Original Research Article

HORMONAL PROFILE EVALUATION IN SWISS MALE MICE ADMINISTERED LAMBDA CYHALOTHIN AND EXTRACTS OF SOME LOCAL SPICES.

Abstract

Aim: This study was aimed at evaluating the hormonal profile of Swiss male mice administered lambda cyhalothin and extracts of some local spices.

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

Location and Duration of Study: This study was carried out in the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Rivers State, Nigeria. GPS 4^{o}47'50"N 6^{o}58'49"E The study lasted for 35days.

Methodology: Forty-two male mice were randomly selected into six (6) groups A-F (n=7/group). Group B were gavage Cyhalothin LCT) alone. Group C, D, and E were gavaged 10mg/kg/bw of LCT and 20mg/kg/bw/day of Tetrepleura tetraptera, Piper guineense, and Xylopia aethiopica respectively, while group F received 10mg/kg/bw of LCT together with the combination of the three spices. All animals were allowed access to cool clean water and standard rat pellet ad libitum. Twenty-four hours to the termination of the experiment, feed was withdrawn from the animals. Blood samples for hormonal profile evaluation were collected through ocular puncture between the hours of 7:00 and 9:00am into plain sample tubes according to the approved protocol of blood collection techniques. Analysis for the quantitative determination of all androgens was carried out using enzyme-linked immunosorbent assay (ELISA), while statistical analysis was carried out using one-way Analysis of Variance (ANOVA) and expressed as their respective units. Where significant differences were found, Pair-wise comparisons conducted with Tukey test using SPSS 20 software.

Results. Result showed a significant (P=0.05) decrease in the concentration of all androgens in group B animals treated with LCT alone. The level of these hormones were also reduced although not as low as the level in group B, with administration of the combination of all three spices in group F. This implies that the consumption of these spices might have had an antagonistic effect on the level of hormone produced when combined. Exposure to lambda cyhalothrin, affects reproductive hormones in males leading to infertility and other reproductive dysfunctions. Using spices such as tetrapleura tetraptera and Piper guineense singly as remedy can ameliorate the toxic effect of insecticide related hormonal changes under the guidance of a doctor or any healthcare professional. However, high and frequent intake of Xylopia aethiopica or the concomitant consumption of these three spices is not advised for males.

Keywords: antioxidant, hormones, insecticides, phytochemicals, spices.

1.0 Introduction

The adverse effects of insecticides occur as a result of their indiscriminate use. Pyrethroid insecticides are synthetic analogues of pyrethrins, which are natural chemicals found in chrysanthemum flowers. The use of pyrethroid insecticides have been documented since 1970s, preliminary evidence suggests that its usage is increasing (Jose *et al.,* 2010), hence the number of human exposures to pyrethroids have increased (Sudakine, 2006) resulting in reproductive toxicity, endocrine disruption and adverse immune system effects.

Environmental agents such as pesticides, initiate free radical production causing various degenerative changes in organisms. However, there are several biological defense mechanisms such as antioxidant enzymes and non-enzymatic antioxidants including carotenoids, vitamin E, Vitamin C, glutathione and Coenzyme Q10 against intracellular oxidative stress present in organisms [3,4,].

Traditionally, some herbs and spices have been used to balance hormone levels although not scientifically proven because remedies may be safe for some yet dangerous for others [5].

Some plant products such as, Nigella seeds has been reported to influence hormonal levels in the body as well as other related functions of the endocrine system. Nigella seed extract contain thymoquinone have helped regulate testosterone, luteinizing and thyroid hormone levels and exhibit estrogenic activity [6] suggesting that nigella seeds can be used as an alternative to hormone replacement therapy during menopause. Also, 600mg of concentrated ashwagandha extract has been reported to daily improve thyroid stimulating hormones in Adults, while others reported its side effects [7].

