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PROTECTIVE EFFECTS OF *BASELLA RUBRA* AGAINST FLUORIDATED PYRETHROID INSECTICIDE INDUCED HEPATO-NEPHRIC TOXICITY IN MALE MICE

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ABSTRACT

The present study was to examine the protective effects of *basella rubra* against fluoridated pyrethroid insecticide i.e Bifenthrin(BF) and Lambda cyhalothirin(LCT) induced hepato-nephric toxicity in male mice.

Methods: Thirty animals were placed in 6 groups (n=5) and given daily treatments for seven days. 1: Vehicle control group (VC) [0.1 mL corn oil (CO) from days 1-7]; 2: BR extract group (*Basella rubra*) [*Basella rubra* extract on day 1–2, rest on day 3 + 0.1mL drinking water on days 4-6]; 3: BF 5mg/kg group [5 mg/kg BF in 0.1mL CO on days 1 & 2, rest on day 3 and followed by 0.1mL drinking water on days 4-6]; 4: BF5mg/kg+BR (BF5+BR) group [5 mg/kg BF in 0.1 mL CO on days 1 & 2, rest on day 3 and followed by BR extract in water for days 4,5,6]; 5: LCT 5mg/kg [5mg/kg LCT in 0.1mL CO on days 1 & 2, rest on day 3 and followed by 0.1mL drinking water on days 4-6, and 6: LCT 5mg/kg+ BR (LCT 5+BR) group [5 mg/kg LCT in 0.1mL CO on days 1 & 2, rest on day 3 and followed by BR extract in water for days 4,5,6]. On day 7, all of the animals were euthanized to retrieve the kidneys and liver for histological evaluation. The dosage was provided by gavage.

Results: BF and LCT exposure to liver showed significant degenerative signs such as portal vein enlargement. The presence of many macrophages and, in rare circumstances, oval stem cells indicate the liver's natural rehabilitation process. In kidney, the BF and LCT caused with glomerular enlargement preceded by glomerular obliteration. The PCTs lost their brush border. Glomeruli were becoming scarce. Tubular tissue segments degeneration Micrometric estimations for the liver revealed that LCT caused decrease in the CSA of the central vein. Width of sinusoidal space increased in BF group while CSA of hepatocyte nucleus, marginal portal vein, marginal bile ductule and CSA of hepatic artery showed no significant variation among all the groups. In kidney , BF and LCT caused decrease in mean CSA of glomerulus, mean number of epithelial cells as compared to VC group.

Conclusions: The concomitant usage of *Basella rubra* for the subjects exposed to pyrethroids appeared to have an ameliorative impact due to its rehabilitory effect against the toxicity of BF and LCT

Key-words: *Bifenthrin; Lambda cyhalothirin; Basella rubra; hepato-nephro toxicity*

INTRODUCTION

Bifenthrin ($C_{23}H_{22}ClF_3O_2$) is third generation chiral synthetic pyrethroid insecticide. Bifenthrin has strong insecticidal properties and is frequently used as a miticide in crops, gardens, and households.[1,2] Like other insecticide Bifenthrin is considered toxic for non-targeted animals.[3] Various studies results show that BF exposure has toxic effect on body vital organs such as liver, kidneys, lungs, and the nervous system.[4-7.] Bifenthrin has a pro-inflammatory action. It is also responsible for liver and renal damage [8]

Lambda cyhalothrin ($C_{23}H_{19}ClF_3NO_3$) which belongs to class 2 of synthetic pyrethriods.[9, 10] Lambda cyhalothrin has wide use in controlling pest. Although Lambda cyhalothrin (LCT) is only slightly harmful to insects, aquatic invertebrates, and mammals, significant toxicity has been noted. In some species of chicks, lambda-cyhalothrin may be fatal at low quantities. [11]

