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Rescuing Effects of Vitamin E and Olive Oil on the Hepato-nephronal Implications of Lambda-cyhalothrin in Chick Embryos

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ABSTRACT

Purpose: The purpose of this study was to investigate the salvaging effects of vitamin E and extra virgin olive oil (EVO) on the hepato-nephronal derangements of lambda-cyhalothrin in chick (*Gallus domesticus*) embryos.

Methods: Freshly laid, 250 fertilized eggs were equally distributed in five groups. 1) Vehicle control Group (VCon): 0.1mL of 5% DMSO in corn oil (2) Lambda-cyhalothrin Group (LCH): 0.1mL of 0.01 mg/kg LCH in 5% DMSO (3) EVO + Lambda-cyhalothrin Group (LCHO): 0.1mL of 5% DMSO + 0.01 mg/kg lambda-cyhalothrin solution in EVO (4) Vitamin E+ Lambda-cyhalothrin+ EVO Group (LCHOE): 0.1mL of 5% DMSO + 0.01 mg/kg lambda- cyhalothrin + 0.1mg/kg Vitamin E solution in extra virgin olive oil (5) Vitamin E + Lambda-cyhalothrin Group (LCHE): 0.1mL of 0.01 mg/kg LCH + 0.1mg/kg Vitamin E solution in 5% DMSO. On 15th day of incubation embryos were recovered and dissected to obtain liver and kidneys for fixation and further processing.

Results: Distinct hepato-histological derangements such as compression of sinusoids, necrosis of hepatocytes, enlargement and partial disintegration of hepatocytic nuclei, enlarged cross-sectional area of central vein and hepatocytes were observed in LCH group. Several nephro-histo-architectural mutilations including necrosis in endothelial lining of bowman's capsule, shrunken glomerulus, enlarged peri-glomerular space, larger lumen size and fibrosis in proximal convoluted tubule and distal convoluted tubule were noted in LCH group. The maximum rehabilitation of LCH induced damages was recorded in LCH+VitaminE+Olive oil group as the micrometric results of both organs, showed much resemblance with control group.

Conclusions: LCH is highly toxic to developing chick liver and kidney. However, the natural products in vitamin E (tocopherols and trocotrienols) and olive oil (oleic acid, phenolic components and squalene) can bring down the risk of possible hepato-renal damages.

Key-words:*chick embryo; Lambda cyhalothrin; olive oil; vitamin E; chick embryo liver and kidney*

This article has been extracted from M.Phil. thesis (Zainab Aslam)

INTRODUCTION

Poultry industry has always dominated for food production worldwide through meat and eggs provision but unfortunately this vibrant economic unit is susceptible to pesticide exposure through various ways[1]. The accumulated pesticide residues in eggs and meat impose risk to developing

embryo but also can be hazardous for consumer health as well.[2] LCH a synthetic type II pyrethroid and is commonly used in agriculture regions and residential areas for pest management.[3,4] Despite being effective against the intended species, it can toxically affect non-target organisms including fish, amphibians, reptiles and mammals.[5,6] It can easily enter the biological membranes and can disturb the normal cellular integration due to its lipophilic nature.[7] It has been found to promote oxidative stress and can disrupt the neuronal membrane ionic conductivity[8,9]. Moreover, it has potential to cause growth retardation, disruption in normal neurological development and even death of developing chick embryos.[10] Since, several antioxidants have been shown to reduce the potential risks caused by many pesticide.[11] Vitamin E and olive oil were chosen in this study to assess their mitigating effects against LCH-induced histopathologies.