The plant, *Xylopia aethiopica* fruits is locally called “Uda” by the Igbo’s in the Southern part of Nigeria. This plant has a wild spectrum of biological activities and have played a crucial role in traditional medicine because of their valuable physiological and pharmacological properties. [8]. The fruits found to contain volatile aromatic oil, fixed oil and rutin has been reportedly used as spices, aqueous decoction especially after child birth, antidiabetic and hypoglycemic agent, probably for its antiseptic, antioxidant property[8,9]

Tetrapleura tetraptera, also used in preparation of pepper soup for mothers after labor to prevent postpartum contraction. Phytochemical analysis reveals the presence of essential oil, saponins, terpenoids, tannins, steroid, flavonoids and alkaloids [10]. The extract of *T. tetraptera* effectively

inhibited the growth of *E. coli, S. aureus* and *Proteus mirabilis* with resultant zones of inhibition of 5.00 – 18.00mm for the leaves, stem, bark and roots [10, 11]. *Piper guineense* (Uziza) are native to tropical regions of central and Western Africa and are cultivated in Nigeria where the leaves are used as a flavor for stew [12]. They contain large amount of beta-caryophyllene which is an anti-inflammatory agent and significant proportion (10%) of myristicin, elemicin, safrole and dillapoil which are believed to have preservative, antibacterial, antifungal and antioxidant properties [13].

Spices are a large group of natural ingredients which includes dried seeds, fruits, roots, rhizomes, bark and flowers plants used in very small quantities as food additives to preserve, improve colour, flavor, aroma and other organoleptic qualities of food. [14]. they have strong biological activities found to exceed those many synthetic agents which can be deleterious to health. [14].

Pyrethroids such as lambda cyhalothrin are often preferred as an active ingredient in homes, offices and agricultural insecticides because they are cost effective and long lasting than natural pyrethrums so invariably domestic animals and man become exposed to it.

Plant derived natural products have received considerable attention in recent years due to the diverse pharmacological properties including antioxidant and antitumor activity [15]. However, some of these plants parts or products can have positive or negative impact on the level of hormones in man. Report on this area of study is rare in literature and so it became imperative to evaluate the hormonal profile of male mice administered extracts of some local spices.

2.0 MATERIALS AND METHODS

2.1 Experimental Location

The study was carried out in the green house of the Department of Animal and environmental Biology, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt. (GPS 4^{o}47'50''N 6^{o}58'49''E). The experiment was conducted from January to February, 2021.

3.3 Experimental Design and Procedure

42 male mice of 7 weeks old (mean weight 20.57±3.35g) were used for the study. The animals were randomly placed into 6 groups (A – F) of 7 mice each. Group A was the negative control because they were not exposed to any treatment. Group B was the positive control and so gavaged lambda cyhalothrin alone. Group C animals were gavaged 10mg/kg/bw/day of lambda cyhalothrin and 20mg/kg/bw/day of aqueous extract of *Tetrapleura tetraptera*, Group D animals were gavaged

10mg/kg/bw/day of lambda cyhalothrin and 20mg/kg/bw/day of aqueous extract of *Piper guineense*, Group E animals were gavaged 10mg/kg/bw/day of lambda cyhalothrin and 20mg/kg/bw/day of aqueous extract of *Xylopia aethiopica* and Group F animals were gavaged 10mg/kg/bw/day of lambda cyhalothrin and 20mg/kg/bw/day of aqueous extract of the combination of all the spices in equal proportions. The mice were housed in plastic cages under standard conditions (12hL: 12hD) and acclimated for 2 weeks prior to the commencement of the experiment. All animals were fed with standard rodent pellet and cool clean water *ad libitum*. The experiment was conducted according to the institutional animal care protocols at the Rivers State Nigeria and followed approved guidelines for the ethical treatment of experimental animals.

Lambda cyhalothrin was purchased from reputable chemical company in Port Harcourt, Rivers State with 95% purity.

3.2 Preparation of Extract

The seeds of *Piper guineense, Xylopia aethiopica* and *Tetrapleura tetraptera* were obtained from Mile 3 market, Port Harcourt. The seeds were cleaned and healthy seeds were selected. The seeds were sun dried and milled into fine powder using a milling machine. The powdered spices were prepared in clean boiled water at 100^0C. This was allowed to cool before administration.

