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Neuro Histo-toxicologic Implications of Lambda Cyhalothrin in Chick Embryo And the Possible Rescuing Effect of Vitamin E And Olive Oil

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ABSTRACT

Purpose: Teratological capacities of lambda-cyhalothrin (LCH) and the rescuing abilities of vitamin E and olive oil in ovo exposure were analyzed in the golden black variety of domestic chicken.

Methods: The study was conducted on fresh fertilized eggs collected and divided into 5 groups as follows: (1) Vehicle Control group (VCon) which received 0.1mL of 5% dimethyl sulfoxide (DMSO) solution in vegetable oil (2) the Lambda-cyhalothrin group (LCH) was administered a 0.1mL of 5% DMSO and 0.01 mg/kg of LCH, all dissolved in vegetable oil. (3) Lambda-cyhalothrin+Vitamin E group (LCHE), was provided with a 0.1mL of 5% DMSO, 0.01mg/kg LCH & 0.1mg/kg of Vitamin E, dissolved in vegetable oil. (4) Lambda-cyhalothrin+ Vitamin E in Olive oil (LCHOE), received 0.1mL of 5% DMSO and 0.01 mg/kg LCH, both dissolved in 0.1mg/kg vitamin E solution (EVOO). Finally, the last group (5) Olive oil +Lambda-cyhalothrin (LCHO) were administered 0.1mL of 5% DMSO, 0.01mg/kg LCH & 0.1mg/kg Vitamin E in EVOO. The embryos were removed from egg shells after 14th day of incubation, weighted and disected for the removal of brains for histological outcomes.

Results: There was significant decrease in embryonic weight, volume and density of brain in LCH group as compared to vehicle control. LCH treated embryos showed complete cell obliterations, microlesions and partial nuclear disintegration (micronuclear formation). Number of different types of neuronal cells (pyramidal, pyramidal-like, multipolar, local circuit) was also decreased in LCH treated embryos. However, vit E and olive oil treated embryos show rescuing effects towards all above mentioned abnormalities.

Conclusions: Type II fluoridated pyrethroid LCH is highly toxic to developing chick brain which may unfortunately be present in chick feed. However, the precious ingredients of olive oil and vitamin E can reduce the risk of neurodevelopmental damages.

Key-words: *Chick embryo; Lambda cyhalothrin; Olive oil; Vitamin E; Chick (embryo) Brain*

INTRODUCTION

Synthetic pyrethroids are analogues of the natural pyrethrin present in the *Chrysanthemum cinerariaefolium* plant. During the last four decades, pyrethroids have been utilized worldwide for pest management in agriculture, forestry, sanitary areas, and households (as mosquito repellent)[1]. Lambda-cyhalothrin (LCH) is a potent, synthetic, type II pyrethroid pesticide and is worldwide used to control a different variety of insect pests in agricultural and domestic fields and public health sectors. Lambda-cyhalothrin is moderately toxic for mammals and highly toxic for fish, bees and aquatic invertebrates at low concentrations[2].

Histopathological alterations observed in the brain of mice following LCH exposure suggest neurotoxic potential of LCH. LCH induces oxidative stress which results in the damage to the brain tissues of mice[3]. The term Vitamin E includes a group of lipid-soluble compounds that possess antioxidant actions[4]. Animal studies have documented the role of vitamin E as a factor essential for important functions and the development of brain and nerves. Vitamin E deficiency presents primarily as neurological and neuromuscular disorders. Alpha-tocopherol can prevent the kainate-induced cell death in the hippocampus thus preserving the synaptic plasticity and function of that brain area. The hippocampus appears to be an elective area for the physiological and even therapeutic effects of this vitamin in the developing brain[5].

Olive oil and its constituent's phenol and its derivatives have shown to treat various ailments due to their remarkable antioxidant properties. Many reports demonstrated that phenolic found in extra virgin olive oil, exert strong antioxidant properties and are able to counteract oxidative stress in brain tissue[6]. This research work was designed to investigate the neurotoxic effects of pyrethroids pesticide lambda-cyhalothrin and rescuing effects of vit E and olive oil in the hippocampus region of chick (embryo) brain.

