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The Ameliorating Effects of Virgin Olive oil in Adrenal Gland of Mice Exposed to Lambda-Cyhalothrin and Cypermethrin

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ABSTRACT

Purpose: Histopathological and micrometric effects of Cypermethrin (CYM) and lambda-cyhalothrin (LCH), the type II pyrethroid insecticides, were explored on adrenals of 6 weeks old sixty male mice (28-30g). Additionally the ameliorative capacity of extra virgin olive oil treatment were also investigated against their pathologies.

Methods: Sixty male mice (28-30g) were randomly distributed in six groups (10 mice each): 1. Negative control group (Cont) (100µL corn oil for two days followed by one day rest (no dose) and 100µL corn oil for next three days), 2. Positive control group (Virgin Olive Oil/VOO) (100µL corn oil for two days followed by one day rest (no dose) and 100µL extra virgin olive oil for next three days), 3. CYM group (100µL of 5mg/kg CYM solution in corn oil for two days, one day rest (no dose) and three day post treatment of 100µL corn oil), 4. LCH group (100µL of 5mg/kg LCH solution in corn oil for days 1-2, no dose on day 3 followed by 100µL corn oil from day 4-6), 5. CYM+VOO group (CYM treatment as in group three followed by extra virgin olive oil treatment as in group two after one day rest) and 6. LCh+VOO group (LCH treatment as in group four followed by extra virgin olive oil treatment as in group two after one day rest). All solutions were given to mice through gavage.

Results: The results showed various characteristic effects of insecticide exposure upon adrenal cortex (lesions in zona fasciculata leaving behind wide empty spaces between adjacent fascicles), medulla (cellular necrosis, vacuolization in cytoplast and nuclear disintegration, enlarged nuclei and cytoplast). In addition to that various significant micrometric alterations were also noted. Extra virgin olive oil significantly rehabilitated the derailments in the adrenal cortex and adrenal medulla.

Conclusions: On basis of present findings it is obvious that the type II pyrethroid insecticides bear cortico-medullary adrenal toxic potentials. As these pathologies were found effectively ameliorated upon OV treatment, it is concluded that such pathological implications are rescuable by the extra virgin olive oil.

Key-words: *Lambda-cyhalothrin, Cypermethrin, adrenal gland, Pyrethroids*

INTRODUCTION

The widespread use of synthetic pyrethroid insecticides, such as Lambda-Cyhalothrin (LCT) and Cypermethrin, to control pests has raised health concerns[1]. Numerous studies link insecticide exposure to adverse outcomes, including endocrine disruption, fertility issues, neurological disorders, and cancer. Despite their efficiency, low toxicity, and rapid degradation, pyrethroids have led to the accumulation of toxins in the environment[2]. Lambda-Cyhalothrin (LCT) is commonly used in agriculture, pest control, and veterinary applications but can be toxic to non-target mammals[3]. It's been shown to induce hepatotoxicity and nephrotoxicity in rodents, with potential oxidative stress due to its lipophilic nature. Additionally, pyrethroids have acute neurotoxic effects in insects. These findings highlight concerns about histopathological impacts, emphasizing the need for further research into their effects on various organisms[4].

Lambda-Cyhalothrin ($C_{23}H_{19}ClF_3NO_3$) and Cypermethrin ($C_{22}H_{19}Cl_2NO_3$) are synthetic chemicals that belong to type II pyrethroid insecticides[5]. Certain histopathological and histochemical studies have indicated that LCH showed tissue-specific dose-related degenerative damage to different organs in goldfish and mice[3-5]. It has also caused reproductive toxicity with irregular seminiferous tubules containing only Sertoli cells.[5] In rats, the oral LD50 was 79 mg/kg in males and 56 mg/kg in females[7,8]. Cypermethrin (CYM) is a broad-spectrum pyrethroid and effective neurotoxin[9]. There have been extensive histomorphological, neurotoxicological, immunological and biochemical reports on CYM toxicity in different species of animals[10-13].