Olive oil is an excellent natural source of antioxidants with a healthy balance of monounsaturated and polyunsaturated fatty acids.[12,13] The chemical makeup of extra virgin olive oil is composed primarily of triglycerides (oleic acid), polysaturated fats (linoleic and linolenic acid) and trace amounts of saturated fats (stearic and palmitic acid).[14,15] In case of chicken, it has found to boost fertility, improved yolk quality, and protection against drug induced toxicity[16,17]. Similarly, vitamin E is the powerful natural antioxidant with the combination of naturally occurring lipophilic compounds (tocopheroles and tocotrienols).[18,19] Both olive oil and vitamin E have also been found to minimize the teratogenic effects of LCH in 14 days chick embryos.[20]

The liver is the principal site of metabolism for pesticides and other environmental toxicants.[21] Kidneys are the organs that are mainly in charge of eliminating toxins and their metabolites such as pesticides. The removal of these products demands high circulatory and metabolic rates. Thus, both of these organs are exposed to hazardous substances and their byproducts at high concentrations.[22] There is a dearth of knowledge on avian toxicity of LCH, particularly in developing chick embryos, that indicate a critical need for more research in this area. Keeping in view all these points this research work was designed to explore the rescuing effects of vitamin E and EVO on histopathological signs of LCH exposure in *Gallus domestics* embryo's liver and kidneys.

MATERIAL AND METHODS

Eggs collection and experimental groups

250 freshly laid, fertilized eggs (34–38g) of golden black variety of *Gallus domesticus* were cleaned, sterilized, dried and randomly assigned to five groups on the basis of in-ovo treatment at zero day of experiment.

1. **Vehicle control Group (VCon):** 0.1mL of 5% DMSO solution in corn oil.
2. **Lambda- cyhalothrin Group (LCH):** 0.1mL of 5% DMSO + 0.01 mg/kg LCH solution in corn oil.
3. **Olive oil + Lambda-cyhalothrin Group (LCHO):** 0.1mL of 5% DMSO + 0.01mg/kg LCH solution in EVO.
4. **Vitamin E + Lambda-cyhalothrin in vegetable oil Group (LCHE):** 0.1mL of 5% DMSO + 0.01 mg/kg LCH + 0.1mg/kg Vitamin E solution in corn oil.
5. **Vitamin E+ Lambda-cyhalothrin in olive oil Group (LCHOE):** 0.1mL of 5% DMSO + 0.01 mg/kg LCH + 0.1mg/kg Vitamin E solution in EVO.

Dose Administration

All the eggs were positioned horizontally. Then 50µL of conc. Hydrochloric acid was poured to a point on egg shell to soften it for easy penetration of needle during dose administration. Eggs of each group received their respective dose in single administration. Once dose has been administrated, window in eggshell was quickly sealed with clean filtered wax to avoid contamination.

Incubation

Eggs were incubated for 14 days at 37±0.5°C with 60% humidity in a 48-eggs capacity Nanchang Vena digital automatic incubator (VA-48). Every day, eggs were candled to monitor the development of the embryos, and those containing dead embryos were discarded.

Embryo Recovery

Embryos were recovered on 15th day. After cracking the eggshell from broader side embryos were separated from egg content carefully with the help of blunt forceps, scissors and camel hair brush. All the embryos were maintained in fixative for 48 hours.

Dissection of embryos

The embryos of each group were weighed and dissected to obtain complete liver and kidneys for histological and micrometric studies. All the organs

were preserved in acidified formyl ethanol after being weighed for subsequent processing.

Histological preparations

For histological studies organs were processed by different steps including dehydration, clearance, embedding, blocking, sectioning and finally staining of organ's sections was done.

Histological observations

Sections of liver and kidney were carefully observed at 100X and 400X magnifications on Labomid CXR2 trinocular research microscope. A 7.2 MP digital camera (Sony DSC W35) was used to take super-macro photos of selected organ sections in order to create histopathological representations. The pathological effects of tissues were emphasized by using CorelDRAW11 to improve digital images of selected liver and kidney sections for color, contrast, cropping, and the inclusion of highlighting signs, as shown in the results section.

Micrometry

From each of digital photographs (100× and 400×), five randomly selected sections of liver and kidney of each group, were used for micrometry. Micrometry of these images was carried out in CorelDRAW11 by using different tools and readings obtained from images of each group.