MATERIAL AND METHODS

Egg collection and dose groups

Fertilized eggs (freshly laid in the preceding night) of the golden-black variety of domestic chicken were collected from poultry breeders in the nearby villages of Sargodha city. Eggs of approximately equal size (weighing 48–52g) were selected and distributed into five groups (n=50 per group):

a. **Vehicle Control (VCon):** the eggs received a precise 0.1mL of 5% DMSO solution in vegetable oil.
b. **Lambda-cyhalothrin (LCH):** was administered 0.1mL of 5% DMSO with 0.0 mg/kg of LCH in vegetable oil.
c. **Olive oil+Lambda-cyhalothrin (LCHO)***:* the eggs received 0.1mL of 5% DMSO combined with 0.01mg/kg LCH in extra virgin olive oil (EVOO).
d. **Vitamin E+Lambda-cyhalothrin in vegetable oil (LCHE):** the eggs received of 0.1mL 5% DMSO and 0.01mg/kg LCH & 0.1mg/kg of Vitamin E solution in vegetable oil.
e. **Vitamin E+Lambda-cyhalothrin in olive oil (LCHOE):** the eggs received of 0.1mL 5% DMSO and 0.01mg/kg LCH & 0.1mg/kg of Vitamin E solution in extra virgin olive oil (EVOO).

Dose administration

Eggs were kept in horizontal position for 5 minutes so that embryos come on the top and did not get damaged by needle that had to be injected. Windows were created by 1cc sterilized syringe. Paper tag labeled with group names were used to seal the window and to tag the eggs of each group as well. Weight of each egg was taken on weight balance and noted before dose administration and after dose administration.

Eggs incubation

Digital incubator with the capacity of 50 eggs was used. Incubator was set at specific humidity and temperature at 37±0.5^{o}C. Incubator automatically rotates the eggs twice a day. Eggs were candled to check the growth of embryos on daily basis and eggs with retarded growth were thrown out. Eggs were incubated for 14 days.

Embryo recovery

Eggs were recovered after 14 day of incubation. Broad end of egg was cut and opened with the help of blunt forceps. Embryo from the top of yolk was separated with the help of camel hair brush and forceps, placed in the petri dish filled with saline solution (0.9%) and then it was kept in fixative, acidified formyl ethanol for 24 hours. This procedure was repeated for all eggs. After 24 hours of fixation heads of embryos were separated with the sharp scalpel. Skin of head was removed and brain was taken out carefully. Brain was again dissected to remove the hippocampus from it.

Histopathology and observation of brains

Hippocampus obtained from all groups were processed for wax embedding. First of all hippocampus was immersed successfully in various alcohol grades for dehydration. Dehydrated hippocampus was was cleared through immersion in xylene for 5-6 hours. Finally, they were placed in molten histological paraffin wax (at 56-58^{o}C) for 3-5 hours for proper embedding and sectioing. Sections of 3µm thickness were obtained on a rotary microtome and stretched on albumenized histological glass slides and stained via E & H and mounted by canada balsam. These slides were finally observed under trinocular research microscope (Labomed CXR2) for histopathological observations mechanically fitted with Sony Cybershot (Model: DSC-W35) 7.2 megapixel digital camera.

Histological studies

Digital micrometry in corelDRAW19, the wire frame calibrations with stage micrometer, highlighting of histological abnormalities, processing of each group selected sections and cropping in Corel PHOTO-PAINT 19 were carried.

Data analysis

The morphometric and histological data was analyzed by applying (Analysis of covariance) ANCOVA and (Analysis of variance) ANOVA tests which were done by using IBM SPSS statistics 22 software.

RESULTS

Morphometric Results

Mean embryonic weight

Statistical analysis of data for mean weight of embryos through one way of ANOVA revealed a highly significant ($p \leq 0.001$) difference among groups. Similarly post hoc analysis by using Tuckey Multiple Range Test (TMRT) also showed a significant ($p \leq 0.05$) decline in LCH and LCHE group as compared to vehicle control however there was no significant ($p > 0.05$) difference between vehicle control, LCHO and LCHOE groups. (Table 1).

Mean weight of chick (embryo) brain

Data for mean weight of chick (embryo) brain was statistically analyzed through Analysis of Covariance (ANCOVA) by taking mean weight of embryo as covariate. Results revealed highly significant ($p \leq 0.001$) difference among groups. Least significant difference based post hoc analysis showed significantly elevated mean values in LCHO, LCHE and LCHOE as compared to vehicle control (Table 1).

