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Embryonic Toxico-pathological Effects of *Origanum vulgare*: Evaluation of the Gross and Histopathological Lesions using a Chick Embryo Model

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ABSTRACT

Background & Objective: Toxico-pathological effects of herbal plants have always been a major concern, but little information is provided on the toxico-pathological effects of *origanum* on the fetus. Due to ethical rules and regulations, no drug experiment on the human fetus is permitted; hence, the chick embryo model is used as an ideal opportunity to study the adverse effects of drugs. This study aims to assess the histopathological effects of the various doses of *Origanum vulgare* (*O. vulgare*) using the chick embryo model.

Materials & Methods: Ross chicken eggs were subjected into four experimental groups (n=7); phosphate buffered saline-injected group and *Origanum*-injected groups that were treated with *O. vulgare* leaf-extract at doses of 25, 50, and 100 mg per kg egg-weight, respectively. After injecting the embryos with the extracts, re-incubation was performed and allowed to develop until day 18, when embryos were evaluated for pathological lesions.

Results: Treated embryos were normal in the *Origanum*-injected groups and no defect was noticed on the body features. The microscopic lesions including congestion and edema were made in the brain, liver, kidney, heart, and lung in a dose-dependent manner.

Conclusion: It can be concluded that *O. vulgare* at a concentration higher than 25 mg per kg is toxic for chick embryo in a dose-dependent manner; therefore, more attention should be given to the toxicity of *O. vulgare* in the period of fetal development.

Keywords: Chick, Embryo, Fetus, *Origanum vulgare*, Pathology

Introduction

The ever increasing demand to treat emergenic diseases has led to the idea of finding new medicine, and herbal drugs have produced promising results **(1-3)**. The *Origanum vulgare* (*O. vulgare*), of the family *Lamiales* grows in many parts of the world. Its medicinal properties include antioxidant, antiemetic, anti-inflammatory, anticancer, anti-fungi, anti-yeast, anti-viral, anti-microbial, larvicidal, enzyme inhibition, and cytotoxic activities **(4-6)**. Today, it is often utilized to treat cardiac diseases, analgesia, atherosclerosis, wound healing, and human skin diseases **(7-10)**. Recent researches indicated that it can destroy some micro-organisms like *Escherichia coli*, *Klebsiella oxytoca*, *Staphylococcus aureus*, *Salmonella*, and *Shigella* **(11-13)**. However, like many other drugs and herbal medicines, *Origanum* has some side effects, such as allergic reactions, alteration in iron absorption, anticoagulant activities, thymol toxicity, and gastrointestinal symptoms **(14, 15)**.

Many researches were conducted to prepare a different type of *Origanum* by products, such as extract, edible jelly and powder. The present study performed to evaluate the toxic effects of the *Origanum* leaves on the chick embryo. Due to its availability and easy manipulation as well as its embryogenesis similarity to human beings, chick embryo has become an attractive model to study the developmental processes **(16-21)**.

Materials and Methods

This experimental study was approved by the local Ethics Committee of Shiraz University of Medical Sciences (November15, 2014 - Approval no.IR.SUMS.REC.1393.8050).

Herbal Plant Extract

The *O. vulgare* leaves were purchased from the Gyahan Darooi Company, Kerman, Iran on October

2015 and authenticated at the Department of Pharmacological Sciences of Kerman University, Kerman, Iran. Traditional Soxhlet extraction was carried out in a standard apparatus **(22, 23)**. For 4 hours, 100 g of *O. vulgare* leaves were soaked in1000 mL of solvent (water/ethanol 80/20v/v). The extracts were filtered *via* filter paper and the remaining volume was completely dried in an atmospheric oven at 60°C.

