

## Original Research Article

### PROTECTIVE ROLE OF SOME LOCAL SPICES ON LAMBDA CYHALOTHRIN INDUCED NEPHROTOXICITY IN SWISS MICE

#### **Abstract**

**Aim:** This study was aimed at evaluating the protective role of some local spices on lambda cyhalothrin induced nephrotoxicity in Swiss mice

**Experimental design:** A completely randomized experimental design using standard methods for analysis.

#### **Place and duration of Study:**

This study was carried out in the Animal house, ~~of the~~ Department of Animal and Environmental Biology of Rivers State University, Nkpolu-Oroworukwo Port Harcourt, Nigeria. GPS 4°47'50"N 6°58'49"E. ~~This~~ study lasted for 35days.

**Methodology:** The experimental animals were separated into 6 groups (A-F) of seven mice each. Group A served as the control and Group B – F served as the test groups. At the end of the 35 day treatment period, feed was withdrawn from the mice 24 hours before termination of the experiment. Blood samples for analysis of kidney function test were collected from each mouse by ocular puncture into plain tubes. The blood was allowed to clot and centrifuged at 2500g to separate the serum from the cells. Total protein was analyzed using the spectrophotometric method of biuret, Bradford and erythrosine – b, albumin was estimated, creatinine and urea was done using enzymatic method. Vital organs were removed and weighed for organosomatic indices. Data were subjected to one way Anova using SPSS 22. Histological sections of the kidney ~~was~~ mounted on slides, stained with hematoxylin and eosin (H&E). Photomicrographs were generated.

**Results:** There is no significant difference ( $P=0.05$ ) between the organ weight and bodyweight in group A compared with the treatment groups. The values of Albumin, total protein, urea, creatinine and total bilirubin increased significantly in group B administered lambda cyhalothrin alone but decreased in groups administered the local spices. Histopathological analysis of the kidney shows the regular structure of the kidney architecture in group A, tubular degeneration and several vacuolations were observed in group B. However, less degeneration of the epithelial cells, regeneration of renal tubular epithelium were observed in groups C- F.

**Conclusion.** Based on the results from liver biomarkers and histological micrographs from this study, pronounced degeneration was observed in the kidney cells exposed to lambda-cyhalothrin only. Moreover, all spices used had a regenerating ability on the kidney tubules. It is advocated therefore that consumption of this spices either singly or in combination should be encouraged especially for those exposed to insecticides poisoning

**Keywords:** antioxidant, biomarkers, insecticides, Nephrotoxicity, spices.

## 1. INTRODUCTION

Pesticides are used all over the world to enhance food production by eradicating unwanted pests, insects and controlling disease vectors[1]. The indiscriminate use over large agricultural and urban areas have been implicated in environmental pollution with adverse health hazards. However, health hazards resulting from human exposure to these insecticides especially indirect consumption through the food chain or as fumigants in developing countries have been a growing concern to researchers across the globe. Lambda Cyhalothrin is a non-systemic [2] synthetic pyrethroids widely used for pest eradication in different areas with humans inadvertently exposed to it. Since the important of Lambda-cyhalothrin in insect control can't be overemphasized, there is great concern about the mechanism of action and its negative impact on the environment and non-target organisms.

Induction of oxidative stress is one of the main mechanisms of many of the pesticides action therefore, estimation of free radical generation ~~vis a vis~~ decreased antioxidant defense has become an important aspect of investigation in mammals [2,3,4,5,6].

Comment [A1]: ?

Several studies have shown that pyrethroids cause kidney injury, alteration in kidney biomarkers and induction of oxidative stress [7, 8, 9,10]. According to [8] an induction of kidney cell damage with a significant increase in the activities of the transaminases in Deltamethrin exposed rats, disruption of renal function causing DNA damages in pubescent female rats has been reported [10]. Exposure to subchronic concentrations of Cypermethrin showed enlargement of sinusoid, shrinkage of glomeruli, congestion of blood vessels with hemorrhage between the groups treated with 5mg/kg/bw,7.5mg/kg/bw, and 10mg of Cypermethrin.

Comment [A2]: Citation?

In the histopathological observation of kidneys exposed to varying concentrations of deltamethrin, there was a significant alterations and lesions within proximal and distal tubules of the kidney of female wistar rats administered subcutaneous injections of deltamethrin at doses 0.003, 0.03 and 0.3mg/kg/bw [9,11]. ~~More so~~ kidney function test of some farm workers showed significant ( $p<0.05$ ) elevation in serum urea and creatinine concentrations of Cypermethrin exposed farm workers compared to the control group. However, uric acid exhibited no significant difference between farm workers and controls ( $p>0.05$ ). Serum total protein, albumin and globulin were significantly increased in farm workers compared to controls ( $p<0.05$ ). In general, serum urea and creatinine concentrations increased with the farm worker's age and work duration [9,11].