Data analysis and statistical application

Analysis of variance (ANOVA), Tukey Multiple Range Test (TMRT) and Analysis of Co-variance (ANCOVA) through software IBM SPSS Statistics (version 25), were used for statistical analysis of micrometric data.

RESULTS

Histological results of liver

One very important and persistent feature of these three groups was the presence of aggregations of hepatoblastic, endothelial and cholangiocytic cells that indicates its pursuit towards the faster recovery of the

The embryonic chick liver histology presents a comparatively complicated picture in comparison with adult liver. In terms that discrete liver lobules are not identifiable because of reticulated and ramified arrangements of sinusoidal network in the developing chick liver. Nevertheless, some features like the central veins, the rounded, elongated solenoids of hepato-blastocytes which appears to be only partially separated from each other are the overwhelming feature of the embryonic chick liver. The variegated sinusoidal spaces are well lined with endothelial and Kupffer cells. The bile ductules, the marginal veins and the hepatic arterioles are not clearly identifiable due to the incomplete architectural differentiation of the hepatic lobules. (Fig: 1A)

The LCH exposure to the developing chick embryos in-ovo was found to induce various mutilations to the commensurate unexposed embryonic hepatic microscopic architecture. These histoarchitectural derangements include the merger of hepatoblastic solenoids at the cost of extreme compression of the sinusoidal spaces. Individual hepatocytic necrosis was very frequent. Moreover, enlargement and partial disintegration of nuclei of hepatocytes was obvious. The central hepatolobular veins were considerably larger in size (Fig: 4 Mean CSA of central vein) than that of the VCon group chick liver histological dispositions. (Fig: 1B)

The simultaneous application of olive oil, vitamin E and olive oil + vitamin E along with LCH in-ovo exposure appears to rescue the developing chick liver from the hepato-histo-pathological signs of LCH alone as mentioned in above paragraph. In terms of the solenoids of hepato-blastocytes were more or less intact. However, the nuclear enlargements and partial disintegrations were persistent with far less severity in the LCH + vitamin E and LCH + olive oil group's embryos. Whereas, the histological slides in LCH + vitamin E + olive oil were almost of the level of differentiation of vehicle control group embryos. Only few hepato-blastocytes were seen having partial enlargements and disintegrations of their nuclei.

Hepato-histo-architecture of the developing chick liver

However, among all these three groups, the best comparable hepato-histological architecture was seen in LCH + vitamin E + olive oil. (Fig: 1C, 1D & 1E).