3.3 Blood Collection

At the end of the 28 day exposure period, the animals were fasted for 24hours following the administration of the last concentrations of the pesticide. Blood samples were collected by ocular puncture according to the approved protocol of blood collection techniques. Blood was collected in plain bottles without anti-coagulant and allowed to clot; thereafter the samples were centrifuged at 3000g for 10 minutes. The clear supernatant separated from the pellet was collected and stored at -4^o C until required for analysis. *Analysis for the quantitative determination of the reproductive hormones were carried out using enzyme-linked immunosorbent assay (ELISA) according to the method described by[16]*

Statistical analysis

Data from Hormonal analysis were subjected to one-way Analysis of Variance (ANOVA); where significant differences were found, Pair-wise comparisons conducted with Tukey test using SPSS 20 software.

3. RESULTS

The effect of oral administration of lambda-cyhalothin and the three local spices used in this study on the production of testosterone level in male mice is shown in figure 1a. The concentration of Testosterone reduced from 1.17±0.08µg/ml in the control group to 1.03±0.13µg/ml in group B with the administration of 10mg/kg/bw/day of lambda-cyhalothrin only. There was a significant (P=0.05) increase in the production of testosterone to 2.13±0.21µg/ml in group C with the addition of *Tetrapleura tetraptera.* A further non-significant increase was observed with the addition of *Piper guineense* at 2.53±0.51µg/ml, a decline in production to 2.49±0.18µg/ml with the addition of *Xylopia aethiopica* and a further decrease to 1.27±0.04µg/ml in group F which received the combination of all three spices.

The effect of oral administration of lambda-cyhalothin and the three local spices used in this study on the Ffollicle stimulating hormone (FSH) production in male mice is shown in figure 1b. The concentration of FSH reduced from 0.27±0.11u/l in the control group to 0.15±0.07u/l in group b with the administration of 10mg/kg/bw/day of lambda-cyhalothrin only. There was a significant increase in the production of Follicle stimulating Hormone to 0.29±0.03u/l in group C with the addition of *Tetrapleura tetraptera,* a further significant increase to 0.36±0.08 u/l with the addition of *Piper guineense*, but a decline in production to 0.22±0.14 u/l with the addition of *Xylopia aethiopica* and a further decrease to 0.19±0.03u/l in group F which received the combination of all three spices.

Fig 1c shows the level of estradiol production in male mice administered lambda-cyhalothrin with the three local spices. The concentration which was initially recorded at 142.23±2.16µg/ml in group A decreased significantly to 92.31±1.21µg/ml in group B. Upon the administration of *Tetrapleura tetraptera*, the level of estradiol increased to 109.12±2.45µg/ml and then decreased steadily to 104.11±0.98µg/ml, 100.12±0.52µg/ml and 96.12±0.66µg/ml in groups D, E, and F with the administration of *Piper guineense, Xylopia aethiopica* and a combination of the spices respectively.

Fig 1d shows the concentration of Progesterone in male mice exposed to lambda-cyhalothin and three local spices for 14days. The concentration was 10.7±0.18µg/ml in the control group but decreased to 7.21±0.92µg/ml in group B. A steady decrease was recorded in groups C,D,E,F with 8.4±0.26µg/ml, 8.6±0.19µg/ml, 8.9±0.22µg/ml and 8.7±0.04µg/ml respectively.

The effect of oral administration of lambda-cyhalothin and the three local spices on the level of Luteinizing Hormone (LH) in male mice is shown in figure 1e. The concentration of LH reduced from 0.57±0.24u/l in the control group to 0.23±0.06u/l in group B with the administration of 10mg/kg/bw/day of lambda-cyhalothrin only. There was a significant increase in the production of LH to 0.58±0.21u/l in group C with the addition of *Tetrapleura tetraptera,* a further significant increase to 0.73±0.12u/l with the addition of *Piper guineense* but a decline in production to 0.34±0.04u/l with the addition of *Xylopia aethiopica* and a further decrease to 0.29±0..08u/l in group F which received the combination of all three spices.