The histological changes induced by 0.32 ppm/kg-bw of Endosulfan in ~~k~~kidney of albino rat was reported with [12]. ~~He reported~~ few histological changes in the cellular structure of unexposed rats or control [12].

However, large capsular space, degeneration and necrosis of renal tubular epithelia were reported in exposed animals with varying health stress conditions finally causing mortality at higher concentration.

There has been an immense effort for researchers to find a potent, natural, antioxidants from indigenous ~~plants and plant parts used as herbs~~, spices, fruits, nuts and vegetables in the reduction of oxidative stress and kidney injury because they are less expensive, readily available and exhibit no adverse side effect.

Comment [A3]: Need to check sentence formation

Comment [A4]: Citation?

Administration of lycopene along with Deltamethrin resulted in the reversal of deltamethrin-induced biochemical changes in kidney and increase in the level of renal antioxidant defense system [5]. Histopathological studies showed lycopene administration markedly reduced the toxicity of deltamethrin and preserve the normal histological architecture of the renal tissue [13,14].

**Comment [A5]:** Sentence case

-However, report also showed that oral administration of *Allium sativum* extract and Vitamin C causes ~~a~~ significant restoration of normal kidney biomarkers activities induced by Deltamethrin [13,14,15]. This researcher maintained that biological defense mechanisms against intracellular oxidative stress induced by pesticides are present in the organism as antioxidant enzymes and non-enzymatic antioxidants like including carotenoids, vitamin E, Vitamin C, glutathione and Coenzyme Q10 [15,16]. Cyhalothrin is known to produce a variety of biochemical and histological changes on non-target organisms with few researchers proffering solution to this damage. The ameliorative effect of pomegranate as treatment for albino rats exposed to lambda-cyhalothrin has been reported [14]

**Comment [A6]:** Punctuation? Sentence formation need to be checked.

**Comment [A7]:** deltamethrin

The potency of medicinal plants depends on their active phytochemical components producing several physiological actions and is responsible for their numerous bioactivities. The phytochemical constituents of the fruits of *Xylopia aethiopica* showed the presence of cardiac glycoside, flavonoids, tannins, phenols anthraquinones, saponins, and steroids but absence of terpenoids and alkaloids proving its antimicrobial and antibacterial activity [15,16,17].

**Comment [A8]:** Sentence need to be more precise with clear explanation

*Tetrapleura tetraptera* fruit is widely used in Nigeria as spice for the treatment and management of various medical conditions such as asthma, hypertension and painful arthritic inflammatory conditions. The analgesic and anticonvulsant effects of 50-800mg/kg of *T. teraptera* in mice [16].

*Piper guineense* is a West African species of pepper. The spice derived from its dried fruit is known as West African pepper, uziza pepper, ashanti pepper with the presence of flavonoids, tannins, anthraquinones, steroids, coumarins, proteins, cardiac glycoside and terpenoids. The leaves and fruits are used as flavor in most dishes due to its assumed medicinal properties. The leaves assist in the treatment of cough, bronchitis, intestinal disease, rheumatism and while the fruits are used as an aphrodisiac and contraction of the uterus [17,18]. Moreover, [19] reported *Piper guineense* possessing phytochemicals with antiplasmodial anti-plasmodial and analgesic potentials.

**Comment [A9]:** Punctuation

Several reports on the adverse effect of pesticides on the increase of kidney diseases and in both children and adults is alarming while the exact causative agent and treatment has not been known. On daily basis, people use plant natural spices without scientific knowledge of its components and effect. There is need to evaluate the effect of lambda -Cyhalothrin, a common pesticide on the kidney using these plants species as supposed remedy. The result of this research will be useful to completely understand the protective role of these spices and reducing the risk of kidney injury.

**Comment [A10]:** .

**Comment [A11]:** Repetition of words in a sentence

**Comment [A12]:** The sentence can be written more clearly

## 2. MATERIALS AND METHODS

### 2.1 Experimental Location

This study was carried out in the Animal house, ~~of the~~ Department of Animal and Environmental Biology of Rivers State University, Nkpolu-Oroworukwo Port Harcourt, Nigeria. GPS 4°47'50"N 6°58'49"E.