Both Kidney and liver show have deleterious effects when exposed to LCT. LCT cause oxidative stress.[12] According to a study, rat liver inflammation and changes in lipogenic genes are brought on by LCT exposure.[13] Other studies revealed the histopathological abnormalities caused by LCT in the mice liver included chromatin and vascular congestion, liver cells deterioration and RBC accumulation.[14] A study conducted on male mice kidney shows that LCT causes tubule degradation, the activation of mononuclear inflammatory cells, and the invasion of blood corpuscles. Bowman's space is clearly changed, with faulty epithelium.[15]

Basella rubra, sometimes known as Malabar spinach, has numerous therapeutic benefits. *Basella rubra* contains a high concentration of protein, vitamins, and minerals, all of which are necessary for human growth.[16] Leaf extract is used to cure catarrh and as a laxative in children with urinary illness. Flowers can be used as an antidote to toxins. While the plant extract is utilized to treat anemia by raising WBC count. [17]

Basella rubra exposure to the kidney did not result in distal convoluted tubule (DCT) and Proximal convoluted tubule (PCT) blockage. There was no glomeruli degeneration or bleeding, or any histological damage. The tubular liner was likewise unharmed. Similar to the liver, it revealed typical hepatic cords with intact hepatocytes and was free of any injury. There were no symptoms of sinusoidal congestion or necrosis.[18]

The current study sought to investigate the ability of *Basella rubra* (BR) to mitigate the hepato-nephro histopathology's caused by BF and LCT exposure in male mice.

MATERIAL AND METHODS

Animal care

Before the experimental process of groups ethical approval was obtained from the ethical committee of Department of Zoology, University of Sargodha.

Thirty mature male mice were divided into six groups, five in each. The animals were kept in controlled environments (humidity, temperature, and light-dark cycles). Throughout the experiment, food and water were freely accessible.

Chemicals used

Sr. No.	Name	Brand
	Chemicals	
1	Lambda cyhalothrin (LCT)	Sigma-Aldrich
2	Bifenthrin (BF)	Sigma-Aldrich
3	Corn Oil	Spectrum
4	Xylene	Lab Alley
5	Hematoxylin	Sigma-Aldrich
6	Eosin	Lab Alley

Preparation of the BF and LCT solution

Includes the following steps: From the 20mg/kg stock solution, 10mL of 5mg/kg LCT and BF solution were prepared.

For animal of 1000g required dose is = 20mg

For each 1g of animal required dose is = 20mg/1000g

For animal of 30g weight required dose is

=20mg/1000g×30 = 0.6mg/30g

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

As a result of dissolving 0.6g of LCT and BF in 100ml of corn oil, we obtained a stock solution of both insecticides containing 20mg/kg. The amount of 20mg/kg solution is then required to prepare 10ml of 5mg/kg LCT and CYP solution by using a following formula.

$$C1V1 = C2V2$$

Basella rubra extract preparation

To begin, fruit from *Basella rubra* was gathered from a nursery near the University of Sargodha. The seeds were separated, and the seedless *Basella rubra* was put in a centrifuge machine, where the fruit extract was extracted.

Plant details of Basella rubra[19]

Treatment groups

(i) Control group (VC): 0.1mL (100µL) of corn oil on day 1 and 2 with rest on day 3 which followed by 0.1mL of drinking water from day 4-6 through gavage.

(ii) *Basella rubra* (BR extract) group: 0.1mL (100µL) of *Basella rubra* fruit extract mixed in water for first two days with rest on day three and 0.1mL (100µL) of drinking water for day 4, 5 and 6.

(iii)Bifenthrin (BF) group :5mg/kg BF solution dissolved in 0.1mL (100µL) of corn oil on day 1 and 2, rest on the day 3, 0.1mL(100µL) of drinking water for the day 4, 5 and 6.

(iv)Bifenthrin+ *Basella rubra* extract (BF+BR) group :5mg/kg BF solution dissolved in 0.1mL (100µL) of corn oil on day 1 and 2, rest on the day 3 receiving 0.1mL (100µL) of *Basella rubra* extract mixed in water for the day 4,5 and 6.