Some studies have shown that antioxidant such as, Vitamin E, isoflavones and L-ascorbic acid prevent the oxidative damage due to CYM intoxicication in rats[14]. Olive oil, sesame oil, black seed oil, corn oil attenuated the physiological disturbances and histopathological alterations induced by diazonone intoxication[15]. The olive tree (*Olea europaea* L.), family: Oleaceae, has been widely accepted as one of the species with the highest antioxidant activity in its oil, fruits, and leaves[16].

Virgin olive oil (VOO) was initially thought to be beneficial due to enrichment of monounsaturated fatty acids (MUFA) especially oleic acid. However, recently two isolated compounds of VOO the oleuropein (OP) and hydroxytyrosol (HL) demonstrated antioxidant and protective actions against several disorders. OP and HL are highly stable[17,18]. They perform antioxidant activity by radical chain breakage (peroxidative chain reactions), anti-oxygen radicals, metal chelators and scavenging free radicals (peroxyl radicals) due to their catecholic structure[19]. The extra virgin olive oil possess excellent nutritional properties against many human pathologies such as oxidative stress, inflammation, cardiovascular diseases, cancer and aging-related illness due to the presence of biophenolic compounds[20]. Keeping in view these benefits this research work was planned to check out the rehabilitations of histopathological signs of CYM and LCH treatment on VOO exposure in mice adrenal gland by histopathological and micrometric studies.

MATERIAL AND METHODS

Placement of Experimental Animals

The current research work was instigated on sixty adult male albino mice having weight almost 28-30g. Animal husbandry of UVAS (University of Veterinary and Animal Sciences) and VIR (Veterinary Institute of Research) Lahore, Pakistan provided animals. Animals were kept in plastic cages (About 11-12 inches; 28-30 cm) in length, 7-8 inches (18-20 cm) in width, and 5-6 inches (13-15 cm) in height) gauzed with net internally. Ten male albino mice per group weighing 28-30g were kept in total groups of six.

Preparation of Stock Solution

0.6g of CYM and LCH is dissolved in 100mL corn oil to get stock solution of 20mg/kg and then 10mL required dose of 5mg/kg of both insecticides was prepared.

Animal Treatment Details

- ***Negative Control (Cont) group****:* was given 100µL corn oil (Rafhan corn oil) for consecutive six days through gavage.
- ***Positive Control (VOO) group:*** was given 100µL corn oil for two days followed by one day rest to allow clearance and mitigate potential effects (no dose) and subsequently, a 100µL dose of extra virgin olive oil was administered for the next three days.
- ***Cypermethrin (CYM) and Lambda-cyhalothrin (LCH) groups****:* were provided with 100µL of 5mg/kg CYM soln. and 5mg/kg LCH soln. respectively for 2 days, rest on 3rd day (no dose) and then 100µL corn oil for three days through gavage.
- ***CYM+VOO and LCH+VOO groups***: received 100µL of 5mg/kg CYM soln. and 5mg/kg LCH soln. for 2 days then given rest for 1 day (no dose) followed by post-treatment of 100µL extra virgin olive oil through gavage for 3 days.

Organ's Excision

On 7th day of study animals were dissected and adrenals were excised in intact form. Organs were fixed in acidified formyl ethanol for 48 hours and then for dehydration in 50%, 70%, 90% and absolute ethanol. Dehydrated adrenals were then cleared in xylene for almost 45 minutes followed by embedding in molten paraffin wax (58ºC-60ºC). Serial transverse sections of 3-4microns thickness were taken using rotary microtome (ERMA TOKYO 422), to ensure comprehensive representation of adrenal gland during histopathological analysis.

Histological Remarks

On trinocular microscope (Labomed CXR$_2$) coupled with a 7.2 mega pixel digital camera (Sony DSC-W35), sections of adrenals were photographed at 100X and 400X magnifications after proper observations. In corelDRAW11 further improvisation of digital photoshots were done for selective sections of adrenals.