### 2.2 Animal Care and Management:

The animal house was properly washed and disinfected, drinkers and food trawlers were equally washed with soap and water. 42 Adult male Swiss mice of mean weight  $20.57 \pm 3.35$ g were purchased from an animal farm in Rivers State, Nigeria. The mice were housed in wire mesh cages under standard conditions (12hrs:12hd) and allowed to acclimatize for 7days before the commencement of the experiment. The mice were fed with standard pellet and clean cool water *ad libitum*. All experiments were conducted according to the institutional protocols of animal care at Rivers State University, Port Harcourt, and the standard procedure for ethical treatment of Laboratory animals.

### 2.3 Chemicals

Lambda-Cyhalothrin was purchased from a reputable chemical store in Port Harcourt. The dosage was calculated based on information of the lethal dose ( $LD_{50}$ ) from literature [20].

### 2.4 Preparation of *Tetrapleura tetraptera*, *Xylopi aethiopica* and *Piper guineese*

*Tetrapleura tetraptera* fruit, *Xylopi aethiopica* and *Piper guineese* seeds were purchased from a reputable market near the study area. The fruits and seeds were sun dried, blended into fine powder and stored for use.

### 2.5 Experimental design

The experimental animals were separated into 6 groups (A-F) of seven mice each. Group A served as the control and received water and standard pellet only. Group B – F served as the test groups. Group B received the 10mg/kg/bw/day of Lambda-Cyhalothrin only, Group C received 10mg/kg/bw/day of

Lambda-cyhalothrin and 30mg/kg/bw/day of *Tetrapleura tetraptera*. Group D received 10mg/kg/bw/day of Lambda-cyhalothrin and 30mg/kg/bw/day of *Piper guineense*. Group E received 10mg/kg/bw/day of Lambda-cyhalothrin and 30mg/kg/bw/day *Xylopiya aethiopica* while Group F received combination of 30mg/kg/bw/day of the three spices with 10mg/kg/bw/day of Lambda-Cyhalothrin.

## 2.6 Blood Collection

After the 35 days exposure of the mice, feed was withdrawn from the mice 24 hours before termination of the experiment. Blood samples for analysis of kidney function test were collected from each rat by ocular puncture modified [21] into plain tubes. The blood was allowed to clot and centrifuged at 2500g to separate the serum from the cells. Serum samples were stored in the freezer at 4°C for further analysis [22]. Total protein was analyzed using the spectrophotometric method of biuret, Bradford and erythrosine – B according to [23,24] Albumin was estimated using the method of [25] modified by [26]. Creatinine and Urea was done using enzymatic method [27,28].

**Comment [A13]:** Description of the methodology can be given

## 2.7 Histopathological analysis of the Kidney

Immediately after dissection of each animal, 0.5g of the kidney was fixed in 10% neutral formalin and sectioned with a digital Rotatory Microtome (AO spencer No. 820) at 5µm. Histological sections mounted on slides was stained with hematoxylin and counter-stained with eosin (H&E) according to [29] modified by [21]. Photomicrographs were generated with a digital microscope Biosphere Miller B with an image processor DN2 – microscopy image processing software [21] at x40 magnification.

**Comment [A14]:** Briefly describe the modification done in your experiment

Data obtained from body weight, organ weight and –biochemical analysis of Kidney biomarkers were subjected to one-way ANOVA and graphs produced using SPSS 22 software.

## 3. RESULTS

### 3.1 Body weight and organosomatic indices of swiss mice co-administered with lambda-cyhalothrin and some local spices

**Comment [A15]:** Instead of some; different local spices can be used

The effect of lambda-cyhalothrin exposure administered with the local spices on the mean weight in relation to their bodyweight is shown in Table 1.

There is no significant difference ( $P>0.05$ ) between the percentage weight of liver in the control group and that in the treatment groups compared to their body weights. Also there is no significant difference in the heart weight in relation to body weight of the animals in group B compared to those in other groups.

The same trend is shown in the spleen (splenosomatic indices), Kidney (Renosomatic indices) and the Seminal Vesicle where there are no significant difference between the group administered cyhalothrin only, the control group and the groups treated with the spices i.e groups C, D, E and F.

