



Evaluation of the Toxicopathological Lesions of *Berberis Vulgaris* Using a Chicken Embryonic Model

Amin Derakhshanfar^{a,b}, Hadi Tavakkoli^{*c}

^a Diagnostic Laboratory Sciences and Technology Research Center, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran. ^b Center of Comparative and Experimental Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. ^c Department of Avian Medicine, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran

Abstract

Toxicopathological effects of herbs have been always a major concern. There is scant information available about the toxicopathological effects of barberry in the fetus. Since the embryogenesis in chicken is similar to human beings. The objective of this study is to evaluate the lesions of the various dosages of *Berberis vulgaris* using a chicken embryonic model. Fertile chicken eggs were divided into four equal treatment groups; phosphate buffered saline-injected group and barberry-injected groups whose individuals were injected with *Berberis vulgaris* fruit-extract at dosages of 10, 50 and 100 mg per Kg egg-weight, respectively. Embryos were re-incubated post-treatment and allowed to develop until day 18, after which they were examined for macroscopic and microscopic lesions. Results demonstrated that embryos were stunted in the barberry-injected groups. Defect in feather growth and general congestion was accompanied by pathological changes in brain, liver, kidney, heart, and lung. Histopathological lesions include congestion, hemorrhage, edema, and micro-thrombosis in the affected organs. Based on findings, it is concluded that *Berberis vulgaris* at the above-mentioned concentrations is toxic to the chicken embryo in a dose dependent manner. Further studies are needed to clarify the toxic effects of this herb on the development of human fetus.

Keywords: Berberidacea, *Berberis vulgaris*, Chicken, Embryo, Fetus, Pathology

Corresponding Author: Hadi Tavakkoli, Department of Avian Medicine, School of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
Tel: +98 (341)3222900
E-Mail: Tavakkoli@uk.ac.ir

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1. Introduction

Herbal remedies are used, as a normative basis, for the maintenance of good health. Increasing reliance on the use of herbs in the industrialized societies has been traced to the extraction and development of various drugs and pharmaceutical products from these plants

[1-3]. Indeed, the demand for new medicines to treat emergent diseases led to the idea of finding new active ingredients. One of the most promising ones is the evaluation of the herbal drugs.

The *Berberis vulgaris* L., Var. *asperma* Don, family *Berberidaceae* plant grows in many parts of the globe. Medicinal properties associated with various parts of the plant include antipyretic, antioxidant, antiemetic, anti-inflammatory, anti-arrhythmic, anti-cholinergic, anti-leishmaniasis, anti-malaria, and sedative action. Today, it is often dispensed in the treatment of cholecystitis, cholelithiasis, jaundice, gastric ulcer, hypertension, arthralgia, rheumatism, dysentery, scurvy, yeast infections, leishmaniasis, malaria, urolithiasis, and opioid treatment [4-7]. Recent researches show that it is also able to kill some microorganisms such as *Escherichia coli*, *Salmonella*, *Shigella*, *Entamoebahistoltytica*, *Vibrio cholera*, *Candidaalbicans*, *Staphylococci*, and *Streptococci* [8-11].

Despite the use of barberry in human medicine, its use is sometimes associated with adverse effects such as nausea, regurgitation, vertigo, convulsion, nosebleed, kidney failure, skin and eye inflammation and decreased blood sugar [12, 13]. It should not be used by children, pregnant women, and nursing mothers [14].

In recent years, in order to make industrial compounds of barberries, many researches have been conducted on preparing different products such as extract, beverages, sauce, jelly, candy, pastilles and edible powder.

Although increasing consumption of barberry compounds is predicted in the human diet and folk medicine, there are few reports, based on scientific observation, in the literature on the teratogenic and toxic effects of these compounds during pregnancy, especially for the fetus. In this regard, the current study aimed to determine the macroscopic and microscopic lesions of barberry fruit in the chicken embryo. Furthermore, this basic embryo-toxicological study was performed in the chicken embryo as a model to investigate adverse effect of barberry for human fetus since the embryogenesis in chicken is similar to human beings [15].