Morphometric and Statistical analysis

Using pre-calibrated digital scale in corelDRAW11 relative thickness of different zones (glomerulosa, fesciculata, reticularis), cross-sectional area of fascicles and number of endocrine cells per fascicle in cortex were measured. In adrenal medulla CSA of chromaffin cells and number of chromaffin cells per unit area of 2500µm^2 were measured using ellipse and rectangle tool in coralDRAW11. Following formula was used for calibrations: CSA = (length X width/4)π

Micrometric data was analyzed by using Analysis of Covariance (ANCOVA), Analysis of Variance (ANOVA) and Tukey Multiple Range Test (TMRT).

RESULTS

Histological Results of Adrenal

The microscopic sections of adrenal gland in negative control and positive control (VOO) treated groups showed all basic features of mouse adrenal histo-architecture. There was complete distinction between cortical and medullary region. The cortical adrenal gland sections can be clearly demarcated into the outer zona glomerulosa showing basophilic properties and small tightly packed cells followed by zona fasciculate existing inner to zona glomerulosa. There was no distinct boundaries between two regions. The fasciculate usually identifiable in fascicular arrangements of endocrine cells of this region. Outer to adrenal medulla and at base of zona fasciculate the characteristic x-zone was also identifiable. Adrenal medulla mostly appears to be consist of chromaffin cells securing the epinephrine and nor-epinephrine granules in their cytoplasm (Fig1: A&B). In both insecticides treated groups that is LCH and CYM little signs of histopathology were observed in zona glomerulosa while the zona fasciculate shows lesions leaving behind wide empty spaces between adjacent fascicles. The x-zone also shows some lesions but most of it was intact. The medullary region have shown empty spaces and signs of cellular necrosis. In terms of enlarged nuclei and cytoplast along with cytoplasmic vacuolization in LCH group whereas appearance of vacuolization in cytoplast and nuclear disintegration were noted in characteristics histopathologies in adrenal medulla in CYM group (Fig1: C&D). In LCH+VOO and CYM+VOO groups rapid rehabilitation of cortical histo-architecture was noted with clear signs of mitosis whereas slight rehabilitation in terms of cellular mitosis

and cellular regeneration were noticed in adrenal medulla. However, signs of cellular degeneration and vacuolization were still persisted in both groups (Fig1: E&F).