(v)Lambda-cyhalothrin (LCT) group:5mg/Kg LCT solution dissolved in 0.1mL (100µL) of corn oil on day 1 and 2, rest on the day 3, 0.1ml (100µL) of drinking water for the day 4, 5 and 6.

vi)Lambda-cyhalothrin+*Basella rubra* extract (LCT+BR) group: 5mg/kg LCT solution dissolved in 0.1mL(100µL) of corn oil on day 1 and 2, rest on the day 3 receiving 0.1ml (100µL) of *Basella rubra* extract mixed in water for the day 4,5 and 6.

Recovery of organs and histological steps

On the 7th day of the experiment, the animals were sacrificed via cervical dislocation in order to obtain the kidney and liver. Organs went processed through fixation, alcohol degradation, tissue clearance and wax embedding (58–60ºC). Finally processed tissues were sectioned via rotary microtome (ERMA TOKYO 422). And stained by H E.

Histological observations and digital micrometry

Digital photographs of both tissues were obtained by using 7.2MP digital camera (Sony DSC-W35) at 100× and 400× magnification. Computerized micrometry was applied for both from the digital snapshots by the accomplished of CorelDRAW11.

Data analysis and statistical application

The micrometric data were analyzed via statistical software SPSS20. ANOVA, ANCOVA, Tukey's Multiple Range Test. Micrometric parameters of liver include cross sectional area (CSA) of hepatocyte nucleus (µm^2), CSA of marginal portal vein (µm^2), CSA of marginal bile ductule (µm^2), CSA of hepatic artery (µm^2), CSA of hepatocyte nucleus (µm^2), mean CSA of central vein (µm^2), width of sinusoidal space (µm^2), area occupied by hepatic triad (µm^2), mean number of uni-nucleate hepatocytes/area. Likewise, the parameters for kidney consist of mean CSA of glomerulus (µm^2), mean CSA of PCT (µm^2), mean number of epithelial cells/areas, mean number of podocytes/areas, Peri glomerular space (µm^2).

RESULTS

Histological results of liver and kidney

The histological section of kidney from the VC group revealed nothing remarkable. At the margins, there was a normal portal triad with a central vein. Similarly, the liver slides from the BR group revealed the similar outcomes, with normal hepatocyte queues. A suitable portal triad was present. Some rehabilitators oval stem cells were found between the sinusoidal voids of the *Basella rubra* group, much like in the control group (Fig: 1A, 2A), (Fig: 1B, 2B). However, the BF and LCT displayed pathological symptoms such as portal vein enlargement and damaged hepatic cords. Hemolysis may be seen in the central vein. Both insecticidal groups, BF and LCT, suffered considerable damage, including apoptotic hepatocytes and blood flow obstruction from the portal triads to the central lobular vein. (Fig: 1C, 2C) (Fig: 1E, 2E). However, groups treated with *Basella rubra*, namely (BR+BF) and (BR+LCT), had rehabilitative effects. This includes the existence of uni- and binucleated nascent hepatocytes. Blood flow from the portal triads to the central lobular vein was improved slightly. Portal vein hypertrophy was partially reversed. In terms of the existence of regeneratory cells and macrophages, liver sections demonstrated rehabilitative activity. (Fig: 1D, 2D) (Fig: 1F, 2F).

Normal blood capillaries and arterioles were visible in the tubular segment. Similar architectural formations were seen in the kidney histological sections of the BR group. The BR group had only a small increase in

glomeruli size. In addition, there is a startling rise in the number of podocytes in the glomerular regions. (Fig: 3A) (Fig: 3B). Nephrotoxicity symptoms were observed in both the BF and LCT groups, with glomerular enlargement preceded by glomerular obliteration. The PCTs lost their brush border. Glomeruli were becoming scarce. Tubular tissue segments have been disturbed as well, showing epithelial deterioration (Fig: 3C) (Fig: 4E) The (BR+BF) and (BR+LCT) groups exhibited rehabilitative activity, which was evident due to an increase in glomeruli size. Endothelial cells tend to cling to tubules that have been repaired. The migration of podocytes was hampered. The majority of glomerular sections and tubular areas were combined. (Fig: 3D) (Fig: 3F)

Micrometric findings for the liver and kidney

Micrometric findings for the kidney showed that the Mean CSA of glomeruli remained significantly ($p<0.05$) lower in BF group than rest of all five groups. Similarly mean CSA of PCT(μm^2) remained significantly ($p<0.05$) higher in VC group than all other groups BR, BF, BR+BF, LCT and BR+LCT. Likewise, the Mean Number of Podocytes/area (8888.62μm^2) remained significantly ($p<0.05$) higher in VC than the rest of experimental groups (Table. 1).