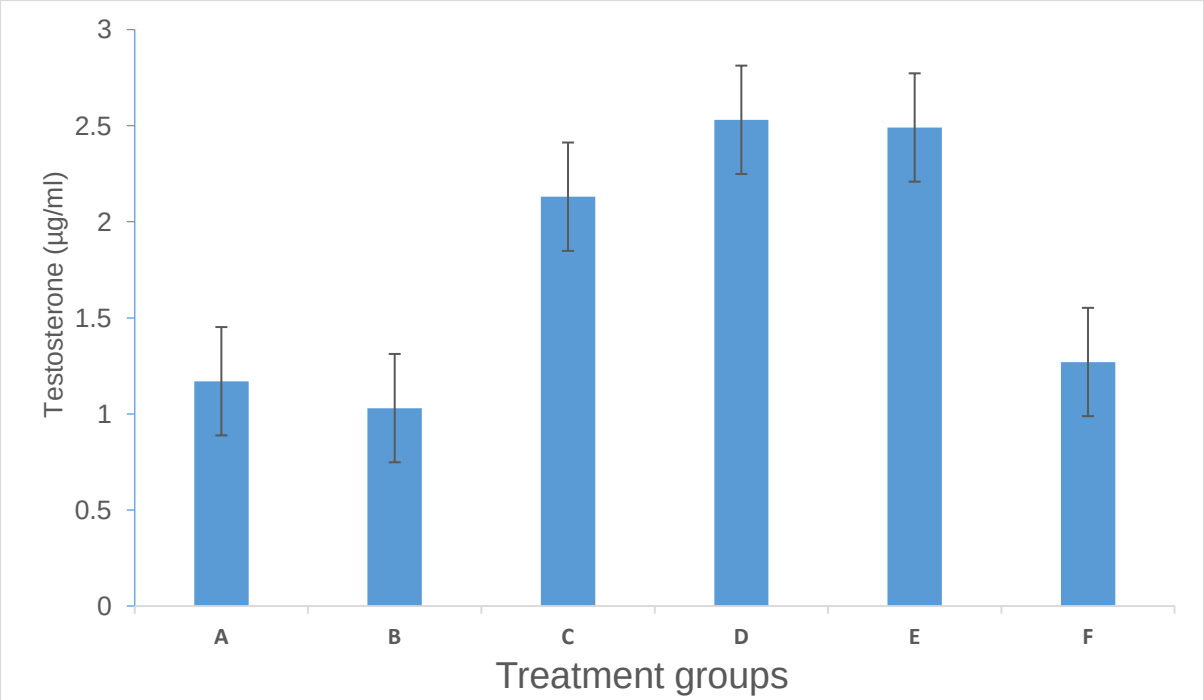

Fig 1a: Testosterone levels in mice exposed to LCT anf some local spices

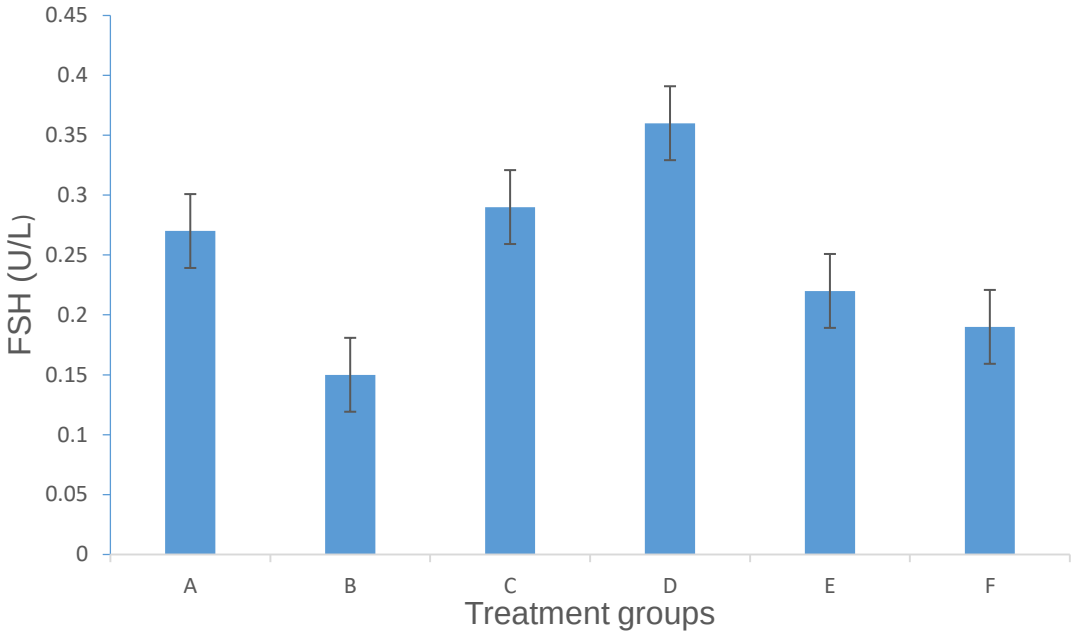

Fig 1b: Follicle Stimulating Hormone levels in mice exposed to LCT and some local spices