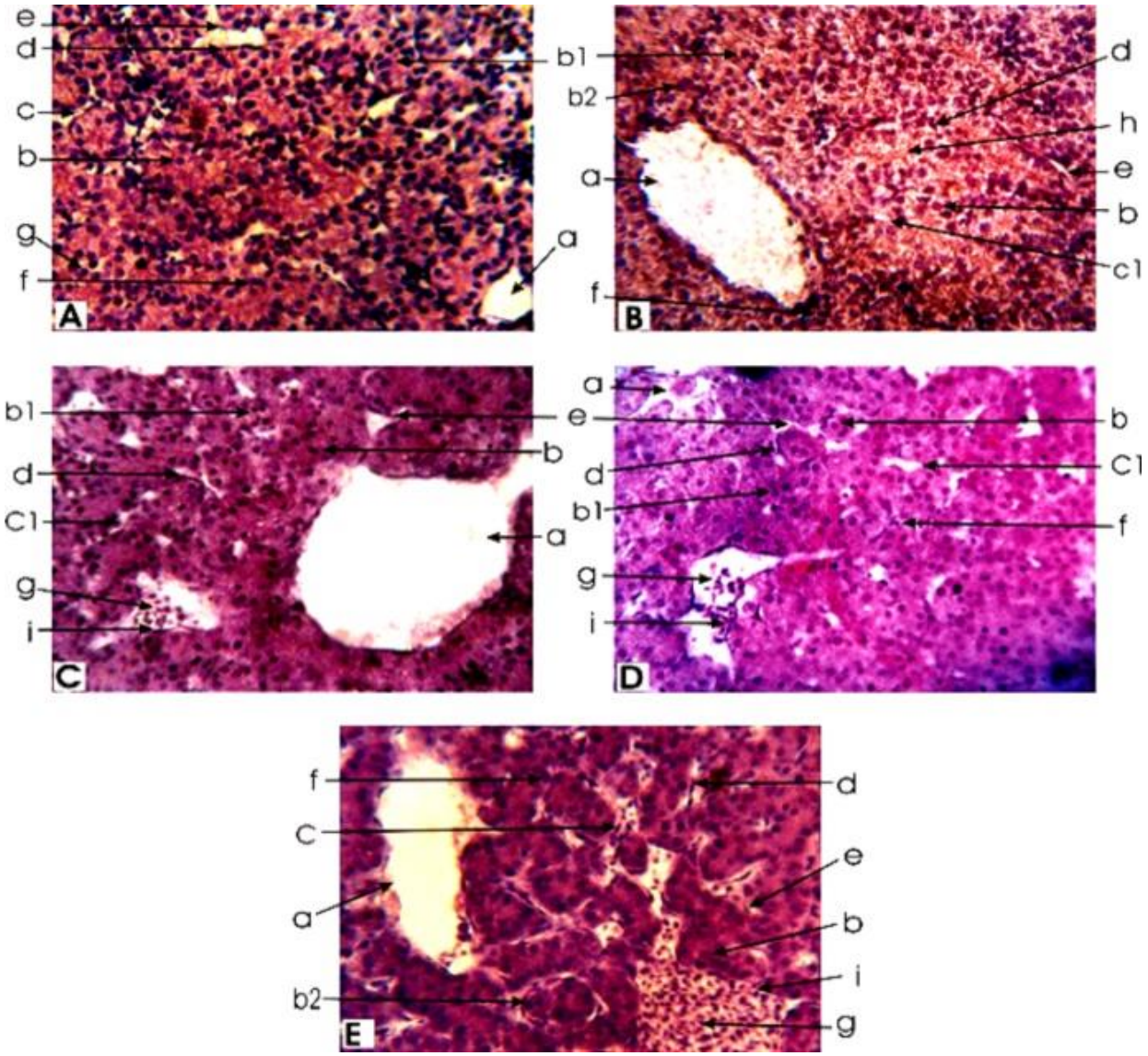

Figure 1: Hematoxylin and Eosin stained histological sections (400X) of chick embryo liver. **A:** VCon, **B:** LCH, **C:** LCHO, **D:** LCHE, **E:** LCHOE, **a:** central vein, **b:**mononucleated hepatocytes, **b1:**binucleated hepatocytes, **b2:**necrotic hepatocytes, **c:** sinusoids, **c1:**reminent of sinusoids, **d:**Kupffer cells, **e:** endothelial cells, **f:** mitotic cell, **g:**hepatoblastic cells, **h:** necrotic mass, **i:**collengocytes

Histological results of kidney

The histological sections of chick embryo kidney of vehicle control group showed typical histo-architectural characteristics, such as intact Bowman's capsule with well-placed glomerulus having thin peri-glomerular space. Proliferating glomeruli and nephrons can also be seen clearly around Bowman's capsule. The random distribution of proximal and distal convoluted tubules can be seen with normal lumen having cuboidal endothelium with central ciliated brush borders. Some arterioles and blood capillaries were also found in between outer margins of adjacent tubules. (Fig: 2A)

On the other hand, in ovo exposure of LCH caused various histo-architectural derangements in chick embryonic kidneys. These histopathological signs

include, necrosis in endothelial lining of Bowman's capsule and secondarily shrunken glomerulus with widened peri-glomerular space as compared to vehicle treated group. Pace of glomerular proliferation was also limited (Fig: 2B; Mean numberof glomeruli per unit area). Megakaryocytes infestations were predominant in this group. Moreover, intracellular peri-tubular fluid accumulation was found in proximal and distal convoluted tubules causing swelling and so increase in tubular wall thickness as well. (Fig: 2B)