Micrometric estimations for the liver revealed that LCT caused decrease in the CSA of the central vein. Width of sinusoidal space (μm^2) increased in BF group while CSA of hepatocyte nucleus (μm^2), CSA of marginal portal vein(μm^2), CSA of marginal bile ductule (μm^2), CSA of hepatic artery (μm^2), area occupied by hepatic triad(μm^2)showed no significant variation among all the groups(Table. 1).

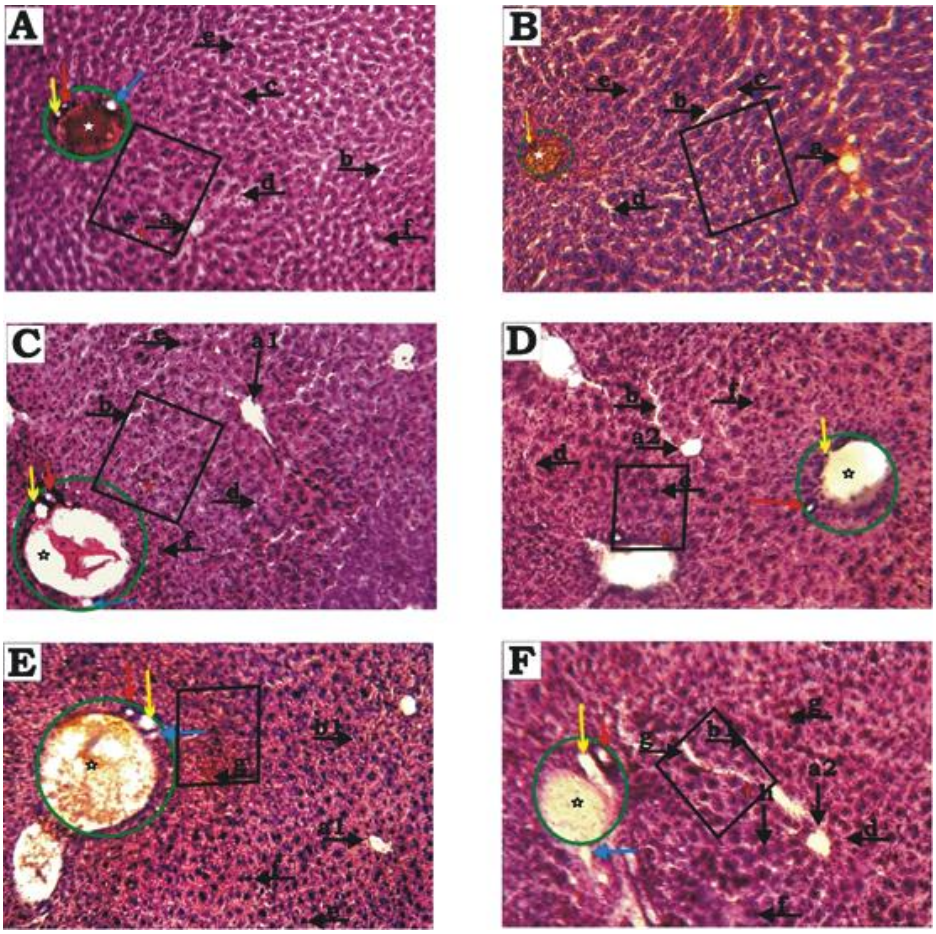

Figure1: Hematoxylin and Eosin stained histological sections (100X) of mice liver.**A**: (VC); **B**: (BR); **C**: (BF); **D**: (BR+BF); **E**: (LCT); **F**: (BR+LCT)(a: Central vein,a1:Narrow damage central hepatic vein,a2:Repaired central hepatic veins, b:sinusoid,b1:choked sinusoids, c:hepatic cords, d:Rehabilitatory oval progenitor cells, e:Uni-nucleated hepatocytes, f:Bi-nucleated hepatocytes, g: Macrophages, Green circle: Portal Triad along Hepatic portal vein, Green Circle+ White Star :Small hepatic portal vein; Green circle+ Black star: Enlarged hepatic