Drug Administration to the Chick Embryos

A total of 28 fertile chicken eggs (Ross 308) with an average egg-weight of 54.8±0.5g were supplied by the Mahan Breeder Company, Kerman, Iran and randomly assigned into four equal treatment groups (n=7). Fertilized chicken eggs were placed at 37.5ºC and 60% relative humidity in a humidified incubator (Belderchin Damavand Co. PLC-DQSH, Tehran, Iran). Experiments were performed on chick embryos until day 18 of the embryonic growth. All treatments were administered as 0.5 mL/egg of a single dose into the yolk sac of each egg. On the 4^{th} day of the embryonic growth, phosphate buffered saline (group 1) or *O. vulgare* leaves-extract at doses of 25 (group 2), 50 (group 3) or 100 (group 4) mg per Kg egg weight, were injected into each group of fertilized chicken eggs, as described previously (20, 21, 24-26). Before injection, the extracts were filtered through a syringe filter (0.22 µm) to remove any micro-organism.

Assessment of Pathological Lesions

Embryos were investigated for gross and histopathological injures on the 18^{th} day of the growing period. At first, embryos were killed by chilling and then examined under the stereo-microscope to study any gross lesions (27, 28). The brain, liver, kidneys, heart, and lungs were fixed in 10% neutral buffered formalin to prepare paraffin-embedded tissues, and then stained with hematoxylin and eosin.

Measurements

The body length and weight of the embryo was calculated *via* scale (Limestone AT-p735, Iran, range 100 g to ± 0.01 g) and caliper (Mitutoyo-500, Japan, range 0.01 mm). The length was measured from front angle of the head to the apex of the tail (uropygial gland).

Statistical Analysis

Statistical analysis was done via SPSS 20 (SPSS Inc., Chicago, IL., USA). The Statistical tests including Fisher's exact test, One-way analysis of variance, and Tukey's tests were used to evaluate the significant among treatment groups. A P-value<0.05 was considered significant.

Results

Macroscopic Findings

The embryos of all experimental and control groups were normal. There was no abnormality on the external body limbs (Figure 1).

Microscopic Findings

In group 2, all the tissues were normal and no microscopic changes were observed (Figure 2). In the embryos of group 3, congestion was seen in the brain, liver, kidneys, heart, and lungs. In the brain and lungs, edema was also seen (Figure 3).

Figure 1. The chick embryos treated with phosphate buffered saline (a), 25 mg/Kg egg-weight of *O. vulgare* leaf-extract (b), 50 mg/Kg egg-weight of *O. vulgare* leaf-extract (c), and 100 mg/Kg egg-weight of *O. vulgare* leaf-extract (d) are normal with no gross abnormality on the body surface.

The lesions in the brain, liver, kidneys, heart, and lungs in the embryos of group 4 were the same as group 3, but the brain edema was much more severe (Figure 4). No microscopic injures were evident in the control embryos. Finally, no significant difference was observed in the embryo weight and the embryo length of the groups that were treated with an *O. vulgare* leaves-extract compared to the control group (Table 1).