Volume of chick (embryo) brain

Data for mean volume of chick (embryo) brain was statistically analyzed through Analysis of Covariance (ANCOVA) by taking mean weight of chick embryo as covariate. Results showed highly significant ($p \leq 0.001$) difference among groups. Least significant difference based post hoc analysis showed significantly decreased mean values in all treatment groups LCH,LCHO,LCHE and LCHOE as compared to vehicle control (Table 1).

Density of chick (embryo) brain

Data for mean density of chick (embryo) brain was statistically analyzed through Analysis of Covariance (ANCOVA) by taking mean weight of chick embryo as covariate. Results revealed ($p \leq 0.05$) significant difference among groups. Least significant ($p \leq 0.05$) difference based post hoc analysis showed significantly decreased mean values in all treatment groups LCH, LCHO, LCHE and LCHOE as compared to vehicle control (Table 1).

Micrometric Results

Number of pyramidal cells/area

Statistical analysis of data for pyramidal neurons through one way of ANOVA revealed a significant difference among groups. Similarly post hoc analysis by using TMRT showed a significant ($p \leq 0.05$) decline in LCH group as compared to vehicle control however there was significant ($p \leq 0.05$) increase in all other treated groups (Table 2).

Number of pyramidal-like cells/area

Statistical analysis of data for pyramidal like neurons through one way of ANOVA revealed a highly significant ($p \leq 0.001$) difference among groups. Similarly post hoc analysis by using TMRT showed a significant ($p \leq 0.05$) decline in LCH group as compared to vehicle control. However there was a significant ($p \leq 0.05$) increase in all other groups LCHO, LCHE and LCHOE as compared to vehicle control (Table 2).

Number of multipolar cells/area

Statistical analysis of data for multipolar neurons through one-way of ANOVA revealed a highly significant ($p \leq 0.001$) difference among groups. Similarly post hoc analysis by using TMRT showed a significant ($p \leq 0.05$) decline in LCH group as compared to vehicle control and a significant ($p \leq 0.05$) increase in all other groups (Table 2).

Number of local-circuit cells/area

Statistical analysis of data for local circuit neurons through one way of ANOVA showed a significant ($p \leq 0.05$) difference among groups. Similarly post hoc analysis by using TMRT showed a significant ($p \leq 0.05$) decrease in LCH, LCHE and LCHOE as compared to vehicle control except LCHO group (Table 2).

Number of neuroglial cells/area

Statistical analysis of data for neuroglial cells through one way of (ANOVA) revealed a highly significant ($p \leq 0.001$) difference among groups. Similarly post hoc analysis by using TMRT showed a significant ($p \leq 0.05$) decrease in LCH group as compared to vehicle control. However there was significant ($p \leq 0.05$) increase in LCHE, LCHO and LCHOE group as compared to vehicle control (Table 2).

Histological Results

Histology of vehicle control group have shown characteristic distribution of the neuroglial cells. Various types of neuronal cells (pyramidal (Figure 1A), pyramidal like, multipolar, local circuit and neurosupportive glial cells were clearly observed and identified. Various precursor neuronal and glial cells at various stages of mitosis were also observed.

In LCH treated embryonic brain (hippocampus) histological sections various toxicological insults were identifiable that include complete cell obliterations, microlesions, partial nuclear disintegration and micronuclear formation (Figure 1B). The cotreatment of vitamin E along with LCH (LCHE) have led to slight improvement in terms of neuroglial cell density however all above mentioned toxicological signs were also obvious in this group embryonic hippocampal sections (Figure 1C).

In LCH and olive oil (LCHO) group the megakaryocytic neuroglial precursor cells were the most obvious signs observed in this group. Additionally it is apparently seen that the neuroglial cells show partial merger with each other forming streaks of such partially merged megakaryocytic neuroglial cells (Figure 1D). Various precursor neuroglial cell obliterations were also observed. Embryonic hippocampus sections in LCHOE

group have shown maximum neuroglial cell density in all the insecticide treated groups. However some megakaryocytic cells and relict indications of streak formation due to partial merger of neuroglial cells along with some neuronal cells obliterations were still observable (Figure 1E).