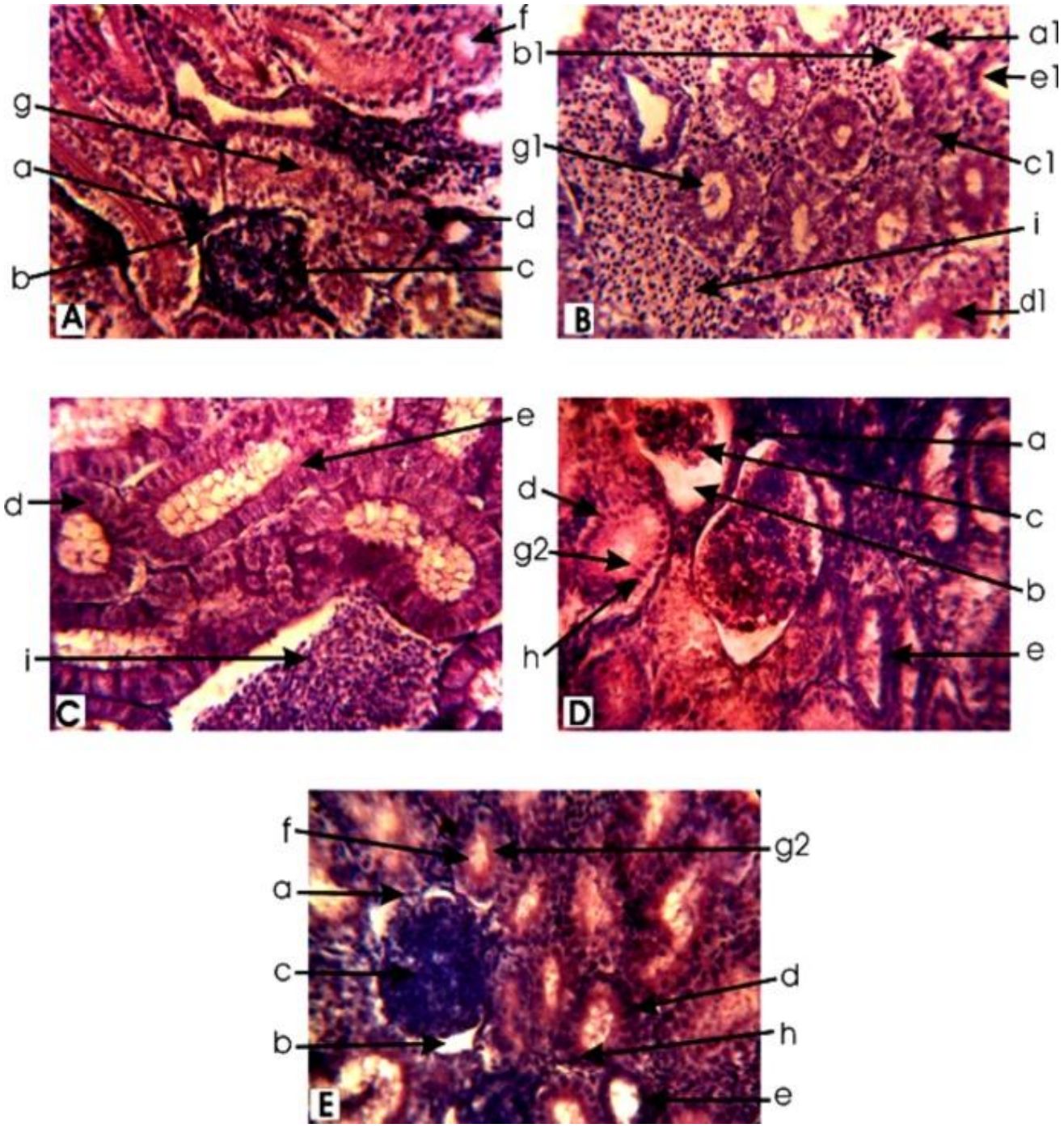

Figure 2: Hematoxylin and Eosin stained histological sections (400X) of chick embryo kidney. **A:** VCon, **B:** LCH, **C:** LCHO, **D:** LCHE, **E:** LCHOE**a:** Bowman capsule, **a1:** necrosis in endothelial lining of Bowman capsule, **b:**periglomerular space, **b1:** widened periglomerular space, **c:** glomerulus, **c1:** shrunken glomerulus, **d:** proximal convoluted tubules, **d1:** damaged proximal convoluted tubules, **e:** distal convoluted tubules, **e1:** damaged distal convoluted tubules, **f:**lumen, **g:**brush border cells, **g1:** damaged brush border cells, **h:**endothelial mitosis, **i:** megakaryocytes.

The co-treatment of olive oil and LCH showed some improvement in histopathological alterations in chick embryo kidney. But this progress was to a lesser extent as the tubular swelling was still present with fibrosis signs and tubules were also showing widened peripheral spaces. These expanded tubular lumens appear to have cellular debris in them. (Fig: 2C)

Vitamin E and LCH treated group also showed partial disintegrations along with rescuing effects of vitamin E. As some tubular fibrosis is still present but with far much less severity than olive oil + LCH group. Glomerular architecture was noted to be improved with Bowman capsule but the space between glomerulus and its respective Bowman capsule was still broader. Endothelial mitosis can also be noted here as a positive sign towards better fate. (Fig: 2D)