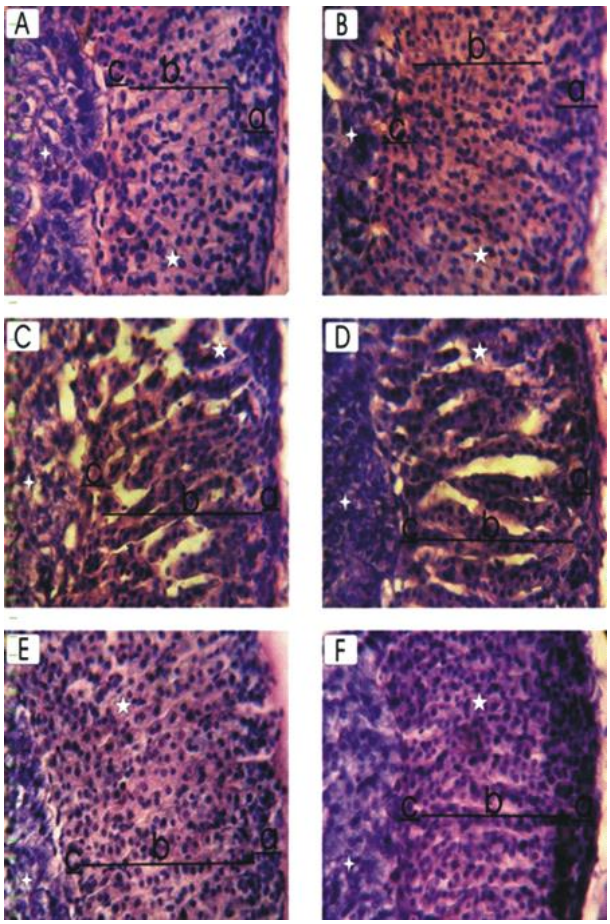

Fig 1: H&E stained histological section (400X) of mice adrenal gland. A: Control group (Cont), B: Olive group (VOO), C: Cypermethrin group (Cym), D:Lambda-cyhalothrin (LCh), E:Cypermethrin+Olive (Cym+VOO), F: Lambdacyhalothrin+Olive (LCh+VOO). Five corner star: adrenal cortex, four corner star: adrenal medulla. a: zona glomerulosa (Z.G), b: zona fasciculate (Z.F), c: zona reticularis (Z.R).

Morphometric Results

Thickness of Zona Fasciculata+x-zone is highly significant (p≤0.0001) among all groups. Further pairwise comparison was done by Least Significant Difference Multiple Comparison as post hoc analysis which revealed no significant (p>0.05) difference between Cont group (165.39±3.43) and VOO group (161.21±3.24), a significant (p≤0.05) increase in LCH (214.71±3.99) and CYM (204.79±3.36) as compared to Cont group and a significant (p≤0.05) decrease in LCH+VOO (193.87±3.35) and CYM+VOO (191.48±3.12) as compared to LCH and CYM group respectively (Table. 1).

Mean CSA of cells in Zona Fasciculata show highly significant (p≤0.0001) difference among all six groups, when statistically analyzed by one way ANOVA. Post hoc analysis by TMRT revealed no significant variation between Cont (94.92±1.93ab) and VOO group (6.57±0.14a). Mean values were significantly increased in LCH (165.49±3.58e) and CYM (145.39±4.35d) as compared to control group. Moreover in LCH+VOO (114.86±4.59c) and CYM+VOO (111.28±4.11bc) groups mean cell CSA was significantly (p≤0.05) lower as compared to LCH and CYM group respectively (Table. 1).

Number of endocrine cells per fascicle revealed highly significant discrepancy (p≤0.0001) among groups. Post Hoc analysis indicated significant (p≤0.05) decrease in VOO (6.57±0.14a) as compared to Cont (6.95±0.15b). Significantly (p≤0.05) elevated mean values were recorded in LCH (9.45±0.15e) and CYM (9.58±0.13e) groups as compared to negative control group. A significant (p≤0.05) mean value decline was obvious in LCH+VOO (8.09±0.13d) and CYM+VOO (7.72±0.13c) groups as compared to LCH and CYM group respectively (Table. 1).

Mean CSA of Chromaffin cells revealed significant difference (p≤0.05) in mean value among groups. Post Hoc analysis by utilization of TMRT showed no significant difference (p>0.05) between Cont (181.41±8.2ab) and VOO (172.56±5.1a) groups. A significant (p≤0.05) and a non-significant difference (p>0.05) increase was shown as compared to control in LCH (203.87±7.4b) and CYM (200.22±5.5ab) group respectively. A non-significant (p>0.05) decline was obvious in LCH+VOO and CYM+VOO (194.40±10.8ab) (190.28±3.5ab) groups as compared to LCH and CYM groups respectively (Table. 2).

Mean CSA of Nucleus of Chromaffin cells showed highly significant difference (p≤0.0001) among groups. Similarly TMRT based Post Hoc analysis indicated significantly (p≤0.05) increased mean value in LCH (28.75±0.89c) and CYM (27.94±0.89bc) group as compared to Cont (23.65±0.79a). In CYM+VOO (25.34±0.82ab) group mean values were significantly (p≤0.05) lower as compared to CYM group. Moreover,

there was no significant difference between Cont, VOO (23.57±0.65a) and CYM+VOO groups (Table. 2).