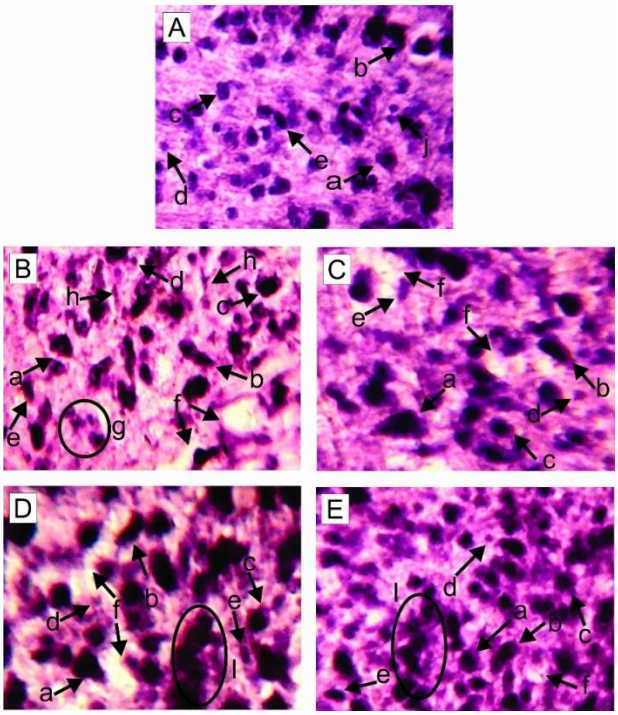

Figure 1: Hematoxylin and Eosin stained histological sections (40X) of chick (embryo) brain (hippocampus). 1A: (vehicle control) 1B: (LCH) 1C: (LCHE) 1D: (LCHO) 1E: (LCHOE) [a: pyramidal neuron, b: pyramidal-like neuron, c: multipolar neuron, d: neuroglial cell, e: local circuit neuron, f: cell obeliteration, g:nuclear disintegration, h: microlesions j: mitosis i: streaks]

Table 1. Variation between measurements of morphometric parameters on 14th day in chick (embryo) brain (VCon: Vehicle control (1A), **LCH:** Lambda-cyhalothrin (1B)**, LCHE:** Vitamin E+Lambda-cyhalothrin in vegetable oil (1C), **LCHO:** Olive oil+Lambda-cyhalothrin (1D), **LCHOE:** Vitamin E+Lambda-cyhalothrin in olive oil)

Morphometric parameters	Mean + SEM				
	VCon	LCH	LCHO	LCHE	LCHOE
Mean weight of embryo (g)*** ɜ	5.93±0.03^{b}	3.17±0.01^{a}	5.80±0.02^{b}	3.75±0.04^{a}	5.24±0.04^{b}
Mean weight of embryo brain (g)*** ε	0.25±0.01^{a}	0.19±0.01^{b}	0.28±0.01^{b}	0.28±0.01^{b}	0.27±0.01^{b}
Mean volume of embryo brain(ml)*** ε	0.32±0.02^{a}	0.15±0.02^{b}	0.22±0.02^{b}	0.25±0.02^{b}	0.21±0.02^{c}
Mean density of embryo brain(g/ml)** ε	1.87±0.02^{a}	0.63±0.02^{a}	1.38±0.02^{ab}	0.77±0.02^{b}	1.85±0.02^{b}

***: (p≤0.05), **: (p≤0.01), ***:(p≤0.001) ε: Analyzed by ANCOVA ɜ: Analyzed by ANOVA**

Table 2. Variation in histometric parameters of chick embryo Hippocampus (**VCon:** Vehicle control, **LCH:** Lambda-cyhalothrin**, LCHO:** Olive oil+Lambda-cyhalothrin, **LCHE:** Vitamin E+Lambda-cyhalothrin in vegetable oil, **LCHOE:** Vitamin E+Lambda-cyhalothrin in olive oil)

Histopathological parameters	Mean + SEM				
	VCon	LCH	LCHO	LCHE	LCHOE
Number of Pyramidal neurons per unit area(µm)**	2.33±0.03^{ab}	1.13±0.02^{a}	2.80±0.03^{b}	3.00±0.04^{b}	2.60±0.05^{b}
Number of Pyramidal like neurons per unit area(µm)***	1.93±0.01^{a}	1.73±0.01^{a}	2.86±0.02^{b}	2.80±0.02^{b}	2.20±0.01^{ab}
Number of Multipolar neurons per unit area(µm)***	2.00±0.01^{a}	1.33±0.02^{a}	2.80±0.02^{b}	3.13±0.02^{b}	3.13±0.01^{b}
Number of Local Circuit neurons per unit area(µm)*	1.86±0.02^{ab}	0.73±0.02^{a}	2.26±0.05^{b}	1.46±0.03^{ab}	1.33±0.02^{ab}
Number of Neuroglial cells per unit area(µm)***	3.93±0.05^{ab}	2.40±0.04^{a}	4.13±0.05^{b}	4.13±0.06^{b}	4.80±0.04^{b}