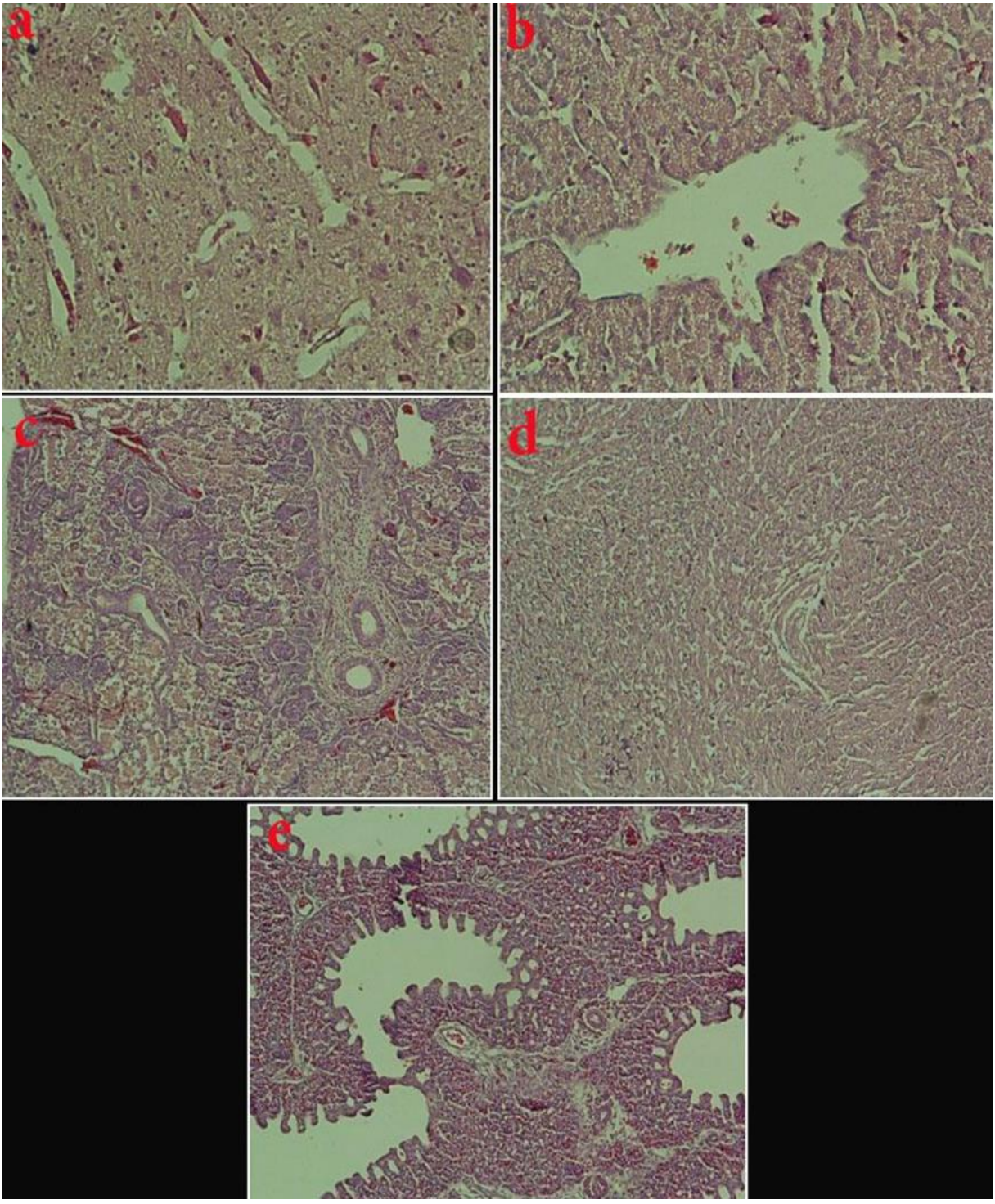

Figure 2. Photomicrograph of the chick embryo treated with 25 mg/Kg egg-weight of *O. vulgare* leaf-extract. (a) Normal structure of the brain is seen (×200, H&E), (b) Normal structure of the liver is seen (×200, H&E), (c) Normal structure of the kidney is seen (×100, H&E), (d) Normal structure of the heart is seen (×100, H&E), (e) Normal structure of the lung is seen (× 100, H&E).