portal vein; Yellow Arrow: Bile ductule that is origin site of progenitor cells; Red Arrow: Hepatic arteriole, Blue Arrow: Lacteal duct, rectangle + red star, Rectangular area zoomed in fig 2

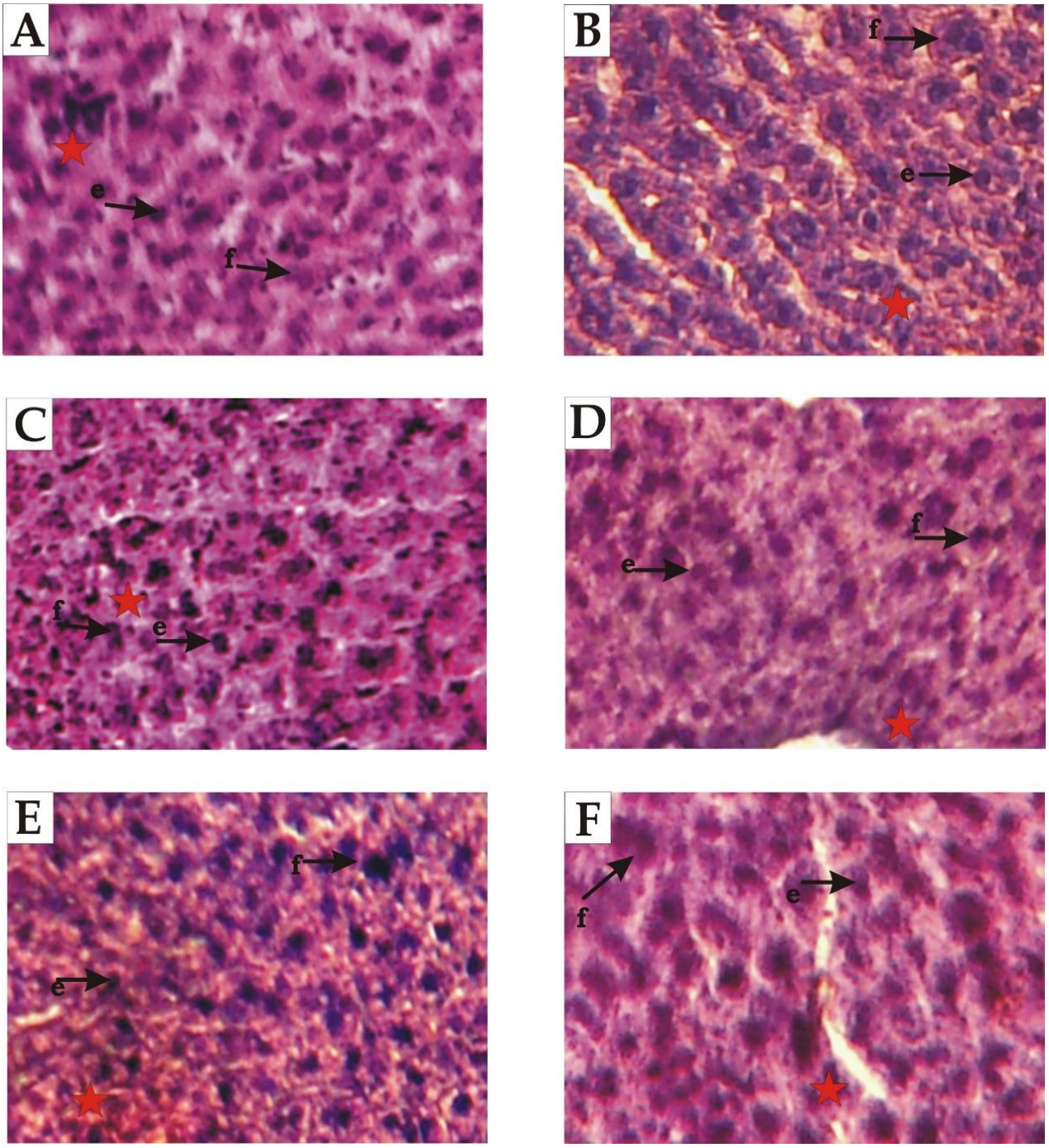

Figure2: Hematoxylin and Eosin stained histological sections (100X) of mice liver cells. **A**: (VC); **B**: (BR); **C**: (BF); **D**: (BR+BF); **E**: (LCT); **F**: (BR+LCT) e:Uni-nucleated hepatocytes, f: Bi-nucleated hepatocytes, red star shows direction of rectangular section