The concurrent administration of vitamin E + olive oil along with LCH exposure appears to better rescue the developing chick kidney from nephro-histo-pathological mutilations. The kidney histology of this group showed maximum features closer to vehicle treated group. As restoration of glomerular size with normal lining of bowman capsule and thin peri-glomerular space can easily be seen here. PCT and DCT also showed their typical architecture with no fibrosis. Presence of normal brush border cells in proximal convoluted tubules and removal of accumulated debris was also noted. Tubular endothelial mitosis can also be observed as a healthy signal. These prominent signs are possibly indicating that toxicological damage of LCH exposure was suppressed almost completely with combined use of vitamin E and olive oil as compare to individual treatment of olive oil or vitamin E along with LCH. (Fig: 2E)

Micrometric results of liver

The mean weight of embryo and mean weight of liver in LCH group decreased significantly ($P \leq 0.05$) as compare to control group and a non-significant ($P > 0.05$) increase was observed in LCHO, LCHE and LCHOE groups with control group. The mean CSA of central vein and mean CSA of hepatocytes had shown significant ($P \leq 0.05$) increase in LCH group but there was no significant difference among LCHO, LCHE and LCHOE with VCon group. A significant decrease was observed in mean number of mono-nucleated hepatocytes per unit area in LCH, LCHO, LCHE and LCHOE as compare to VCon. In case of mean number of bi-nucleated hepatocytes per unit area and mean number of kupffer cells per unit area, a significant decline was recorded in both LCH and LCHO groups but LCHE and LCHOE groups had shown non-significant ($P > 0.05$) difference with VCon group (Table 1).

Table 1. Variation between histological measurements of chick embryo's liver (**VCon, LCH**, **LCHO**, **LCHE** and **LCHOE**)