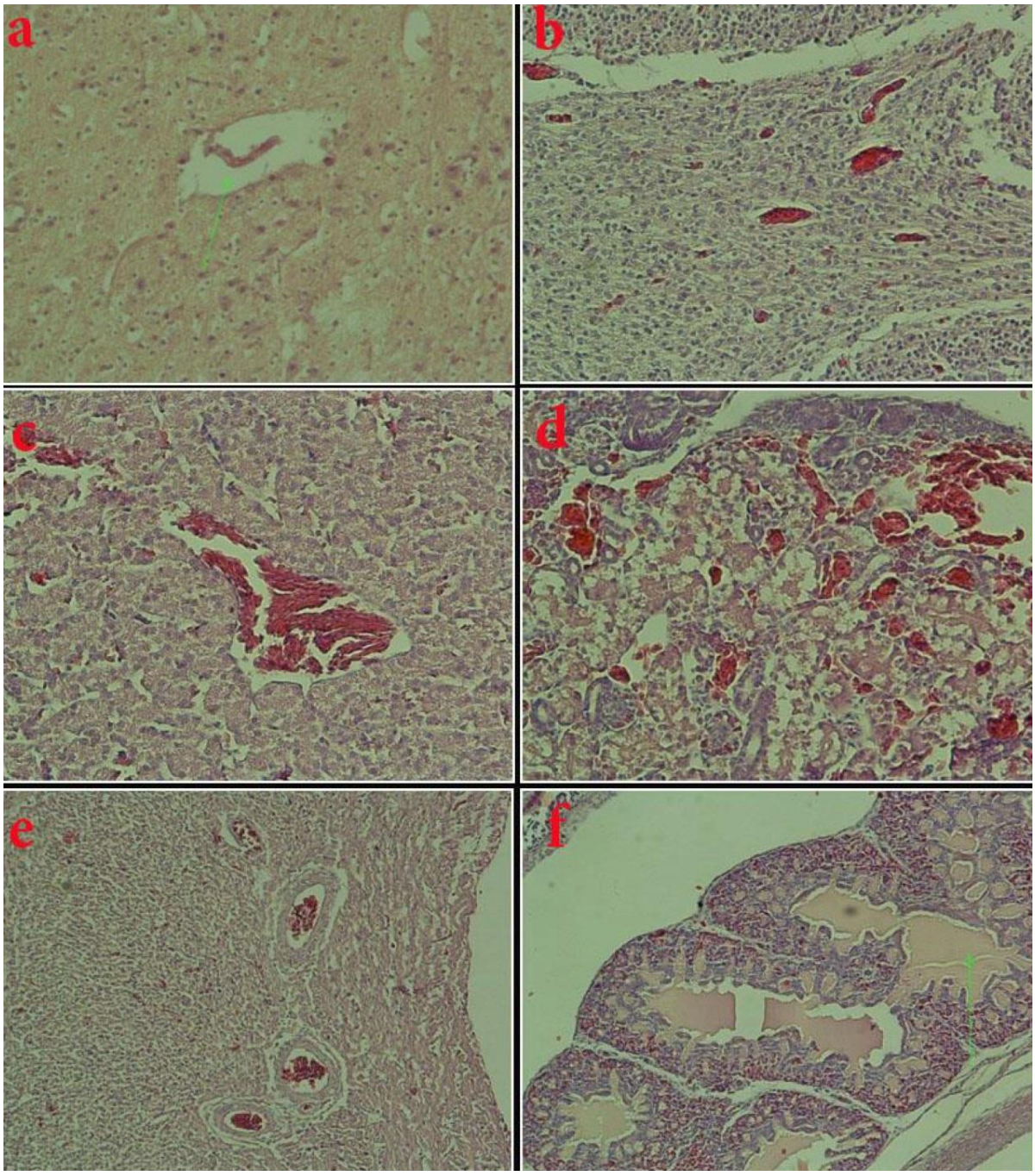

Figure 3. Photomicrograph of the chick embryo treated with 50 mg/Kg egg-weight of *O. vulgare* leaf-extract. (a) Dilation of the Virchow- Robin space (arrow) is seen in the brain (×200, H&E), (b) Congestion is seen in the brain (×200, H&E), (c) Congestion is seen in the liver (×200, H&E), (d) Renal congestion is seen (×200, H&E), (e) Congestion is seen in the heart (×100, H&E), (f) Pulmonary edema (arrows)is seen (×100, H&E).