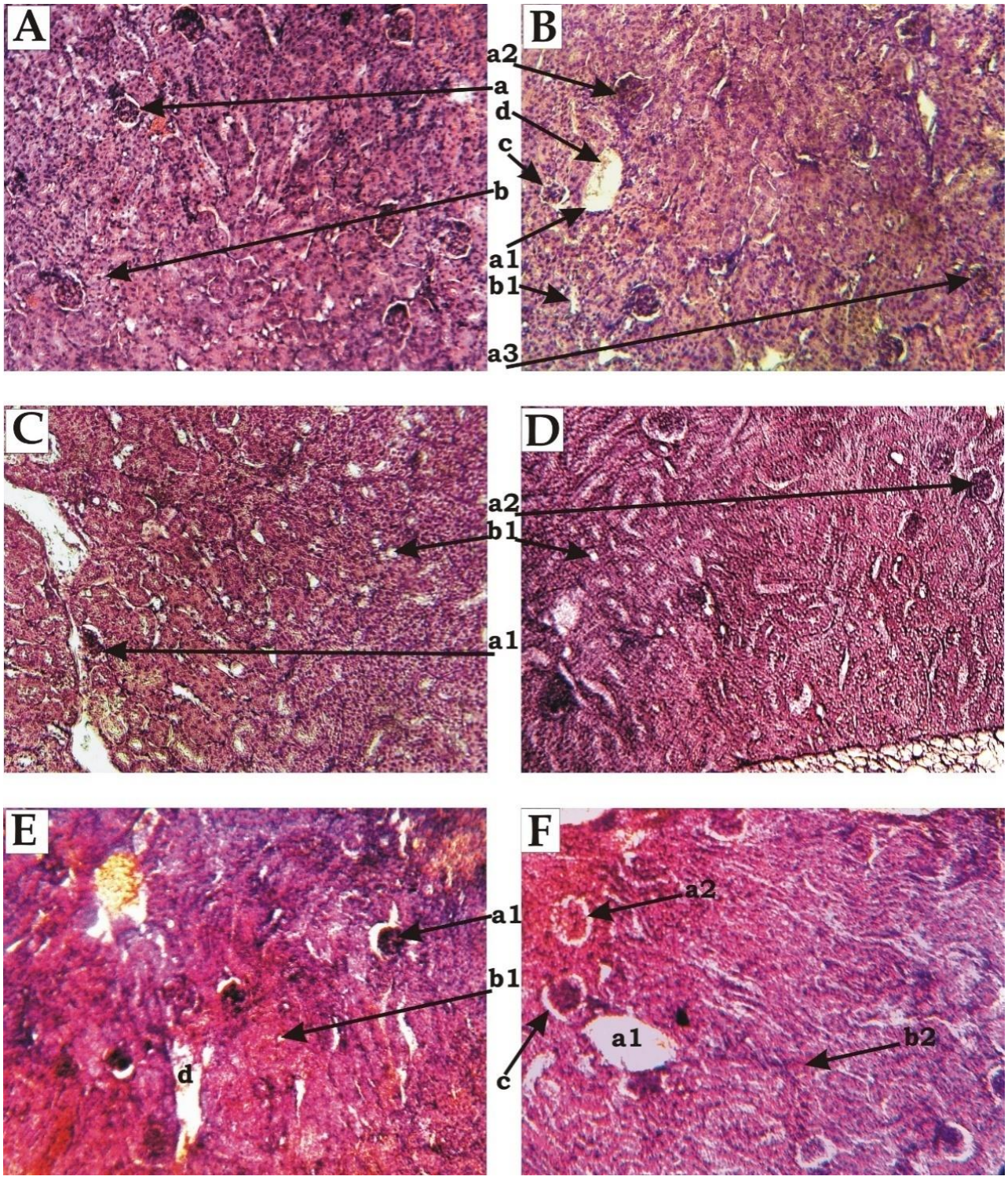

Figure3: Hematoxylin and Eosin stained histological sections of kidney (100X).**A**: (VC); **B**: (BR); **C**: (BF); **D**: (BR+BF); **E**: (LCT); **F**: (BR+LCT) **a**: normal glomerulus, **a1:** obliterated glomeruli, **a2**:regenerative glomeruli, **a3**: shrunken glomeruli, **b:** normal tubules, **b1**: damaged tubules, **b2**: repaired tubules, **c**: bowman's capsule, **d**: fibrosis, **e**: stem cells or podocytes in bowman's capsule membrane, **e1**: movement of podocytes into glomeruli, **f**: endothelial cells, **g**: bowman's space, **h:** capillaries

Table 1 : Shows Micrometric parameters related to Liver and Kidney

Micrometric Parameters	Mean±SEM					
	VC	**BR**	**BF**	**BR+BF**	**LCT**	**BR+LCT**
Mean CSA of Glomerulus (μm^2)*	512.62 ±25.35^{c}	492.63 ±25.14^{bc}	422.36 ±26.03^{b}	461.37 ±25.21^{bc}	341.758 ±25.62^{a}	473.46 ±25.87^{bc}
Peri glomerular space (μm^2)	196.34 ±23.9^{a}	243.18 ±23.73^{ab}	214.56 ±23.32^{a}	255.89 ±24.58^{ab}	241.38 ±24.35^{ab}	282.96 ±24.02^{b}