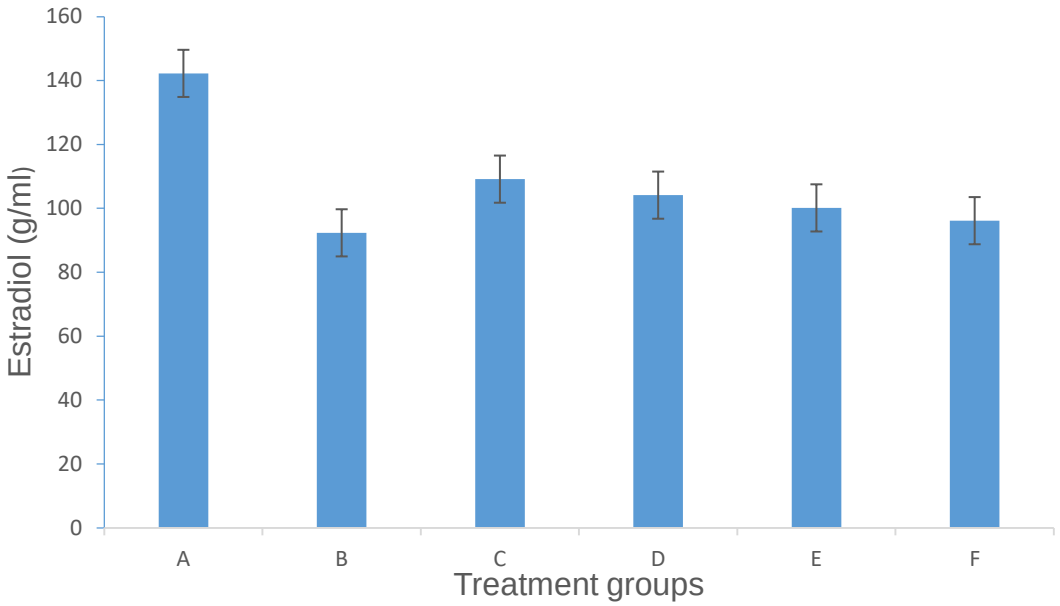

Fig 1c: Estradiol levels in mice exposed to LCT and some local spices

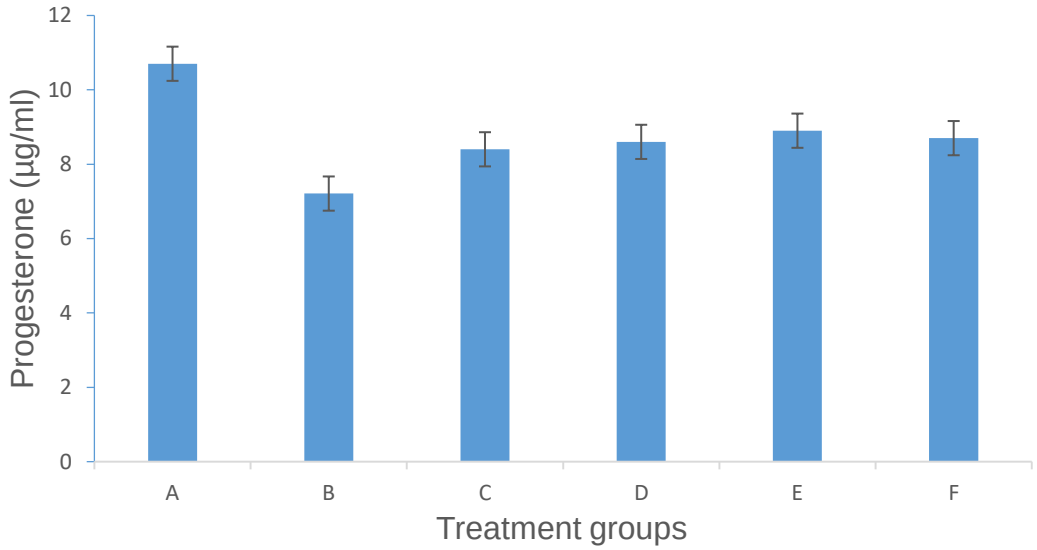

Fig 1d: Progesterone levels in mice exposed to LCT and some local spices

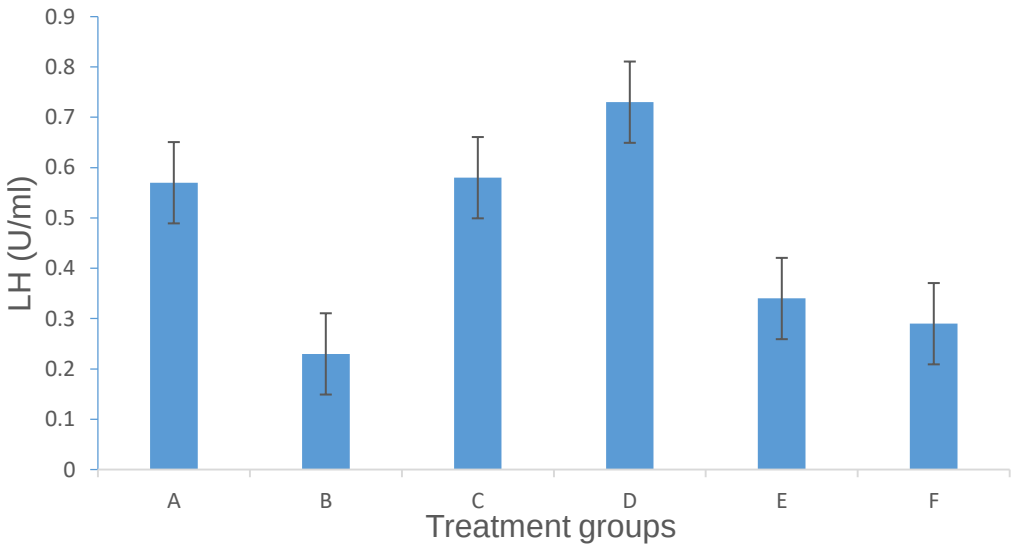

Fig 1e: Luteinizing hormone levels in mice exposed to LCT and some local spices

4. Discussion

Hormones are chemical messengers produced by glands in the endocrine system that trigger communication between cells. They are the bedrock of the endocrine system, which regulates reproduction, growth, mood swing and others. Environmental contaminants, pollutants as well as pesticides have been shown to possess the ability to interfere in the functioning of the endocrine system thereby exhibiting an anti-androgenic activity responsible for the increased incidence in various hormonal imbalance, male infertility and sexual disorders [17, 18,19,20,21]. In this study, administration of Cyhalothrin only as seen in group B resulted in a decrease in the concentration of progesterone, follicle stimulating hormone, luteinizing hormone, estradiol and testosterone - the main androgen responsible for the initiation and maintenance of spermatogenesis in mammals. The decreased in hormonal levels may lead to disruption in spermatogenic processes leading to infertility. The mechanism of action mimicking the action of natural pyrethrins which are anti-androgenic and disrupts the transcription of androgen-dependent genes reflected in the reduction of the production of testosterone [3,4,17,20]. [22] has previously reported that insecticide exposure increased free radical and oxidative stress generation in exposed animals. Therefore the decreased in all the androgens recorded in this study could be from the generation of free radicals and induction of oxidative stress by LCT

According to [23], all the common household insecticides including raid, baygon, significantly decreased the levels of serum testosterone, LH, in exposed rats compared with the control.