Table 1. Effect of in-ovo injection of *O. vulgare* leaf-extract on embryo-weight/egg-weight and the body length of the chick embryo

	Control group	*O. vulgare* injected group (mg/Kg egg-weight)			P value
		25	50	100	
Embryos weight / egg-weight (g)	0.75 ± 0.11	0.75 ± 0.23	0.74 ± 0.89	0.57 ± 0.16	> 0.05
Body length (mm)	78.45 ± 4	77.87 ± 6	77.73 ± 7	77.54 ± 5	> 0.05

*Values are mean ± SD

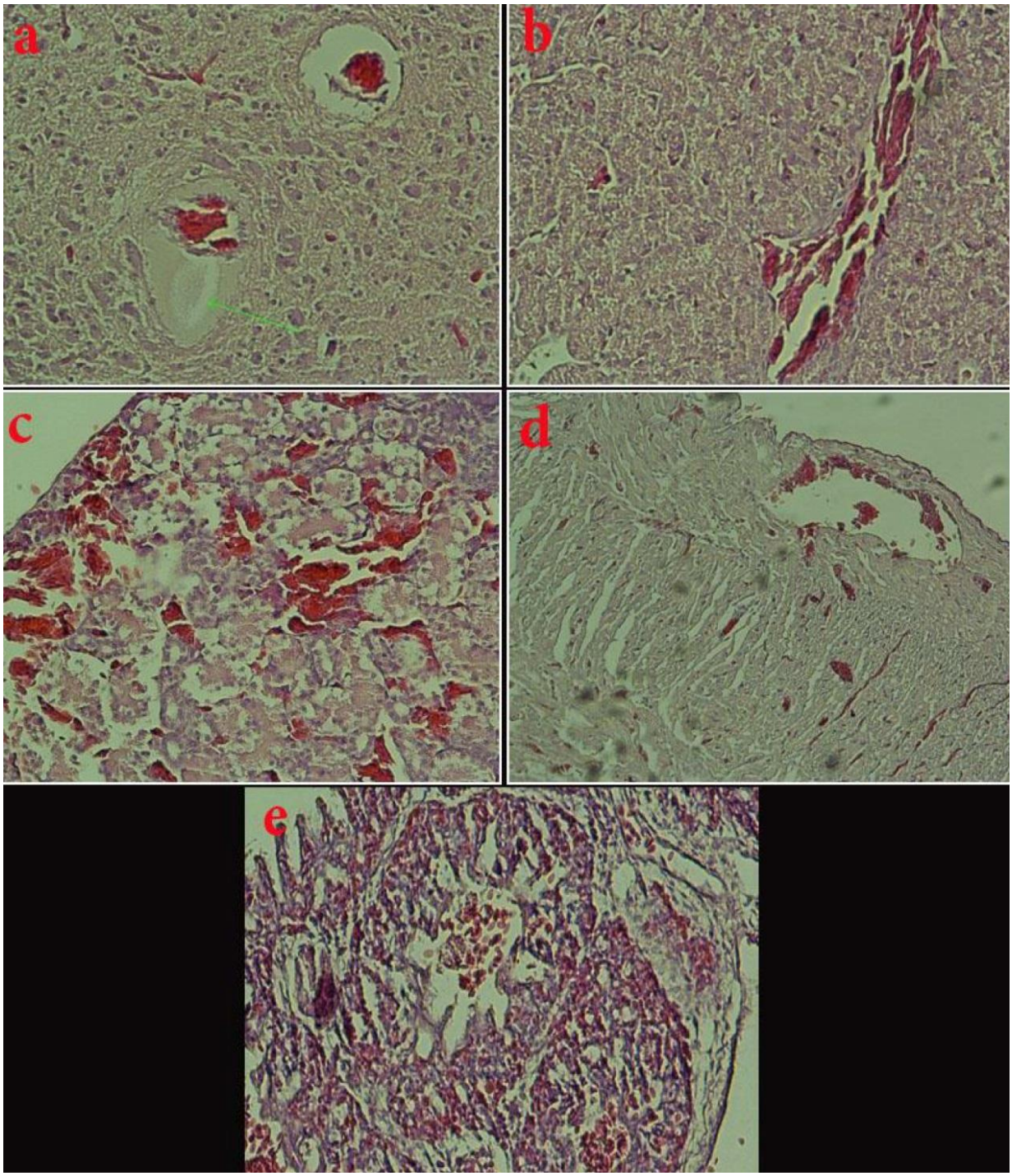

Figure 4. Photomicrograph of the chick embryo treated with 100 mg/Kg egg-weight of *O. vulgare* leaf-extract. (a) Congestion and edema in Virchow- Robin space (arrow) are seen in the brain (×100, H&E), (b) Severe congestion is seen in the liver (×200, H&E), (c) Severe congestion is seen in the kidney (×100, H&E), (d) Severe congestion is seen in the heart (×100, H&E), (e) Severe congestion is seen in the lung (×100, H&E).