Parameters	Mean±SEM				
	VCon	**LCH**	**LCHO**	**LCHE**	**LCHOE**
Weight of embryo (g) ***	5.93±0.31^{b}	4.16±0.27^{a}	5.24±0.44^{ab}	5.31±0.27^{ab}	5.63±0.29^{b}
Weight of liver(g)* ¥	0.164 ±0.015^{c}	0.090 ±0.018^{a}	0.119 ±0.017^{b}	0.137 ±0.016^{bc}	0.158 ±0.016^{bc}
Mean CSA of central vein (µm^2)*	5187.58 ± 689.60^{a}	8174.22 ± 903.78^{b}	5602.69 ± 536.63^{ab}	5556.24 ± 568.61^{ab}	5161.98 ± 727.21^{a}
Mean CSA of hepatocytes(µm^2) *	217.40± 23.67^{ab}	287.10 ± 17.77^{b}	223.73 ± 11.04^{ab}	211.38± 13.94^{a}	209.19 ± 22.03^{a}
Mean number of Mono-nucleated hepatocytes per unit area(26000µm^2) ***	30.87 ± 1.58^{b}	22.87 ±0.77^{a}	23.33± 1.12^{a}	26.40 ±0.64^{a}	25.80±1.07^{a}
Mean number of Bi-nucleated hepatocytes per unit area(26000µm^2) **	19.2±0.76^{c}	14.33±0.91^{a}	15.53± 1.03^{ab}	17.33±0.85^{abc}	18.4±0.79^{bc}
Mean number of Kupffer cells per unit area(26000µm^2) *	2.2±0.45^{b}	0.87 ±0.21$^{a.}$	0.93± 0.21^{a}	1.33±0.31^{ab}	1.6±0.32^{ab}

*: $p \leq 0.05$, **: $p \leq 0.001$, ***: $p \leq 0.0001$,

¥ is analyzed by ANCOVA

Micrometric results of kidney

The mean weight of kidney significantly decreased in LCH, LCHO, LCHE and LCHOE groups as compare to VCon. A significant ($P \leq 0.05$) decline was recorded in mean CSA of glomeruli of LCH group but LCHO, LCHE and LCHOE groups had shown non-significant ($P > 0.05$) difference with VCon group. In case of mean CSA of Bowman capsule there was no significant difference among all groups as compare to VCon group. A significant decrease was noted in LCH group for mean CSA of PCTs but LCHO, LCHE and LCHOE had shown non-significant difference with VCon group. Mean CSA of DCTs declined significantly in LCH and LCHE groups but no significant difference was noted in LCHO and LCHOE as compare to vehicle control group. Similarly, non-significant ($p > 0.05$) difference with control group was measured in all groups for mean number of glomreuli per unit area and mean no. of DCTs per unit area. However, mean no. of PCTs per unit area had shown significant ($P \leq 0.05$) difference among LCH, LCHO and LCHE but LCHOE was non-significant with VCon group.

Table 2: Variation between histological measurements of 14 days chick embryo's kidney (**VCon, LCH**, **LCHO**, **LCHE** and **LCHOE**)

Parameters	Mean±SEM				
	VCon	**LCH**	**LCHO**	**LCHE**	**LCHOE**
Weight of kidney(g)* ¥	0.041 ±0.002^{c}	0.024 ±0.003^{a}	0.032 ±0.002^{ab}	0.027 ±0.002^{ab}	0.034±0.002^{b}
Mean CSA of glomeruli (μm^2)*	18254.61±911.78^{b}	13687.92±769.49^{a}	16093.53±953.64^{a} ^{b}	15030.91±899.95^{a} ^{b}	16398.66±950.68^{a} ^{b}
Mean CSA of Bowman capsule (μm^2)	19836.89±899.21^{a}	16795.74±798.83^{a}	18223.83±830.93^{a}	17255.02±862.76^{a}	18578.90±883.72^{a}
Mean CSA of PCTs(μm^2) *	9888.29 ± 543.21^{b}	7284.54 ± 555.88^{a}	8608.53±628.46^{ab}	8342.16±445.41^{ab}	9629.67 ± 642.09^{b}
Mean CSA of DCTs (μm^2)*	7653.15±498.29^{b}	5319.03±423.19^{a}	6243.92±415.75^{ab}	6942.65±397.70^{ab}	7072.71±379.48^{b}
Mean number of Glomeruli per unit area (410000 μm^2)	1.70±0.54^{a}	0.90±0.28^{a}	1.0±0.33^{a}	1.10±0.38^{a}	1.3 ±0.36^{a}
Mean number of PCT per unit area (410000μm^2) ***	20.5±1.22^{c}	10.3±1.09^{a}	15.10±1.21^{ab}	14.6±1.48^{ab}	16.40±1.31^{bc}
Mean number of DCT per unit area (410000 μm^2) ***	11.3±0.87^{b}	5.4±0.54^{a}	6.8±0.47^{a}	6.5±0.43^{a}	7.8±0.66^{a}

