Mitigating properties of vitamin E and olive oil on the teratomorphogenic impacts of lambda-cyhalothrin in developing chick embryos

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A B S T R A C T
Embryonic toxicity of lambda-cyhalothrin (LCH) and the mitigating properties of extra virgin olive oil (EVO) and vitamin E were investigated in golden black variety of chick embryos. Fifty fertilized eggs distributed in 5 groups received their respective group treatments at zero days of incubation were recovered after 14 days of incubation and fixed in fixative for 48 h for further studies. Results showed that treatment with LCH caused embryonic death, growth retardation and developmental abnormalities such as limbic developments, reduced muscular growth, and embryonic cataract. Post treatment with vitamin E and EVO alone significantly improved all these developmental defects. Morphometric readings also showed that embryos treated with combined dose of vitamin E and EVO had more resemblance towards the control group embryo. These findings suggested that the use of vitamin E and EVO together during pregnancy have the potential to curtail these accidental, environmental and workplace exposures of LCH.

1. Introduction

Lambda-cyhalothrin (a Type II pyrethroids) is also known as a potent agent for biochemical distortion, oxidative damage and nervous disruption [6,14]. Due to lipophilic nature it readily gets absorbed in biological membranes resulting in neurotoxicity, paralyses and death. Oxidative damage in cell leads to the production of mutagenic compounds which promote atherogenic activity or inflammation resulting in cancer, diabetes, stroke, brain injuries and kidney diseases [11]. Furthermore it has been found that prenatal exposure of LCH leads to impaired learning and memory in developing embryos [25]. Various antioxidants have been proved to overcome the potential harms of different insecticides [1,2] so, in this study vitamin E and olive oil are used to find out their mitigating properties against the teratogenic impacts of LCH.

Vitamin E exhibits anti-oxidant potentials against lipid peroxidation reactions in cellular membranes [9]. Its treatment during gestation has revealed protective role against miscarriages and supportive effects for normal development of central nervous system in human fetus [23,30]. Its beneficial impacts on immunity and fertility has also been revealed in a study on rat model [7,28]. Similarly its co-treatment with cypermethrin (CN) has been found to reduce the chances of CN induced embryonic disruptions in Gallus domesticus embryo [3]. While olive oil is a rich source of monounsaturated oleic acid [34]. The major component of olive oil are triacylglycerols constituting 98–99 % of it while some other acids like palmitic acid, linoleic acid and stearic acid are also present in it [19]. Olive oil also contains phenolic compounds that occur in the form of simple compounds like hydroxytyrosol and tyrosol as well as complex compounds like oleuropein and secoiridoids. Phenolic acids, lignans and flavonoids are also present in olive oil [16,27]. EVO treatment during gestation was found to reduce the incidence of embryonic death in rats [31]. Similarly, it has been reported to show protective effects against the harmful teratological effects of insecticides like cypermethrin [3].

As poultry is a growing sector and meets the demand of meat and eggs for the whole world, it has also shown a susceptibility to the insecticide residues present in commercially available poultry feed. The accumulation of these residues in meat and eggs not only threaten consumer health but also impose risks of developmental abnormalities in poultry animals [21]. Unfortunately, insufficient knowledge exists regarding teratogenicity of various insecticides especially LCH in birds which depicts intense need of research in this field. Therefore, present
1902 study was performed to investigate teratogenic potentials of LCH in Gallus domesticus embryos and ameliorative potentials of vitamin E and EVO against LCH induced embryonic defects. Chick embryos were used as they fulfill all the criteria’s needed in teratological studies i.e. ease of accessibility of the embryo, minimal expenditure of time and money, possibility of experimenting on a large scale for statistically valid results and whole animals are also not required.

2. Materials and methods

2.1. Egg collection and experimental groups

Freshly laid fertilized eggs (250) of golden black variety of common chick (Gallus domesticus) were collected and brought in the lab, within 24 h, from the villages nearby Sargodha city. Eggs of approximately equal weight (34–38 g) were selected and randomly distributed in five groups (50 each) depending upon solutions injected in eggs (control group; 0.1 mL of 5% DMSO solution in corn oil (Corn oil is used to nullify any toxic effect of DMSO and to maintain consistency among all the groups as DMSO alone can’t be given in high concentrations). (ii) Lambda-cyhalothrin Group (LCH); 0.1 mL of 5% DMSO + 0.01 mg/kg lambda-cyhalothrin solution in corn oil. (iii) Olive oil + Lambda cyhalothrin Group (LCHO); 0.1 mL of 5% DMSO + 0.01 mg/kg lambda-cyhalothrin solution in extra virgin olive oil. (iv) Vitamin E + Lambda-cyhalothrin in corn oil group (LCHOE); 0.1 mL of 5% DMSO + 0.01 mg/kg lambda-cyhalothrin + 0.1 mg/kg Vitamin E solution in extra virgin olive oil.