***: (p≤0.05), **: (p≤0.01), ***:(p≤0.001) ε: Analyzed by ANCOVA ɜ: Analyzed by ANOVA**

DISCUSSION

Hippocampus has functions related to memory, emotions, thoughtfulness, decisions, correlation and judgment[7]. Being one of the most important part of brain many of the developmental studies have focused on many of the developmental and differentiation aspects of the development of the limbic system (in general) and hippocampus (in particular)[8]. Although blood brain barrier (BBB) usually protects brain and neuronal cells from toxic exposures in adults. However, at early developmental stages, the BBB either don't exist or may be in the rudimentary form this is because the any kind of in utero exposure with environmental toxicants (insecticides like LCH) may led to severe damages to the developing nervous system that ultimately may end up with abortions, miscarriages, premature births and post-natal survival risks[9].

In present study, the developing chick embryos were exposed to lambda-cyhalothrin in ovo. The macrometric preparations of the developing chick brain have shown various signs of toxicity such as reduction in mean embryonic weight[10]. Weight of LCH treated embryos decreased here but according to a study of bifenthrin exposed rats there was no effect on brain weight[11]. Volume and density of brain decreased in LCH treated embryos. However, vitamin E and olive oil has rescuing effects to all above mentioned macrometric damages[12,13]. There was significant decrease in number of neurons in LCH treated brain hippocampus and in the other study various toxicological signs find out in hippocampus due to LCH exposure[14].

The histological examination of the vehicle control group's embryonic hippocampus serves as an essential reference point. The findings reveal a characteristic distribution of neuroglial cells and the presence of various types of neuronal cells, including pyramidal, pyramidal-like, multipolar, local circuit, and neurosupportive glial cells. Additionally, various precursor neuronal and glial cells at various stages of mitosis were observed, indicative of healthy neurodevelopment. In contrast, LCH treatment led to a spectrum of toxicological insults within the embryonic hippocampus. Complete cell obliterations, microlesions, and partial nuclear disintegration were identified, collectively indicating substantial damage to neural tissue. These findings corroborate previous research that has highlighted the neurotoxic effects of LCH and other insecticides[15]. Such toxicological insults can disrupt normal neural development processes, potentially leading to long-term consequences[16].

The co-administration of vitamin E alongside LCH demonstrated a slight improvement in neuroglial cell density. This suggests that vitamin E may possess some neuroprotective properties against LCH-induced damage. These findings align with prior studies

indicating the antioxidant and neuroprotective potential of vitamin E[17]. However, it is noteworthy that despite this improvement, toxicological signs remained apparent, underscoring the need for more robust neuroprotective strategies. Notably, the LCH and olive oil group exhibited the presence of megakaryocytic neuroglial precursor cells. The group treated with LCH, olive oil, and vitamin E displayed the highest neuroglial cell density among the insecticide-treated groups. This suggests that both vitamin E, an antioxidant, and olive oil may play a protective role in mitigating the adverse effects of LCH exposure on neuroglial cells.

Previous studies have also highlighted the neuroprotective properties of antioxidants. For instance, a research conducted in 2021 demonstrated that antioxidants can help counteract oxidative stress and protect neural cells from damage[18]. Likewise another study found that antioxidant supplementation was effective in reducing the harmful effects of environmental toxins on neuronal health[19].

Olive oil, known for its potential health benefits, has been associated with antioxidant and anti-inflammatory properties. Some studies suggest that olive oil may offer protection against oxidative stress and neuroinflammation, both of which can be induced by pesticide exposure [20, 21].

While vitamin E and olive oil offer some level of protection in this study, it is important to recognize that they may not be the sole solution to combat the complex effects of LCH exposure. These findings suggest that a comprehensive approach to neuroprotection, potentially involving multiple antioxidants like vitamin E and the inclusion of olive oil, should be considered to fully safeguard neural cell development and function in the face of pesticide exposure.

CONCLUSIONS

LCH may be used with extreme care at least on the food materials and work places etc to avoid its exposure to the pregnant females to all the possible extent. Simultaneously, it is recommended that olive oil and vitamin E may be used in combination at very low doses for neurodevelopmental protection of the embryos in pregnancy.

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CONFLICT OF INTERESTS

None

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