**Comment [A16]:** was

**Comment [A17]:** repeated meaning as same trend was used previously

**Table 1: Effect of coadministration of the spices on organosomatic indices of male mice exposed to Cyhalothrin.**

**Formatted:** Line spacing: single

GRPS	BODY (g)	WT	Hepatosomatic (%)	Cardiosomatic (%)	Spleenosomatic (%)	Renosomatic (%)	Sem.Vesicle (%)
A	27.40±3.06		4.79±0.26	0.55±0.08	0.39±0.05	1.45±0.08	0.61±0.21
B	28.61±2.36		4.74±0.34	0.52±0.05	0.39±0.07	1.59±0.20	0.53±0.17
C	25.22±2.53		4.82±0.32	0.11±0.03	0.51±0.12	1.62±0.08	0.60±0.11
D	26.93±2.47		5.57±0.56	0.52±0.08	0.67±0.20	1.54±0.17	0.73±0.20
E	30.94±2.33		5.05±0.53	0.49±0.15	0.56±0.16	1.47±0.29	0.69±0.12
F	26.32±1.72		4.51±0.39	0.52±0.17	0.45±0.16	1.35±0.25	0.56±0.23
<b>P&gt;0.05</b>	<b>0.03</b>		<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.04</b>	<b>0.02</b>

**Comment [A18]:** It is in capital letters and others are in sentence case; Provide uniform format in tittle

\*Values are Mean±SD

**Comment [A19]:** Foot note need to be clear

### 3.2 Potential Nephrotoxicity by analysis of kidney biomarkers

The effect of lambda-cyhalothrin co-administered the three local spices in this study on some kidney biomarkers are shown in fig 1A-1D. The value of albumin in the control was 43.3g/dl and increased significantly ( $P=0.05$ ) to 47.01g/dl in group B, administered Cyhalothrin only. The administration of the three spices alongside Lambda-Cyhalothrin in groups C, D, E, F however recorded a non-significant values non-significant value of 45.11g/dl, 42.03g/dl, 41.14 g/dl and 45.1g/dl respectively when compared to with the control group.

The concentration of Total Protein recorded in the control group was 69.12 g/dl. When compared to those in other groups, the value increased significantly ( $P=0.05$ ) to 82.13g/dl in group B with administration of cyhalothrin only. The values of Urea (UR), Creatinine (CR), and Total Bilirubin (TB) followed the same trend with a significant increase ( $P=0.05$ ) in the group exposed to Cyhalothrin but decreased in groups C-F that were co-administered the spices.