Mean Cytoplasmic area of chromaffin cells showed significant difference (p≤0.05) among groups. However, Post Hoc analysis by TMRT exhibited no significant difference (p>0.05) of all groups with each other (Table. 2).

Number of chromaffin cells/unit area One way ANOVA showed no significant difference (p>0.05) between Cont (13.60±0.26d) and VOO (13.50±0.14d) groups. Data analysis showed high significance (p≤0.0001) among groups. Similarly Post Hoc analysis by TMRT presented highly significant (p≤0.0001) decrease in mean value of CYM (9.70±0.28a) and LCH (10.53±0.23ab) as compared to control. A non-significant increase was observed in LCH+VOO (11.20±0.21bc) as compared to LCH group. Similarly significant (p≤0.05) increase was observed in CYM+VOO (11.60±0.23c) as compared to CYM group (Table. 2).

Table 1. Micrometric Variation of the mean thicknesss of zona fasciculate, CSA of fascicles & number of endocrine cells/fascicle of mice adrenal cortex. Values are Mean±SEM (n=60)

Micrometric parameters	Mean + SEM					
	Control	VOO	CYM	LCH	CYM+VOO	LCH+VOO
Mean thickness of Zona Fasciculata+x-[***]	165.39±3.43^{a}	161.21±3.24^{a}	204.79±3.36^{c}	214.71±3.99^{c}	191.48±3.12^{b}	193.87±3.35^{b}
CSA of cells in Fascicles(μm^2) [***]	94.92±1.93ab	90.14±6.02a	145.39±4.35^{d}	165.49±3.58^{e}	111.28±4.11^{bc}	114.86±4.59^{c}
Number of Endocrine cells per Fascicle[***]	6.95±0.15^{b}	6.57±0.14^{a}	9.58±0.13^{e}	9.45±0.15^{e}	7.72±0.13^{c}	8.09±0.13^{d}

*: (p≤ 0.05), significant **:(p≤0.001), very significant ***:(p≤0.0001) highly significant, $^{a\ b\ c\ d}$: Anyone two groups not sharing a lower case letters differ significantly from each other

Table 2. Micrometric Variations of the CSA of chromaffin cells, nucleus of chromaffin cells, cytoplasmic areas of chromaffin cells and number of chromaffin cells of mice adrenal medulla. Values are Mean±SEM (n=60)

Micrometric parameters	Mean + SEM					
	Control	VOO	CYM	LCH	CYM+VOO	LCH+VOO
CSA of Chromaffin cells(μm^2) [*]	181.41±8.2^{ab}	172.56±5.1^{a}	200.22±5.5^{ab}	203.87±7.4^{b}	194.40±10.8^{ab}	190.28±3.5^{ab}
CSA of Nucleus of Chromaffin cells(μm^2) [***]	23.65±0.79^{a}	23.57±0.65^{a}	27.94±0.89^{bc}	28.75±0.89^{c}	25.34±0.82^{ab}	27.37±0.62b^{c}
Cytoplasmic area of Chromaffin cells(μm^2) [*]	157.76±7.4^{a}	148.99±4.5^{a}	172.28±4.7^{a}	175.11±6.6^{a}	169.06±9.9^{a}	162.91±2.9^{a}
Number of Chromaffin cells per unit area[***]	13.60±0.26^{d}	13.50±0.14^{d}	9.70±0.28^{a}	10.53±0.23^{ab}	11.60±0.23^{c}	11.20±0.21^{bc}

*: (p≤ 0.05), significant **:(p≤0.001), very significant ***:(p≤0.0001) highly significant, $^{a\ b\ c\ d}$: Anyone two groups not sharing a lower case letters differ significantly from each other