Group D animals exposed to extract of *Piper guineense* had the highest level of follicle stimulating hormone, luteinizing hormone and testosterone. Worthy of note, however, was the observation that the concentration of all other androgens including FSH, LH, Progesterone and Estrogens were significantly reduced although not as low as the level in group B, with administration of the combination of all three spices in group F. This implies that combination of these spices may have had an antagonistic effect on the level of hormone produced when used together. Other spices such as Nigella seeds extract which contain thymoquinone have helped regulate testosterone, luteinizing and thyroid hormone levels and exhibited estrogenic activity [5]. [7] also reported that 600mg of concentrated ashwagandha extract daily improved thyroid stimulating hormones in Adults, while others reported its side effect. The role of Coenzyme Q10 as an effective antioxidant to boost hormonal level production especially the level of androgens in rats exposed to cypermethrin was also reported [24].

CONCLUSION AND RECOMMENDATION

Based on these findings, exposure to lambda cyhalothrin, affect reproductive hormones in males leading to infertility and other reproductive dysfunctions. Using spices such as *tetrapleura tetraptera* and *Piper guineense* singly as remedy can ameliorate the toxic effect of insecticide related hormonal changes under the guidance of a doctor or any healthcare professional. However, high and frequent intake of *Xylopia aethiopica* or the concomitant consumption of these three spices is not advised for males.

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.

ACKNOWLEGEMENTS

The following people are acknowledged for their various contributions in ensuring the completion of this research: Victoria Owhor, Okachi Ngozi, Kasarachi Beauty.

COMPETING INTERESTS

Authors have declared that no competing interests exist

REFERENCES

1. Jose, J.P., Megan, K.W., Gayanga, W. (2010). Measurement of pyrethroid, organophosphate and carbamate insecticides in human plasma using isotope dilution gas chromatography –high resolution mass spectrometry. *Journal of chemotherapy B*.,analytical technologies in the biomedical and life sciences, 878 (27):2554-62.

2. Sudakine, D.L. (2006). Pyretheroids. *Clin.Toxicol.,* 44:31.

3. Orlu, E. E. and Obulor, A.O (2021). Impact of Coenzyme Q10 on Hormonal Profile in Male Sprague-Dawley Rat Exposed to Sub-Chronic Concentrations of Cypermethrin. Asian Journal of Biology. 12(3): 1-9.

4. Obulor AO, Orlu EE. Protective role of lycopene on hormonal profile and post testicular functions of male rat exposed to sublethal doses of Cypermethrin. Journal of Advance in Biology and Biotechnology. 2019; 21(4):1-9.

5. Johnkennedy, N., Adamma, E., Austin, A., Chukwunyere, N.E. (2011). Influence of Xylopia aethiopica fruits on some hematological and biochemical profile. Al Ameen J. med. Sci. 4(2): 191-196.

6. Khani, S., Abdollahi, M., Khalaj, A., Heidari, H., and Zohali, S. (2021). The effct of hydroalcoholic extract of Nigella sativa seed on dehydroepiandrosterone-induced polycystic ovarian syndrome in rats: an experimental studies. Int J Reprod Biomed, 19(3). 271-282

7. Sharma, K.a., Basu, I., and Singh, S. (2018).Efficacy and safety of Ashwagandha root extract in subclinical hypothyroid patients: a double-blind, randomized placebo-controlled trial. Journal of alternative medicine 24(3)

8. Aneyaw, Y., and Owusu-Ansah, E. (1998). Morphohistological studies of two plant species used in ethnomedicine, Journal of Herbs, Spices and Medicinal plants 5(4): 60.65.