2.2. Dose administration

Eggs were washed, air dried and sterilized by cotton swabs soaked in 70% alcohol. For easy penetration of syringe’s needle during dose administration, a small area of egg shell at egg center was softening through a drop of concentrated HCL. To avoid any injury to developing embryos all egg were placed in horizontal position for 5 min before dose administration so that embryos can rise on top. Relevant group treatment was injected in yolk sac through soften egg shell area. Dose was administered on 0 day of study in all groups. After the dose administration, the window was sealed properly by using melted wax to avoid contamination. Before putting in incubator, weight of each egg was measured by electrical weight balance.

2.3. Incubation

Eggs were incubated for 14 days in an automatic incubator (Nanchang Vena egg incubator, VA-48) at 37.5 °C temperature and 60 % humidity. Candling was done on daily basis and eggs with retarded or stunted growth were discarded.

2.4. Embryo collection

Embryos were recovered after 14th day of incubation. For this purpose eggs were weighed and round portion of the shell from broad side of each egg was removed by using forceps and scissors. Embryo being lighter than the yolk came to the top of the yolk. The egg content was placed in the petri dish filled with saline solution and the embryo was separated from the yolk by using forceps and camel hair brush. Extracted embryos were fixed in the fixative (mixture of 90 mL alcohol and 10 mL saturated formaldehyde) for 48 h.

2.5. Morphometry and data analysis

Morphological measurements such as weight of embryos, crown-rump lengths, fronto-occipital lengths, biparietal distances, length and width of eyes, lengths of beak, anti-brachium, brachium, shank and all the digits of both left and right hindlimbs of each and every embryo belonging to all groups were taken using vernier caliper (with no zero error). Statistical analysis of morphometric data was performed by applying ANCOVA and ANOVA test by using IBM SPSS Statistics 23 software.

3. Results

3.1. Chick eggs fertility index

Analysis of the data for the numbers of unfertilized eggs, fully grown eggs, dead embryos at pre-hemopoietic stage, post-hemopoietic stage and late organogenesis stage was done using the Chi-square test (Table1).

3.2. Morphological results

Control group embryos showed all normal morphological features such as development of fore limbs and hind limbs, beak and eye development, down development and normal increase in size.

In LCH reduction in size of the embryos, embryonic cataract and opening of the eye were the most common abnormalities. Other deformities including beak reduction or anathia, skewed spine, hemorrhagic spots on the body and muscular dystrophy were also obvious. Some appendicular defects were also seen such as meromelia and amelia of forelimbs along with some hindlimb deformities which include focomelia, torted shank and abnormally developed or complete absence of digits (Fig. 1).

Olive oil and vitamin E used as rescuing agents somewhat provided barrier against the drastic effect of LCH. The embryos in LCHO group only showed minor reduction in size while the embryos belonging to LCHOE group showed muscular dystrophy and some other abnormalities with less severity than LCH group such as size reduction, lower beak reduction, skewed neck and phocomelia. LCHOE group showed the most promising results such as the mean body size and morphological features were almost similar to that of control group embryos (See Fig. 1).

3.3. Morphometric results

The Mean embryo weight of LCHO and LCHOE groups was significantly increased as compared to LCH group. However, there was no significant (p > 0.05) difference between control, LCH and LCHOE groups. For crown-rump length, fronto-occipital length, biparietal distance and circumference of eye, significantly (p < 0.05) decreased mean values were seen in LCH group as compared to control group and LCHOE group. Mean length of left arm anti-brachium, brachium and shank of LCHOE group was significantly higher than LCH group. LCHE group showed muscular dystrophy and some other abnormalities with less severity than LCH group such as size reduction, lower beak reduction, skewed neck and phocomelia. LCHOE group was significantly higher than LCH group.

Similarly significantly declined mean length of right arm anti-brachium, brachium and shank was measured in LCH group as compared with the control group. No significant (p > 0.05) difference

Table 1

| Parameters                     | Groups         |
|--------------------------------|----------------|
|                                | Control | LCH | LCHO | LCHE | LCHOE |
| Total eggs                     | 50      | 50  | 50   | 50   | 50    |
| Mortality rate at Pre-hemopoietic stage | 6       | 11  | 8    | 11   | 8     |
| Mortality rate at Post-hemopoietic stage | 2       | 3   | 2    | 3    | 2     |
| Mortality rate at late organogenesis | 3       | 7   | 6    | 4    | 3     |
| Fully grown eggs (15th day)    | 22      | 10  | 15   | 12   | 20    |
| Unfertilized eggs              | 17      | 19  | 19   | 20   | 17    |

The chi-square statistic is 12.5301. The p-value is 0.706751. The result is not significant at p < 0.05.
was recorded in mean length of right thumb, left thumb and middle finger among groups. However significantly decreased ($p \leq 0.05$) mean length of left index and little finger was recorded in LCH group as compared to the control group (Table 2).