Discussion

Nowadays, herbal remedies are considered in primary healthcare programs. In spite of various properties, their side effects still need to be investigated **(1-3)**. The present study shows that systemic alterations can be induced by in-ovo inoculation of chick embryo, using the leaf-extraction of *O. vulgare*. The most important changes induced by the leaf-extract of *O. vulgare* was microscopic lesions. Various histopathological injuries were seen in the brain, liver, kidneys, heart, and lungs' tissues, and the

severest lesion was noticed in embryos which received the high dose of the extract (equal/higher than 50 mg/Kg).

The histopathologic alterations indicated that the chick embryo is susceptible to the application of *O. vulgare* leaf-extract during the embryonic development. Amongst the histopathological lesions, congestion and edema were noticed in the brain which has to be given attention, due to *O. vulgare* leaf-extract side effect on the central nervous system. Interestingly, histopathological injures were observed in embryos without any gross lesions, even the ones treated with a high dose of *O. vulgare* (50 and 100 mg extract/Kg egg-weight). Thymol and carvacrol are the major biochemical in *Origanum* with proven antioxidant and antimicrobial properties **(29)**. Carvacrol has shown to have anti-proliferative property in HeLa cells (**29**), but thymol has beneficial effects on the antioxidant features of the brain in rat **(30)**. Thymol and carvacrol have also anti-inflammatory activity on human macrophages (THP-1) **(31)**. The use of oregano's essential oils provides a promising perspective on the prevention of cell disorders **(32)**, but its high concentration (higher than 30 µg/mL) can cause a reduction in cell viability **(31)**. Genotoxic effect of *Origanum* and carvacrol have been described elsewhere **(33)**. Furthermore, *Origanum* toxicity on some animals and insects has been reported **(34-37)**. For example, Nasr *et al.* (**38**) showed that *O. vulgare* caused significant changes in physiological parameters in diamondback moth. It was also shown that *Origanum* possessed anti-angiogenic activity **(39)**. Thus, it can be inferred that the congestion in the embryo is through its activity on the cardiovascular system. Different alterations have been reported after an application of *Origanum* compounds in animal models. Administration of aqueous extract of *O. vulgare* in a mouse model caused a retardation in the embryo development **(40)**.

In this investigation, alterations were observed after in-ovo administration of *O. vulgare* leaf-extract, which could be the result of major or active chemical compounds present in this plant. Origanum phytochemical analyses exhibited various volatile and aroma organic compounds including linalool, ocimene, caryophyllene, germacrene, bisabolene, and spatulenol **(41, 42)**. Another hypothesis for the alterations induced by *O. vulgare* leaves-extract could be due to the apoptotic effect of this plant. For instance, Savini *et al.* **(43)** showed that *O. vulgare* could induce apoptosis in the colon cancer cells (caco2) of human. In addition of the *O. vulgare* active chemicals, other metabolites might also be correlated with the adverse histopathological effects. Furthermore, specific inherent activities of leaf-extract have been postulated to be associated with its effects. Therefore, new studies are warranted to discover *O. vulgare* biochemical composition and mechanisms in the embryo.

Conclusion

Histopathological lesions that had occurred in a dose-dependent manner during *O. vulgare* administration suggest the susceptibility of chick embryo to the *O. vulgare* leaf-extract. Injures might be due to different pathways including the effects of chemical compounds, apoptotic effect, biochemicals, and mechanisms that contribute to the inherent properties. Therefore, more attention should be given to the toxic effects of *O. vulgare* during fetal development period.

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Conflict of Interest

Authors declared no conflict of interests.

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