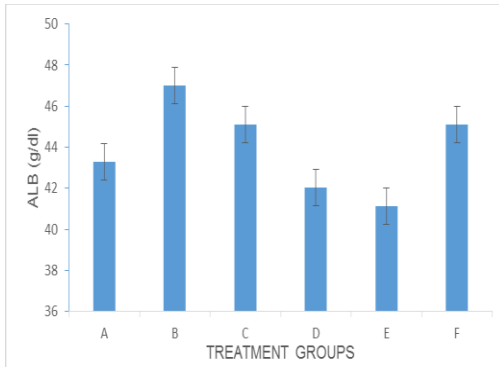


Fig 1A: Concentration of Albumin (ALB g/dl) in the treatment groups

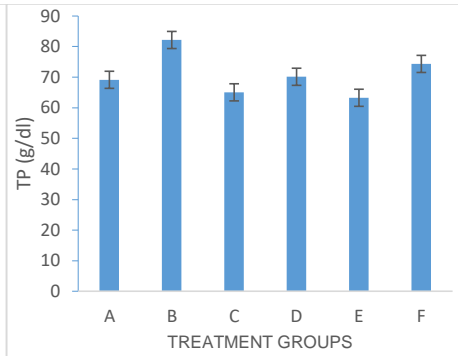


Fig 1B: Concentration of Total protein (TP g/dl) in the treatment groups

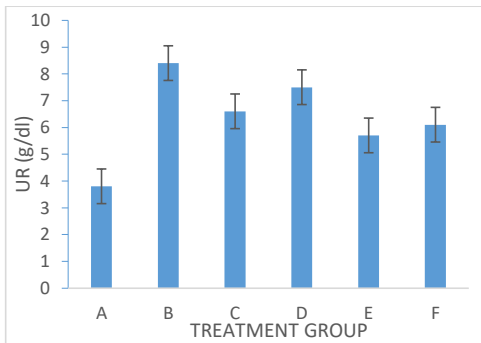


Fig 1C: Concentration of Urea (UR g/dl) in the treatment groups

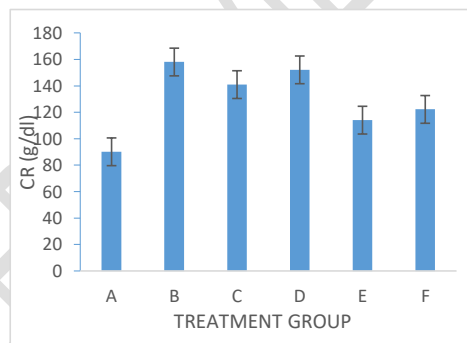


Fig 1D: Concentration of Creatinine (CR g/dl) in the treatment groups

UNDER REVIEW

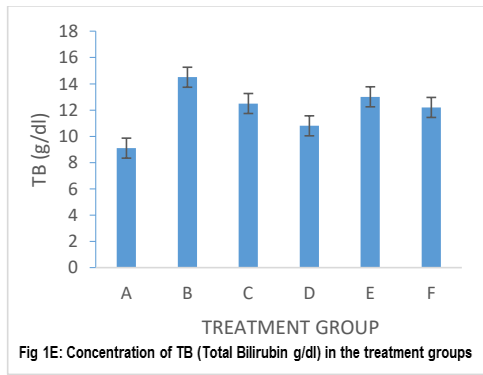


Fig 1A-1E: concentration of Albumin (1A), Total Protein (1B), Urea (1C), Creatinine (1D) and Total Bilirubin (1E) in the treatment groups

**Comment [A20]:** Fig 1: Analysis of kidney markers in treated group (A) Concentration of albumin; (B) Concentration of total protein, .....

### 3.3. Histopathological analysis of the kidney of Swiss mice exposed to lambda-cyhalothrin and local spices

Histopathological analysis of the kidney of male mice exposed to lambda-cyhalothrin and the three local [spices](#) are shown in Plate 1A-F. Plate 1A shows the regular structure of the kidney architecture showing epithelial cells, Bowman capsule's space regular structure of capillaries, tubules and glomeruli of animals in the control group. Close examination of the micrograph in Plate 1B shows tubular degeneration and several vacuolations, alteration in Bowman's, space impaired epithelium Mononuclear inflammatory cells (+), infiltration of blood corpuscles. Plate 1C shows less degeneration of the areas of renal cortex containing corpuscles, presence of vacuolation in the group co-administered *Tetrapleura tetraptera*. Plate 1D shows less degeneration of the epithelial cells and restoration of renal tubular epithelium in the group co-administered *Xylopiya aethiopica*. Plate 1E shows full restoration of the unique renal tubular epithelium in the group co-administered *Piper guineese*. Plate 1F shows fully regenerated renal epithelium with Bowman's capsule space and normal cellular architecture in the group co-administered the combination of the three spices.

**Comment [A21]:** Instead of plate; figure can be used

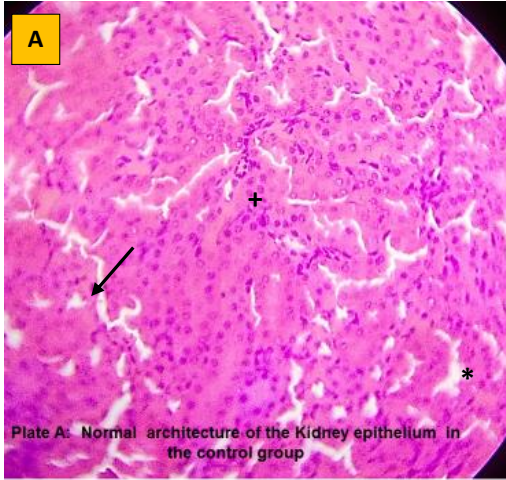


Plate A: Normal architecture of the Kidney epithelium in the control group

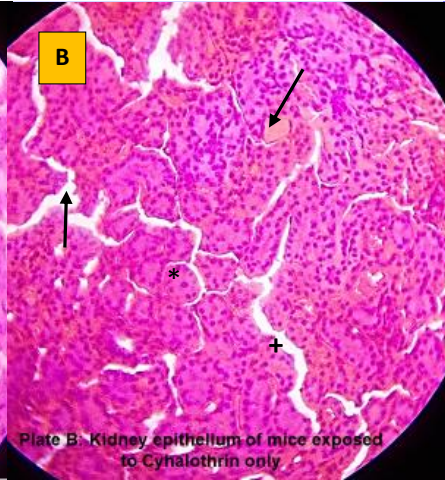


Plate B: Kidney epithelium of mice exposed to Cyhalothrin only

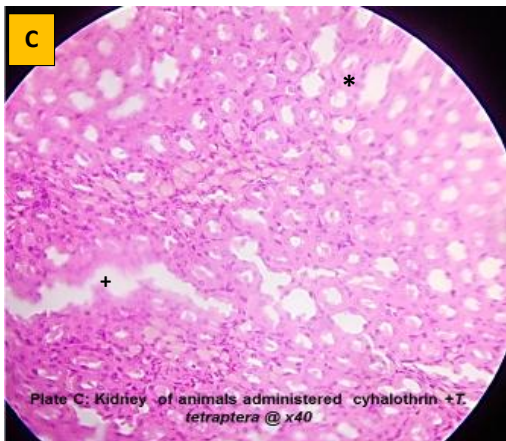


Plate C: Kidney of animals administered cyhalothrin + *I. tetraptera* @ x40



PLATE D: Kidney epithelium of animals exposed to Cyhalothrin + *P. guineensis* @40