4. Discussion

4.1. Lambda-cyhalothrin treatment

Teratogenic potentials of certain pyrethroids insecticide have been reported in various studies. Ahmed et al. [3] observed that as the processes of embryonic development are highly delicate and slight variations in the chemical and physical environment can derail them, the cypermethrin (CN) exposure resulted in developmental abnormalities such as growth retardation, rudimentary beak, microphaly, forelimb micromelia, and hind limb amelia. Another similar developmental study conducted on chick embryos indicated that pyrethroids such as bifenthrin with multiple fluoridations exhibit high teratogenic potentials and are found to be toxic to various body organs, including kidneys, liver and lungs [21]. Unfortunately, lambda-cyhalothrin has also shown teratogenic and mutagenic potentials in experimental animal studies [4,5,10]. As LCH is a fluoridated pyrethroid, it was suggested that the teratogenic potential may partially be attributed to multiple fluoridation in its composition [29]. In rainbow trout, high concentration of lambda-cyhalothrin resulted in decreased motility rate and life span of spermatozoa [15,22]. A similar study including mosquito fish and zebra fish revealed that lambda-cyhalothrin has potential of causing cytotoxic and genotoxic effects [33]. Additionally, Al Malahi et al. [4]; found that treatment with lambda-cyhalothrin caused damaging or altering the sperm morphology and showed strong teratogenic and mutagenic effects in mice. Very little information is present regarding the effects of lambda-cyhalothrin on birds and no proper evidence is found for accumulation of lambda-cyhalothrin in tissues or eggs of birds [10].

In present study, the in-ovo exposure of LCH caused various departures from normal proceedings of embryonic development in chick embryo. LCH exposure (Fig. 1) resulted in embryonic death, growth retardation and developmental abnormalities of various morphological features such as limbic development, reduced muscular growth, and embryonic cataract. Additionally the development of down feather and claws was also highly effected. These findings indicated that embryonic tissue protein synthesis and deposition might have been grossly decreased with the exposure of LCH. These findings were in line with most of the studies which suggested that LCH exposure causes depletion of proteins by causing the destruction or necrosis of cellular function and

![Fig. 1. A: Control (C), B: LCH, C: LCHO, D: LCHE, E: LCHOE, a: Normal Embryonic Eye formation with eye lids, a1: Embryonic Cataract and wide Open Eye, a2: Rudimentary Eye without eyelids, b: Normal Beak, b1: Enlarged Lower Beak, b2: Reduced Lower Beak & Anathia, c: External Auditory Meatus, d: Normal Neck, d1: Skewed Neck, e: Normal Fore-limbs, e1: Fore-limb Meromelia, f: Normal Hind-limbs, f1: Small Hind-limb with Torted Shank, g: Normal Hind-limb Digits, g1: Reduced Hind-limbs or Amelia, g2: Phocomelia, h: Proper Down Development, h1: No Down Development, i: Hemorrhagia, i1: Multiple Hemorrhagic Spots, j: Suspected Anterior Neurpore Opening, k: Rafe of Neural Crest & feather growth alongside the rafe (Spina bifida), m: Muscular Dystrophy.](image-url)
and are more prone to oxidative damage. Additionally, the embryonic tissues and organs is naturally much higher than the adult tissues calcals in the developing embryos as the metabolic rate of the differenti