CSA of marginal portal vein (μm^2)	263.77 ± 45.56^{a}	265.82 ±45.45^{a}	295.18 ±45.26^{a}	284.28 ±45.33^{a}	298.24 ±45.50^{a}	294.37 ±45.31^{a}
CSA of marginal bile ductule (μm^2)	569.52 ±76.45^{a}	529.13 ±76.53^{a}	677.54 ±76.41^{a}	577.98 ±76.53^{a}	697.91 ±76.41^{a}	676.66 ±76.67^{a}
CSA of hepatic artery (μm^2)	475.90 ±83.58^{a}	544.18 ±83.71^{a}	551.03 ±83.86^{a}	535.99 ±83.58^{a}	545.70 ±83.62^{a}	549.83 ±83.71^{a}
Area occ-upied by hepatic triad	261.04 ±37.19^{a}	241.15 ±45.421^{a}	294.78 ±39.29^{a}	274.82 ±46.05^{a}	295.57 ±44.52^{a}	272.08 ±39.52^{a}

Micrometric variations in the various hepato-nephric parameters. CSA=cross-sectional area (length × width/4*3.14), VC= Vehicle control group, CO=corn oil, BR extract group (*Basella rubra*), BF 5mg/kg group =bifenthrin 5 mg/kg, BF5mg/kg+BR= bifenthrin 5 mg/kg+ *Basella rubra* extract, LCT 5mg/kg=Lambda cyhalothirin 5 mg/kg, LCT 5 mg/kg+ BR group= Lambda cyhalothirin 5 mg/kg+ *Basella rubra* extract All values represent the group means ± SEM (standard error of the means)