**Comment [A22]:** Remove plate description in all images

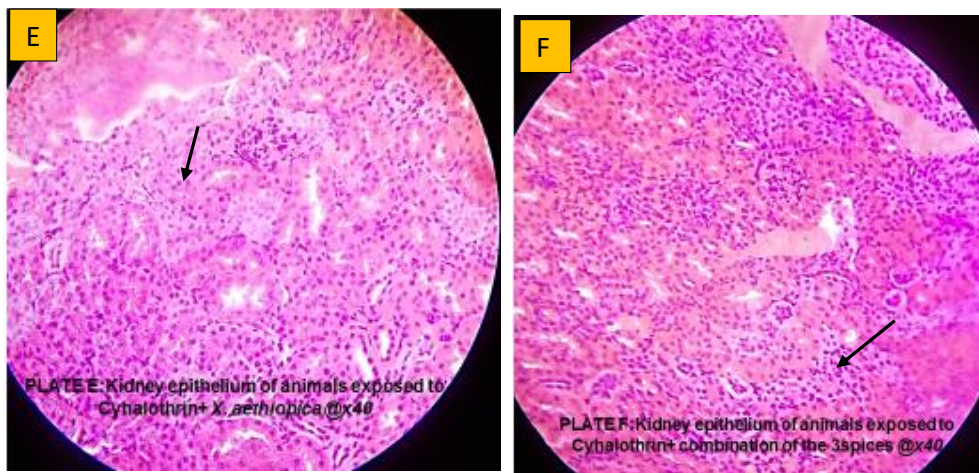


Plate 1A: Histopathological analysis of kidney of mice in the control group showing the regular structure of the kidney architecture showing +epithelial cells \*Bowman capsule's space regular structure of capillaries, tubules and glomeruli. Plate 1B: shows tubular degeneration and several vacuolations, alteration in Bowman's, space impaired epithelium mononuclear inflammatory cells (+), infiltration of blood corpuscles. Plate 1C shows less pronounced changes in areas of renal cortex containing corpuscles and associated tubules co-administered the spices, presence of epithelial vacuolation\*. Plate 1D shows less degeneration of the epithelial cells and restoration of renal tubular epithelium. Plate 1E shows full restoration of the unique renal tubular epithelium. Plate 1F shows fully regenerated renal epithelium with Bowman's capsule space and normal cellular architecture.

**Comment [A23]:** Figure -2

**Comment [A24]:** Repetition of result. The writings in the image can be removed and provided as figure caption.

#### 4. DISCUSSION

The kidney is a specialized organ that selectively removes toxic substances from the body thereby maintaining its internal environment (Homeostasis). The non-significant differences observed in the organ and bodyweight of the experimental animals shows that lambda cyhalothrin is not a systemic toxin. Therefore, it can silently destroy the vital organs of the body without conspicuous changes in the bodyweight. This finding is also supported by [2]. Cyhalothrin is a synthetic pyrethroid implicated in the induction of oxidative stress by elevation of serum total bilirubin (TB) which was significantly ( $p < 0.05$ ) higher in group B administered Cyhalothrin only than the control. The level of Urea, total protein, Albumin and creatinine were significantly ( $p < 0.05$ ) elevated in group B (fig 1). This is at variance with studies by [29] who reported a no significant difference in the Creatinine level of rats exposed to 20mg/kg/bw/day of Cypermethrin. The increase in the level of Urea in group B indicates the diminished ability of the kidney to filter its waste product from the blood and excrete them. Moreover, [2,5,6,30] also reported a significant elevation of Total Bilirubin and Creatinine known to be associated with kidney pathology including tubular necrosis in rats exposed to Deltamethrin, Cypermethrin and lambda- Cyhalothrin respectively. The histological micrographs of the kidneys of mice in the group administered lambda-cyhalothrin only shows

**Comment [A25]:** Citation?

**Comment [A26]:** Check sentence case

**Comment [A27]:** Check the sentence case

**Comment [A28]:** Sentence formation need to be checked

pronounced degeneration of the tubules, several vacuolations, and widened tubular lumen, shows tubular degeneration and several vacuolations, alteration in Bowman's space, impaired epithelium, mononuclear inflammatory cells(+) and infiltration of blood corpuscles Plate 1C-1F: show less pronounced changes in areas of renal cortex containing corpuscles and associated tubules co-administered the spices. The decrease in all the kidney biomarkers evaluated and regeneration of the renal tubules in the groups co-administered *Tetrapleura tetraptera*, *Xylopia aethiopica*, *Piper guineense* singly or in combination (Plate 1F) shows the antioxidant and regenerating ability of these spices against lambda-Cyhalothrin induced kidney injury.