*: $p \leq 0.05$, **: $p \leq 0.001$, ***: $p \leq 0.0001$,

¥ is analyzed by ANCOVA

DISCUSSION

The present histopathological study dealt with the toxic exposure of LCT on embryonic chick liver and kidneys. The main feature of interest for this fluorinated pyrethroid is its frequent usage and availability. The systemic exposure of LCT in experimental animals including chick embryos and in humans can ultimately result in embryo lethality.[23] As the growth of an embryo is a very sensitive process that is readily disrupted by minute chemical or physical environmental changes and less differentiated embryonic tissues lack the capacity to properly metabolize the insecticide and eliminate its metabolites.[24]So they are more prone to all the hazardous substances out there. Physiologically, both liver and kidney are crucial organs for maintenance of homeostasis and metabolic regulation in the body.[25,26] Hence, many experimental studies have focused on these organs from various aspects either developmental or histopathological.

In current study, the histological examination of Vehicle control group's liver and kidney serves as an essential reference point. These findings revealed all features of a healthy liver including round central vein

surrounded by solenoids of hepato-blastocytes along with endothelial and kupffer cells in sinusoidal spaces. The Vcon group represented the normal histology of kidney with Bowman's capsule, proliferating glomeruli, PCTs, DCTs and some blood vessels among tubules. In contrast, the in-ovo LCH treatment resulted in many derangements in hepatic microscopic architecture including extreme sinusoidal compression, hepatocytic necrosis, and larger hepato-lobular veins. Similarly, spectrum of nephronal derangements was observed including necrosis in endothelial lining of Bowman's capsule, shrunken glomerulus, enlarged peri-glomerular space, fibrosis in PCT and DCT, larger lumen size, and cellular fluid accumulation etc. Significant changes in several micrometric estimations also supported these histological findings. Previous Studies reported similar patterns of histopathological changes in liver and kidney of chicks, mice and rats when exposed to LCT.[27-29] These finding aligns with the past work in which newly hatched chicks were exposed to other pyrethroids.[30,31] All of these alterations could be attributed to oxidative stress produced upon insecticide exposure.

It was very interesting to note that EVOO and vitamin E have immense rescuing potential as all these disintegrations became less severe in LCHO, LCHE and LCHOE groups. The high concentration of oleic acid and phenolic compounds in olive oil makes it an excellent antioxidant to prevent inflammation and oxidative stress to rescue the damaged organ[32]. Vitamin E with its most active ingredient called α-tocopherol involved in the process of cellular integrity by initiating stabilization processes in cell.[33] Olive oil treatment in chicken against cypermethrin exposure also showed protective effects. [34]

However, the histological slides in LCHOE group indicates the far better rescuing potential of olive oil and vitamin E when introduced in combined form, as the hepato-nephric histological observations of this group were similar with Vcon group. These features are the clear evidences of cellular pursuance towards recovery of the histo-architecture of the developing chick liver and kidney Vitamin E works in concert with the valuable components of olive oil, oleic acid and oleocanthal, to produce improved outcomes and increased rescue competency. This could be attributed to these both ameliorative agents, as they increase the body's anti-oxidant state and combat ROS in order to shield the body from oxidative harm.[35,36]

CONCLUSIONs

These findings indicate that halogenated insecticides must be avoided or used with the utmost caution to save the threat of toxicity to non-target animals including poultry. However, the olive oil and vitamin E can serve as a natural rescue from such pathological implications of insecticides as they have shown hepato-nephronal protective potential in this study.

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CONFLICT OF INTERESTS

None

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