of the seminiferous tubule reveals a highly significant ($p \leq 0.0001$) difference between groups. The control group has the highest spermatogonial cells, while Bifenthrin has the least spermatogonial cells per unit area. Br group also have large number of spermatogonial cells compared to insecticides as well as insecticides plus Br groups. In comparison to the insecticides treated groups, the Lct+Br and BF+Br treated groups showed significantly increased the number of spermatogonia per unit area (Table 2).

Mean number of leydig cells per unit area

The number of leydig cells per unit area was highest in the control group (14.04±0.32), followed by the Br group (12.02±0.24). On the other hand, BF has the fewest leydig cells (9.00±0.19), followed by the Lct group (10.00±0.18), while Lct+Br (11.04±0.16) and BF+Br (10.72±0.16) have fewer leydig cells than the control but more than the insecticide-treated groups (Table 2).

CSA of seminiferous tubules

The mean CSA of seminiferous tubules in the control and Br groups was significantly less (277.75µm²±11.96) and (229.96µm²±10.49) than in the other insecticide treated groups. However, Lct had the highest CSA of seminiferous tubule (670.17µm²±23.96), followed by Bf (590.17µm²±14.74). Lct+Br and BF+Br groups, on the other hand, demonstrated CSA differences between Con and insecticide-treated groups (289.51µm²±10.53) and (408.83µm²±16.43) respectively (Table 2).

Table 1. Phytochemicals of *Basella rubra* Fruit

Phytochemicals of *B.rubra* fruit	**Functions**
Sinapic Acid	Perform better scavenging agent
Ferulic Acid	Work as antioxidant
Coumaric Acid	Anti-oxidant and anti-inflammatory
Chlorogenic Acid	Antioxidant activities
Myricetin	Anti-oxidant and anti-inflammatory
Quercetin	Anti-oxidant and anti-inflammatory
Luteolin	Antioxidant
Apigenin	Anti-inflammatory and antioxidant
Kaempferol	Antioxidant defense against free radicals
Vitamin A	Essential for spermatogenesis
Vitamin C	Protect spermatogenesis

Table 2. Micrometric results of various parameters of testes (CSA= cross sectional area)

Micrometric Parameters	Groups					
	CO	Br	Lct	BF	Lct+Br	BF+Br
ł Mean animal weight at day 07 (g) **	31.5±0.3^{c}	30.4±0.5^{bc}	28.3±0.5^{a}	30.4±0.5^{bc}	28.6±0.3^{ab}	29.6±0.41^{abc}
ł Mean testes weight (mg) ***	76.4±1.6^{d}	66.9±1.33^{c}	46.6±1.5^{a}	44.5±1.3^{a}	53.8±1.45^{b}	57.7±1.28^{b}
łMean number of spermatogonia per unit area (110.25 µm^{2})	14.0±0.3^{e}	12.0±0.24^{d}	10.0±0.1^{b}	9.0±0.19^{a}	11.0±0.16^{c}	10.7±0.1^{bc}
ł Mean number of leydig cell per unit area (110.25 µm^{2}) ***	11.4±0.2^{d}	9.5±0.20^{d}	5.9±0.20^{a}	6.0±0.21^{a}	7.0±0.20^{b}	8.0±0.20^{bc}
ł CSA of seminiferous tube µm²***	277.75± 11.96^{a}	229.96± 10.49^{a}	670.17± 23.96^{d}	590.17± 14.74^{c}	289.51± 10.53^{a}	408.83± 16.43^{b}

ł: analyzed by ANOVA, *** highly significant, **: very significant, *: significant, no star: no significant difference. ^{abc} the mean values in a row not sharing a common superscript differ significantly (p≤0.05) with each other.

DISCUSSION

Male reproductive toxicology is becoming a hot topic in the modern era, particularly because environmental chemicals and toxins such as insecticides are causing severe damage to the male reproductive organs, resulting in male infertility[9,10]. Male infertility is primarily mediated by xenobiotics via disruption in hormonal regulation, as many of these insecticides have been found to mimic the proestrogenic effect[11-13] may regulate the testosterone synthesis and secretion by means of increasing oxidative stress leading to cellular necrosis in the testicular interstitial tissue[14].

Pyrethroids use in the domestic and agricultural sectors has increased in the last two decades because they are thought to be safer than other pesticide classes such as the organophosphate and carbamate groups[15-17]. Pyrethroids are classified into two groups. Permethrin, Bifenthrin, and fenvalerate are examples of type I pyrethroids without alpha cyano moiety, while cypermethrin, lambda-cyhalothrin, and deltamethrin are examples of type II pyrethroids with alpha cyano moiety[18]. Class I pyrethroids have a basic structure of cyclopropane carboxylic ester. These include allethrin, bifenthrin, permethrin, phenothrin, resmethrin, tefluthrin, and tetramethrin. Class II pyrethroids have a cyano group and cause choreoathetosis and salivation. These include cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, fenvalerate, fenpropathrin, flucythrinate, flumethrin, fluvalinate, and tralomethrin[19,20].

The increasing dependency has called upon the use of type II pyrethroids because of their better efficiency and control of pest insects in the last decade. Unfortunately, these type II pyrethroids insecticides have also been found toxic to the non-target organisms disturbing the endocrine system[21]. Exposure to pyrethroids for longer durations even at doses that are far below the residual levels present in the food consumed will result in severe damage to general physiological processes as well as reproductive function[22-25].

In the present study, the relative testicular histopathology and micrometry of two type II pyrethroids insecticides were compared. Bifenthrin is simply chlorinated were as lambda-cyhalothrin contains fluoride ions. In present study, we have noted the pyrethroid related damage to the interstitial tissue. Both fluoridated pyrethroid

insecticide LCT and BF caused enlargement of tubular sections mainly through extracellular fluid retention in the tubule as well as the interstitial spaces. The spermatogenesis was found severely damaged in both the cases. It seems that the mechanism of toxicological action of both insecticides similar. In both insecticides treated groups obvious retention of extracellular fluid inside seminiferous tubular sections was noted. Indicating the chances of retention of traces of LCT and BF inside the tubules that may keep on inflicting damage to spermatogenesis for longer period of time.

In both LCT+BR and BF+BR groups, the rehabilitative activities in terms of partial regeneration of interstitial tissue and the spermatogenic activity indicates that extract of *Basella rubra* is responsible to correct the oxidative stress related changes through its antioxidant properties making rapid corrective changes in the histopathological signs seen in both Lct and Bf treated groups. The micrometric observations are also very well in line and support the histological changes enamerated above.

CONCLUSION

On the basis of these findings, it is concluded that type II pyrethroid insecticides are potential disruptor of reproductive health. Pathological conditions were partially corrected with post-treatment of *B.rubra* extract in both cases that indicates the rehabilitative and rescuing potential of *Basella rubra.*

ACKNOWLEDGEMENTS

We are thankful to the University of Sargodha and University of Chakwal for providing the required facilities for this research work.

FUNDING

Not Applicable.

CONFLICT OF INTERESTS

None

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