**Comment [A29]:** Repeated words

**Comment [A30]:** The obtained result has to be compared with previous studies. No need to repeat the results

**Comment [A31]:** alone

## CONCLUSION AND RECOMMENDATION

Based on the results from liver biomarkers and histological micrographs from this study, lambda-cyhalothrin induced pronounced changes and degeneration in the kidney cells exposed to lambda-cyhalothrin only. Moreover, all spices used had a regenerating ability on the kidney tubules. It is advocated therefore that consumption of this spices either singly or combination should be encouraged especially for those exposed to insecticides poisoning.

**Comment [A32]:** Need more clarity

## ETHICAL APPROVAL

The experiment was conducted according to the institutional animal care protocols at the Rivers State University Nkpolu-Oroworukwo, Port Harcourt, Rivers state, Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

**Comment [A33]:** Ethical approval number?

## REFERENCES

1. Aslan, A., Cemek, M., Eser, O., Altunbas, K., Buyukokurog'lu, M. E., Cosar, M., Bas, O., Ela, Y., Fidan, H. 2009. Does dex medetomidine reduce secondary damage after spinal cord injury? An experimental study, *European Spine Journal*. 18:336–344.
2. Titus, L.K., Orlu, E.E and Obulor, A.O. (2019). Evaluation of the Therapeutic Role of *Citrullus lanatus* and *Annona muricata* Fruit Extracts on Cyhalothrin-induced Toxicity. *Journal of Advances in Biology & Biotechnology*. 22(4): 1-10,
3. Abdollahi, M., Mostafalou, S., Pournourmohammadi, S., Shadnia, S. 2004. Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to Malathion. *Comparative Biochemistry and Physiology Part C*. 137:29–34.
4. Abu El-Saad, A.M.(2011). Lycopene protects against Deltamethrin-induced Oxidative renal dysfunction in rats. *Egyptian Journal of Experimental Biology*. 7(2): 111-121.