DISCUSSION

The present histopathological study dealt with the toxic potentials of two commonly used type II pyrethroids on adrenals. Hormones of adrenal medulla (epinephrine & nor-epinephrine) deals with fight or flight situations[21,22]. In present study it was seen that chromaffin cells granules (hormonal storage) were rapidly depleted on insecticides treatment. The most logical explanation of their depletions and extendedly the vacuolization and apoptosis of individual chromaffin cells must logically be consequence of elevated toxic stress upon insecticide exposure. In LCH and CYM groups in a similar way the lesions seen in adrenal cortex must also be related to the consequence of metabolic stress on cells of zona

fasciculata for the secretion of glucocorticoids to cope with the toxic stress of the insecticidal exposure in both LCH and CYM treated groups. Interesting micrometric alterations in both adrenal cortex and adrenal medulla must also be seen in the same context. Remarkably, a study conducted in a similar context, that demonstrated analogous histopathological and micrometric changes in the adrenal medulla and cortex when exposed to Bifenthrin (Bif)[23]. This congruence between their results supports the contention that the stress response triggered by Bif exposure indeed leads to an elevated catecholaminergic response, a concept that we also observe in our current study.

Additionally, our study's findings on adrenal responses correlate with previous research on the effects of pesticides on other organs, such as the kidney.previous Study reported similar patterns of histopathological changes, including hemorrhage, tubular dilation, desquamation of tubular cells, inflammatory cell infiltration, and tubular enlargement, induced by lambdacyhalothrin exposure[24]. Likewise, histopathological changes in rat kidneys exposed to LCT includes tubular necrosis, enlargement, inflammatory cell infiltration, and hemorrhage[25]. These alterations were attributed to the accumulation of free radicals resulting from increased lipid peroxidation by cyanides and aldehydes in renal tissues of LTC-treated rats. Similarly, exposure of Cypermethrin on rabbit kidney cause histopathological changes[26].

It was very interesting to note that VOO bears an immense potential in terms of rescue and rehabilitary activities in adrenal cortex and adrenal medulla as observed in LCH+VOO and CYM+VOO groups which must be attributable to the precious antioxidant bioactives such as oleic acid, oleuropein, hydroxytyrosol and ligstroside. This intriguing discovery is strongly reinforced by pertinent scientific literature. VOO status as an exceptional antioxidant, capable of safeguarding tissues, proteins, lipids, and DNA from the damaging impact of oxidative stress, underscores its significance. This is attributed to its straightforward, efficient, and secure dietary application across a wide spectrum of concentrations further emphasizes its pivotal role[27-30].

The finding of present study shows that both LCH and CYM are toxic to the adrenals. The LCH indicates slightly more persistent histo-architectural derailments as compared to CYM. The most logical reason for this persistency may lie in the presence of organoflouride in LCH because the presence of fluoride in LCH is the only difference between chemical composition of LCH and CYM. Nevertheless, the rehabilitative capability of the VOO upon the histo-architectural derailments caused by the two insecticides on adrenals indicates its endocrine potentials.

CONCLUSIONS

The study indicates that the pyrethroids usually believed to be least toxic insecticides should be revisited and they must be used with care and closely assessing expected benefits with that of possible toxic effects especially LCH. However, VOO can serve as a natural rescue from such possible toxic implications of insecticide exposure at work places.

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Not Applicable.

CONFLICT OF INTERESTS

None

REFERENCES

[1] Alrawe SA, Alzubaidy MH. Acute and sub-acute toxicity effects of lambda-cyhalothrin in chicks. Iraqi J Vet Sci 2022; 36(1): 191-200. DOI: 10.33899/IJVS.2021.129674.1678

[2] Boumezrag A, Hemida H, Boumezrag FA, Smail F, Cisse S. Pathological and biological effects of treatments with lambda-cyhalothrin in rabbits. Iraqi J Vet Sci 2021; 35(3): 443-450. DOI: 10.33899/IJVS.2020.126977.1425

[3] Salako AF, Amaeze NH, Shobajo HM, Osuala FI. Comparative acute toxicity of three pyrethroids (Deltamethrin, cypermethrin and lambda-cyhalothrin) on guppy fish. Sci Afr 2020; 9: e00504. DOI:10.1016/j.sciaf.2020.e00504