DISCUSSION

When compared to other types of pesticides, pyrethroids are noted for their exceptional insecticide efficacy and ease of biodegradability.[20] Pyrethroids are thought to be non-toxic to mammals, although many investigations have shown toxicological signs of pyrethroid toxicity in various animal taxa. Toxicity is caused by these pesticides capacity to penetrate biological membranes. As a result, they concentrate in lipid-rich internal tissues such as body fat, epidermis, liver, kidneys, and the central nervous system.[21] Fluoride ingestion in excess causes free radical injury and oxidative damage in a range of tissues. Fluoridated pyrethroids, specifically lambda cyhalothirin and bifenthrin cause oxidative damage in mice. This is due mostly to an excess of free radicals and reactive oxygen species.[22]Meanwhile, the therapeutic potential of plants has been acknowledged by practically all previous civilizations. The roots, stalks, leaves, flowers, fruits, and seeds of the plant all have therapeutic benefits.[23] The plants medicinal properties are new area of research because of their economic practicality, minimal toxicity, and broad pharmacological action.[24] The current investigation was designed to assess the efficacy of *basella rubra* against fluoridated pyrethroid insecticide-induced hepato-nephric damage in male mice.

The levels of oxidative stress are highly related with the LCT metabolites like 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane carboxylic acid (CFMP), 3-phenoxybenzoic acid (3-PBA), and 4-hydroxyphenoxybenzoic acid (4-OH-3-PBA). The accumulation of CFMP and 3-PBA in the liver has been linked to a decline in antioxidant defense's, which promotes oxidative stress.[25]

In this experimental study, the LCT and BF exposure caused hepatocyte size to increase which was accompanied by sinusoidal space dilation, and central veins enlargement, as well as cellular necrosis, portal triad injury, which caused a blockage in blood flow from the portal triads to the central lobular vein, and inflammatory cells also emerged in this course. These all changes corelate with previous studies.[26,27] Damaged and unevenly organized hepatic cords with nuclear distortions were included among the hepato lobular damage. There was also hypertrophy in the portal vein, as well as apoptotic hepatocytes and hemolysis in the central lobular vein. Unfortunately, investigation is needed to be made in regard of hepato-lobular damage caused by exposure to floridated pyrethroids.

The histopathological changes through which kidney went upon exposure to LCT and BF are that the number of glomeruli was reduced, and glomerular complete annihilation was seen. Deterioration was observed in tubular tissue sections. The brush boarder of PCT was also harmed. The glomerular filtrate accumulated in Bowman's capsule, causing it to expand. Micronuclear structures of linked podocytes were seen in various sites. Same adverse effect was reported in previous studies .[22,28] A parallel investigation on the adrenal gland yielded similar results, with zona fasciculata exhibiting cellular hypertrophy.[29]

Basella rubra extract accelerated the healing of histological abnormalities in the liver and kidney caused by exposure to BF and LCT. It was obvious due to the presence of a large number of regeneratory cells and macrophages. Blood pooling was reduced with *Basella rubra*. Nascent mononucleated hepatocytes also emerged likewise nephro-regenerative abilities were also paced by *basella rubra*. The aggregation of podocytes and the adhesion of endothelial cells around restored tubules were obvious indicators of glomeruli rehabilitation.

Phytocomponents analysis of extracts of *Basella rubra* show that Caryophyllene and Linoleic acid have hepatoprotective, nephroprotective properties[30].These properties have been approved in previous literature.[31,32,33] Antioxidants from the leaf portion of *Basella rubra* are a good substitute for artificial antioxidants. Additionally, a larger percentage of saponin has positive benefits on blood cholesterol, cancer, bone health, immune system stimulation, and anti-inflammatory properties.[34]The phytochemical components were found to be responsible for rectifying the hepato-nephric histopathologies produced by floridated pyrethroid exposure (BF and LCT).

CONCLUSIONS

The results illustrated that both floridated pyrethroid BF and LCT exposure caused histological and micrometric modifications in the liver and kidneys of mice. This study showed that *Basella rubra* extract had protective properties against the toxicity of fluoride pyrethroids such as lambda cyhalothirin and bifenthrin.

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