**Comment [A34]:** Recent citation is required

**Formatted:** Centered

**Comment [A35]:** Need to follow uniform format

5. Orlu, E. E. (2014). Deltamethrin-induced Alterations in sperm morphology and spermatogenesis impairment in Adult. Sprague-Dawley Rats. *Research Journal of Applied Sciences, Engineering and Technology*, 7 (11), 2324-2331
6. Obulor, A. O & Orlu, E. E. (2018). Inhibitory Activity of lycopene on Cypermethrin-induced hepatotoxicity and liver injury in male Sprague Dawley Rats. *Journal of pharmacy and Biological Sciences*: 13; 63-73.
7. Sangha, G.K., Kaur, K., Khera, K. & Singh, B.(2011). Toxicological effects of Cypermethrin on female rats. *Toxicology International*, 18(1), 5-8.
8. Huang, C. & Li, X. (2014). Maternal Cypermethrin Exposure during the Perinatal Period Impairs Testicular Development in C57BL Male Offspring. *Public Library of Science ONE*,9(5), e96781. doi:10.1371/journal.pone.0096781.
9. Grewal, K. K., Sandhu, G. S., Ranjit, K., Brar, R. S. & Sandhu, H. S. (2010). Toxic Impacts of Cypermethrin on Behavior and Histology of Certain Tissues of Albino Rats. *Toxicology International*, 17(2), 94–98.
10. Farag, A.T., El-Aswad, A.F.& Shaaban, N.A. (2007). Assessment of reproductive toxicity of orally administered technical dimethoate in male mice. *Reproductive Toxicology*, 23(2), 232–238.
11. Mamum, A., Illa, J., Haque, F., and Ferdousi, Z., (2014). Histological study of the effect of cypermethrin on liver and kidney tissues of mice model. *Journal of pharmacy and biological science*. 9(5). 121-128
12. Sabiha, K. (2014). Study of histopathological changes induced by endosulfan in kidney of albino rats. *Journal of drug delivery and therapeutics*. 4(3).121-128.
13. El-Gerbed, M.S.A. (2014).Protective effect of lycopene on deltamethrin-induced histological and ultrastructural changes in kidney tissue of rats. *Toxicology and Industrial Health*,30(2), 160–173.
14. Yasmin, E., Abdel-Mobdy, Emam. A., Abdel-Rahim. (2015). Toxicological influences of LCT and evaluation of the toxicity ameliorative effect of pomegranate in albino rats. *Global Veterinaria*, 14(6):913-921.
15. Illusnaya, O.A.F., Odunbaku, O.A., Adesetan, O.T., Amosun, O.T. (2012). Antimicrobial activity of fruit extracts of *Xylopiya aethiopica* and its combination with antibiotics against clinical bacterial pathogens. *Journal of Biology, Agriculture and Healthcare*, 2(9); 1-9.
16. Ojewole. I.A.O and Adewumi,O.C. (2004). Anti-inflammatory and hypoglycemic effects of *Tetrapleura tetraptera*. *Journal of Ethnopharmacology*, 95,177-182.
17. Ekpo, I.A., Osuagwu, A.I., Agbor, A.B., Okpako, E.C and Ekanem, B.E (2013) "Phytochemical composition of *Afromomum melegueta* and *Piper guineense* seeds," *Journal of Current Research in Science*, vol. 1, 24–27.
18. Ejele, A.E., Duru, I.A., Oze, R.N., Iwu,I.C and Ogukwe, C.E. (2013): "Composition of antimicrobial potential of *Piper guineense*, *Ocimum gratissimum* and *Newboudia* leaves extracts," *International Research Journal Biochemistry and Bioinformatics*, 2(2), 35–40.
19. Kabiru, A.Y., Ibikunle, G.F., Innalegwu, D.A., Bola, B.M and Madaki, F.M. (2016). In vivo antiplasmodial and analgesic effect of crude thanol extract of *Piper guineense* leaf extract in Albino mice. *Scientifica*, ID 8687313, 6pp
20. Pesticide fact sheet number 171:KARATE (PP321); U.S. Environmental protection Agency, Office of Pesticide Programs, U.S.Government printing Office: Wahington,DC, 1988
21. Obulor, A. O & Orlu, E. E. (2018). Inhibitory Activity of lycopene on Cypermethrin-induced hepatotoxicity and liver injury in male Sprague Dawley Rats. *Journal of pharmacy and Biological Sciences*: 13; 63-73.
22. Orlu, E. E. & Gabriel, E. U. (2011). Liver and Plasma Biochemical profiles of male Gravid *Clarias gariepinus* (Burch. 1822) Broodstock exposed to Sublethal concentrations of aqueous leaf extracts of *Lepidagathis alopecuroides* (Vahl). *American Journal of Scientific Research*, 26, 106-115.
23. Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*: 72;248-54.

**Comment [A36]:** Some Journal name are in italics while others are in regular font

24. Soedjak, H.S. (1994). Colorimetric micromethods in protein determination with erythrosine-B. *Analytical Biochemistry*, 220, 142-148.
25. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). Total protein estimation by Lowry's method. *Journal of Biology and Chemistry*, 193, 265-270.
26. Dumas, B.T., Watson, W.A. & Biggs, H.G. (1971). Albumin standards and measurement of serum-albumin with bromocresol green. *Clinical Chimica Acta Journal*, 31, 87-92.
27. Atasayar, S., Gurer-Orhan, H., Orhan, H., Gurel, B., Girgin, G., Ozgunes, H. (2009). Preventive effect of aminoguanidine compared to vitamin on cisplatin-induced Nephrotoxicity in rats. *Experimental Toxicology and Pathology*, 6, 23-32.
28. Abu-El-Zahab, H.S., Amer, M.M., Awadallah, R., Abdel-Aal, W.E., El-Khyat, Z.A. (1993). Physiological and histopathological studies on long-term inhalation of adult alino rats to pyrethroids. *J. Egypt Ger Soc. Zool*, 11, 311-334.
29. Issam, C., Intissar, G., Fatma, B., Yahia, H.M., Samir, H., Zohra, H and Hassen, B. (2012). Oxidative Stress, Biochemical and Histopathological Alterations in the Liver and Kidney of Female Rats Exposed to Low Doses of Deltamethrin (DM): A Molecular Assessment. *Biomed Environ Sci*, 25(6): 672-683.
30. Ahmed M & Nasr H M. (2009). Dimethoate-induced changes in biochemical parameters of experimental rat serum and its neutralization by black seed ( *Nigella sativa* L.) oil, *Slovak J Anim Sci*, 42: 87-94.

UNDER PEER REVIEW