9. Essien, E.U., Izunwanne, B.C, Aremu, C.Y. & Eka, O. U. (1994). Significance for human of the nutrient contents of the dry fruits of *Tetrapleura tetraptera. Plant Food for Human Nutrition*, 45, 47-51.

10. Okoronkwo, N. E. & Echeme, J. O. (2012). Cholinesterase and microbial inhibitory activities of *Tetrapleura tetraptera*. *Journal of Applied and Natural Science,* 4, 156 – 163

11. Klin-Kabari,D.B. (2011). Effects of extracts from three indigenous spices on the chemical stability of smoke dried cat fish (*clarias lezera*) during storage. *African Journal of Food, Agriculture, Nutrition and development*, 11,5-9

12. Birt, D.A. (2006). Phytochemicals and cancer prevention: from epidemiology to mechanism of action. Journal of the American Dietetic association. 106: 20-24

13. Young,N.S. and Mohammed, M.E. (2019). β-Caryophyllene as a potential protective agent against myocardial injury: the role of tol-like receptors''. Molecules. 24(10). 1929

14. Suhaj,M.(2006). Spice antioxidant isolation and their antiradical activity: A Review. Journal of food composition and analysis, !9, 531-537.

15. Karthikumar, S., Vigneswari, K., Jegatheesan, K. (2007). Screening of antibacterial and antioxidant activities of leaves. *Eclipa prostrate. I Scientific Research and essay. 2(4): 101-104.*

16. Manafa, P.O., Mouneke,I.G., Ekuma-Okereke, O., Ebugosi, R.S., Ogbuowelu, O.S., Okocha, E.C., Nwene, K.E., Manafa, V.I., Manafa, C.C.(2019). Levels of testosterone, progesterone and follicle stimulating hormone in male sickle subjects in Nnamdi Azikwe University Teaching Hospital, Nnewi. Acta Scientific Medical Sciences, 3(11):11-20.

17. Hu, JX., Li, Y.F., Li, J., Pan, C., He, Z., Dong, H.Y., Xu, L.C.(2013) Toxic effects of cypermethrin on the male reproductive system: with emphasis on the androgen receptor.*J ApplToxicol.,*33(7):576-85.

18. Bhushan, B., Pande, S., Saxena, N. & Saxena, P.N. (2013). Serum biochemical responses under stress of Cypermethrin in albino rat. *Environmental and Experimental Biology,* 11, 81-89.

19. Bretveld, R.W., Thomas, C.M., Scheepers, P.T., Zielhuis, G.A. & Roeleveld, N. (2006). Pesticide exposure: the hormonal function of the female reproductive system disrupted. *Reproductive Biology and Endocrinol*ogy, 4(30), 245-255.

20. Abou El-Magd., S. A., Laila, M.E.,Sabik. & Shoukry,A.(2011). Pyrethroid toxic effects on some hormonal profile and biochemical markers among workers in pyrethroid insecticides company.*Life Science Journal.,*8 (1) 311-322.

21. Vinggaard, A.M., Nellemann, C., Dalgaard, M., Jorgensen, E.B. and Andersen, H.R.(2002). Anti-androgenic effects in vitro and in vivo of the fungicide prochloraz. Toxicological Science. 2002; 69:344–353.

22. Sahinoz, E., Aral, F., Dogu, Z., Koyuncu, I. & Yuksekdag, O. (2019). The protective effects of curcumin onorganophosphate insecticide chlorpyrifos-induced oxidative stress and DNA damage in Oncorhynchus mykiss. Turkish Journal of Fisheries and aquatic Sciences, 20(3), 185-195.

23. Airaodion, A.I., Ngwogu, A.C., Megwas. A.U., Ekenjoku, J.A. & Ngwogu, K.O. (2019). Effect of common household insecticides used in Nigeria on Rat male reproductive hormones, 2(1), 1-8.

24. Orlu, E.E & Obulor, A.O. (2021): Impact of Coenzyme Q10 on Hormonal Profile in Male Sprague-Dawley Rat Exposed to Sub-Chronic Concentrations of Cypermethrin, 12(3), 1-9