[4] Hussain SI, Khwaja S, Zahid M, Karim A, Aziz Z, Nisar S, Abbasi HN. Effect of biosal deltamethrin and lambda-cyhalothrin on the activity of GOT, GPT and total protein contents in two fodder pests *Hermolaus modestus* and *Hermolaus ocimumi*. Braz J Biol 2021; 84:e253948. DOI: 10.1590/1519-6984.253948

[5] Thatheyus AJ, Selvam ADG. Synthetic pyrethroids: Toxicity and biodegradation. Appl Eco Env Sci 2013; 1(3): 33-36. DOI: 10.12691/aees-1-3-2

[6] Al-Sarar AS, Abobakr Y, Bayoumi AE, Hussein HI, Al-Ghothemi M. (2014). Reproductive toxicity and histopathological changes induced by lambda-cyhalothrin in male mice. Environ Toxicol 2014; 29(7): 750-762. DOI: 10.1002/tox.21802

[7] Li M, Wang J, Lu Z, Wei D, Yang M, Kong L. NMR-based metabolomics approach to study the toxicity of lambda-cyhalothrin to goldfish (*Carassius auratus*). Aquat Toxicol 2014; 146: 82-92. DOI: 10.1016/j.aquatox.2013.10.024

[8] Elhalwagy ME, Abd-Alrahman SH, Nahas AA, Ziada RM, Mohamady AH. Hepatopancreatic intoxication of lambda cyhalothrin insecticide on albino rats. Int J Clin Exp Med 2015; 8(5): 7297-7305.

[9] Ben Abdallah F, Fetoui H, Zribi N, Fakhfakh F, Keskes L. Quercetin attenuates lambda cyhalothrin-induced reproductive toxicity in male rats. Environ Toxicol 2013; 28(12): 673-680. doi: 10.1002/tox.20762

[10] Oularbi HK. Biochemical and histopathological changes in the kidney and adrenal gland of rats following repeated exposure to lambda-cyhalothrin. Italy J Xenobiotics 2014; 4: 224. *doi:*10.4081/xeno.2014.224

[11] Tomar M, Kumar A, Kataria SK. Evaluation of Acute toxicity of Lambda Cyhalothrin in *Mus musculus* L. Indian J Exp Biol 2015; 53(8): 551-555.

[12] Tian YT, Liu ZW, Yao Y, Zhang T, Yang Z. Effects of alpha-and theta-cypermethrin insecticide on transient outward potassium current in rat hippocampal CA3 neurons. Pest Biochem Physiol 2008; 90(1): 1-7. DOI: 10.1016/j.neuro.2009.01.001

[13] Pronk MEJ, Speijers GJA, Wouters MFA, Ritter L. Cypermethrin and alpha-cypermethrin. Food Additive Series 1996: 38.

[14] Kumari V, Kaur B, Sandhu HS. Experimental cypermethrin toxicity in buffalo calves. Indian J Anim Sci 2002; 72(8): 658-660.

[15] Hussain S, Khan MZ, Khan A, Javed I, Asi MR. Toxico-pathological effects in rats induced by concurrent exposure to aflatoxin and cypermethrin. Toxicon 2009; 53(1): 33-41. doi: 10.1016/j.toxicon.2008.10.008.

[16] Magar RS, Shaikh A. Effect Of MalathionToxicity On Detoxifying Organ Of Fresh Water Fish *Channa Punctatus.* Int J Pharm Chem Biol Sci 2013; 3(3): 723-728.

[17] Raina R, Verma PK, Pankaj NK, Kant V, Prawez S. Protective role of L-ascorbic acid against cypermethrin-induced oxidative stress and lipid peroxidation in Wistar rats. Toxicol. Environ. Chem 2009; 92(5): 947-953. DOI:10.1080/02772240903174781

[18] Al-Attar AM, Elnaggar MH, Almalki EA. Protective effect of some plant oils on diazinon induced hepatorenal toxicity in male rats. Saudi J Biol Sci 2017 24(6):1162-1171. doi: 10.1016/j.sjbs.2016.10.013.