Table 2

| Mean morphometric measurements of 14 days chick embryos. | Mean + SEM | Control | LCH | LCHO | LCHE | LCHOE |
|----------------------------------------------------------|------------|---------|-----|------|------|-------|
| Mean weight of embryos (g)**+*                           | 5.99±      | 4.41±   | 4.83±| 4.38±| 4.19±|       |
| Mean length of crown-rump (mm)**+*                      | 1.05±      | 0.72±   | 0.52±| 1.62±| 1.11±|       |
| Mean length of fronto-occipital (mm)**+*                | 14.52±     | 12.67±  | 13.22±| 13.01±| 13.2±|       |
| Mean distance of biparietal (mm)**+*                    | 11.43±     | 10.84±  | 10.99±| 10.92±| 11.7±|       |
| Mean eye width (mm)**+*                                 | 10.56±     | 10.26±  | 9.91±| 9.99±| 10.41±|       |
| Mean length of little finger (right) (mm)**             | 0.11±      | ±0.12±  | ±0.12±| ±0.12±| ±0.12±|       |
| Mean length of little finger (left) (mm)**              | 0.12±      | ±0.12±  | ±0.14±| ±0.14±| ±0.12±|       |
| Mean length of index finger (right) (mm)**             | 0.18±      | ±0.19±  | ±0.16±| ±0.16±| ±0.16±|       |
| Mean length of index finger (left) (mm)**              | 0.18±      | ±0.18±  | ±0.18±| ±0.18±| ±0.18±|       |
| Mean length of thumb (left) (mm)**                      | 0.15±      | ±0.16±  | ±0.15±| ±0.15±| ±0.14±|       |
| Mean length of thumb (right) (mm)**                     | 0.15±      | ±0.16±  | ±0.15±| ±0.15±| ±0.14±|       |
| Mean length of middle finger (left) (mm)**             | 0.29±      | ±0.30±  | ±0.29±| ±0.29±| ±0.27±|       |
| Mean length of middle finger (right) (mm)**            | 0.19±      | ±0.19±  | ±0.20±| ±0.20±| ±0.18±|       |
| Mean length of index finger (left) (mm)**              | 6.17±      | 5.73±   | 5.28±| 5.21±| 5.76±|       |
| Mean length of index finger (right) (mm)**             | 0.19±      | ±0.19±  | ±0.19±| ±0.19±| ±0.19±|       |
| Mean length of little finger (left) (mm)**             | 5.90±      | 5.21±   | 5.19±| 5.49±| 6.27±|       |
| Mean length of little finger (right) (mm)**            | 0.22±      | ±0.23±  | ±0.23±| ±0.23±| ±0.19±|       |

* P < 0.05, **: P < 0.001 * *: P < 0.0001, : Analyzed by ANCOVA ++: Analyzed by ANOVA.

4.2. Vitamin E and extra virgin olive oil treatment

Vitamin E and extra virgin olive oil being an ideal antioxidant protect tissues, proteins, lipids and DNA from oxidative stress because of their easy effective and safe dietary administration in large range of concentrations [12,20,26]. Various studies performed on rabbits and mice have shown that treatment with vitamin E alone caused significant increase in body weight and enhanced the immune functions [32,35]. In the present study, vitamin E alone was not that sufficient against LCH and resulted in muscular dystrophy and some other abnormalities with less severity. Similar results were indicated by Ahmed et al. [3], while studying the role of vitamin E against the etiologies of CN exposure on chick embryos. In contrast to this, the treatment with extra virgin olive oil showed protective effects against LCH and only showed minor reduction in size as shown in Fig. 1. In this context the combined treatment of vitamin E and EVO was found to provide successful rescuing properties and helped in overcoming the drastic teratological outcomes of LCH. Along with the general suppression of the embryonic toxicity, the combined vitamin E and EVO treatment was also found to significantly improve the lambda-cyhalothrin exposure effects on embryonic growth retardation. These improvements in the growth parameters on vitamin E and EVO exposures along with the insecticide indicate that vitamin E in particular and the various precious antioxidants present in extra virgin olive oil have helped the developing embryo by providing a shield against reactive oxygen species that are usually produced in the developing tissues and organs on insecticide exposure [17,20]. The results of the present study suggest that use of EVO and vitamin E in the diets of farms animals and in avirias, will increase the production rate of many expensive birds and provide protection against the exposure of most of the insecticides. Similarly, if given during pregnancy, they have the potential to curtail accidental, environmental and work place exposures to various noxious environmental agents like insecticides on the developing human embryos.

5. Conclusion

From the present results, it is clearly indicated that LCH treatment interferes with the normal avian developmental processes resulting in various malformations along with embryonic growth retardations. It can also be concluded that concurrent administration of vitamin E and extra virgin olive oil to lambda-cyhalothrin-treated animals ameliorates these deformities. Present study has highlighted therapeutic effects of vitamin E and EVO in minimizing the teratogenic effects of lambda-cyhalothrin exposure.

CRediT authorship contribution statement

Kashif Sadaf: Investigation, Data curation, Writing – original draft, Formal analysis, Resources. Khawaja Raees Ahmad: Conceptualization, Methodology, Resources, Supervision, Validation. Syeda Nadia Ahmad: Writing – review & editing, Visualization. Urooj Kanwal: Investigation, Resources. Sadia Suleman and Zainab Aslam: Formal analysis. Iram Inayat and Saira Siddique: Writing – review & editing. Muhammad Ali Kanwal: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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Data Availability
Data will be made available on request.

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Declaration of interests
The authors declare that they have no conflict of interest.

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