[19] Visioli F, Galli C. Biological properties of olive oil phytochemicals. Crit Rev Food Sci Nutr 2002;42(3): 209-221. doi: 10.1080/10408690290825529.

[20] Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S, La Guardia M. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. Nutr Res Rev 2005; 18(1): 98-112. doi: 10.1079/NRR200495.

[21] Achat S, Tomao V, Madani K, Chibane M, Elmaataoui M, Dangles O, Chemat F. Direct enrichment of olive oil in oleuropein by ultrasound-assisted maceration at laboratory and pilot plant scale. Ultrason Sonochem 2012;19(4): 777-786. doi: 10.1016/j.ultsonch.2011.12.006.

[22] Zoidou E, Magiatis P, Melliou E, Constantinou M, Haroutounian S, Skaltsounis AL. Oleuropein as a bioactive constituent added in milk and yogurt. Food Chem 2014;158**:** 319-324. doi: 10.1016/j.foodchem.2014.02.137.

[23] Javid I, Nasir M, Suleman S, Ikram S, Mumtaz A, Kanwal MA, Ahmad KR. Adrenal histo-toxicological impacts of bifenthrin (a chloro-fluoridated-pyrethroid) are reversed on *Nigella sativa* seed oil treatment in mice. Fluoride 2019;52(1): 66-76.

[24] Ahmed A. Change in acetylcholine activity and some blood parameters in adult sheep dipped in deltamethrin. Iraqi. J Vet Sci 2021; 35(2):301-304. DOI:10.33899/ijvs.2020.126813.1385.

[25] Fetoui H, Garoui EM, Zeghal N. Lambda-cyhalothrin-induced biochemical and histopathological changes in the liver of rats: Ameliorative effect of ascorbic acid. Exp Toxicol Pathol 2009; 61(3):189-96. DOI: 10.1016/j.etp.2008.08.002

[26] Vardavas AI, Stivaktakis PD, Tzatzarakis MN, Fragkiadaki P, Vasilaki F, Tzardi M, Datseri G, Tsiaoussis J, Alegakis AK, Tsitsimpikou C, Rakitskii VN, Carvalho F, Tsatasaki AM. Long-term exposure to cypermethrin and piperonyl butoxide cause liver and kidney inflammation and induce genotoxicity in New Zealand white male rabbits. Food Chem Toxicol 2016; 94:250-9. DOI: 10.1016/j.fct.2016.06.016

[27] De Santis S, Clodoveo ML, Corbo F. Correlation between chemical characterization and biological activity: an urgent need for human studies using extra virgin olive oil. Antioxidants 2022;11**(**2): 258. https://doi.org/10.3390/antiox11020258

[28] Jiménez-Sánchez A, Martínez-Ortega AJ, Remón-Ruiz PJ, Piñar-Gutiérrez A, Pereira-Cunill JL., García-Luna PP. Therapeutic properties and use of extra virgin olive oil in clinical nutrition: a narrative review and literature update. Nutrients 2022;14(7): 1440. doi: 10.3390/nu14071440.

[29] Presti G, Guarrasi V, Gulotta E, Provenzano F, Provenzano A, Giuliano S, Monfreda M, Mangione MR, Passantino R, San Biagio PL, Costa MA, Giacomazza D. (2017). Bioactive compounds from extra virgin olive oils: Correlation between phenolic content and oxidative stress cell protection. Biophys Chem 2017; 230: 109-116. doi: 10.1016/j.bpc.2017.09.002.

[30] Frankenhaeuser M, Lundberg, U. Sympathetic-adrenal and pituitary-adrenal response to challenge. Biol psychiatry higher nervous activity 1985: 699-704.