2. Materials and Methods

2.1. Hatching Eggs

A total of 40 fertile chicken eggs (Ross 308) with the average egg-weight of 54.4 ± 0.8 g were purchased from the Mahan Breeder Company, Kerman, Iran. In this company, the breeder birds were kept and grown up under the standard condition. The birds were reared in a clean floor house covered by wood shavings and were exposed to a 16:8 light:dark cycle. The house was provided with heaters to adjust the environmental temperature according to the age of the birds. Diets were formulated based on corn and soybean meal to meet the requirements according to nutrient requirements of Ross 308 broiler breeder chicken [16].

2.2. Herbal Plant Extract

Berberis vulgaris L. var. *asperma* Don (*Berberidaceae*) fruits (Cat. No. 134B) were

purchased from the GyahanDarooi Company, Kerman, Iran in October 2014 and authenticated at the Department of Pharmacological Sciences of Kerman University, Iran. Traditional Soxhlet extraction was carried out in a standard apparatus by standard methods for 4 h on 100.0 g *Berberis vulgaris* fruit with 1000 ml of solvent (water/ethanol 80/20 v/v). The extracts were filtered and concentrated with recovery solvent using distillation until around 20 ml volume remained. The crude extract solutions were obtained at a temperature of 60 °C or lower to remove the solvents, and completely dried in an atmospheric oven. The extraction yields 58% (w/w), calculated per weight of primary material.

2.3. Experimental Protocol

Eggs were incubated at 37.5°C and 60% relative humidity. The eggs were randomly assigned to four equal treatment groups, 10 eggs each, as follows: group 1: phosphate buffered saline injected group (control group) in which embryonated eggs were injected with sterile phosphate buffered saline of 0.5 ml/egg into the yolk sac on day 4 of the incubation period. The eggs of groups 2, 3 and 4 were treated with *Berberis vulgaris* fruit-extract at dosages of 10, 50 and 100 mg per Kg egg-weight, respectively. Embryos received treatment by direct injection into the yolk sac according to the standard techniques [17]. Embryos were re-incubated post-treatment and allowed to develop. The viability of the embryos was checked throughout the incubation period by candling. At the end of

the experiment, on day 18, the embryos were evaluated for macroscopic and microscopic lesions. The experiment was performed according to the suggested European ethical guidelines for the care of animals in experimental investigations.

2.4. Macroscopic Evaluation of the External Body Features

At the end of the experiment, the embryos were humanely killed by placing on ice and then the eggs were opened at the wider end [18]. After washing in normal saline solution, embryos were observed under stereomicroscope to study any gross abnormalities on the external body surface. The membranes and yolk sac were also inspected.

2.5. Microscopic evaluation of the Internal Organs

The tissues, including brain, liver, kidney, heart and lung were sampled and fixed in 10% neutral buffered formalin. Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 µm thicknesses were cut using a microtome (Slee-Germany) and stained with hemotoxylin and eosin and studied under light microscope.

2.6. Measurements

The average embryo-weight/egg-weight in grams and the average body length in millimeters of each group were computed. Body weight was measured by a digital scale (Sartorius TE 1535, Germany, with a range up to 150 g reading to ± 0.001 g). The body

length was measured by a digital caliper (Mitutoyo, Series 500, Japan, with resolution of 0.01mm) from the front border of the head to the base of the tail including the tip of the uropygial gland.

2.7. Statistical Analysis

Statistical analysis was performed using SPSS version 20. The Fisher's exact test was used to determine the significant differences in lesion occurrence between experimental groups. One-way analysis of variance followed by Tukey's test was applied to assess the significance of differences in embryos weight and body length. A p-value of <0.05 was considered as statistically significant.

3. Results and Discussion

3.1. Macroscopic Evaluation of the External Body Features

The embryos in groups 2 and 3, which were

treated with *Berberis vulgaris* fruit-extract at dosages of 10 and 50 mg per Kg egg-weight, respectively, were normal as well as embryos in the control group and there was not any abnormality in color, feather, limb and other external body features Figure 1(a & b). However, the embryos in group 4 (treated with 100 mg *Berberis vulgaris* fruit-extract/Kg egg-weight) were stunted and under developed. In this group, the embryos were characterized by defect in feather formation and the general congestion of the body surface Figure 1(c & d).

3.2. Microscopic Evaluation of the Internal Organs

In the embryos of group 2 (treated with 10 mg *Berberis vulgaris* fruit-extract/Kg egg-weight), congestion and hemorrhage were seen in lung. In addition, heart, kidney, and liver were congested. In the brain, in addition to



Figure 1. (a and b) the chicken embryos treated with 10 and 50 mg/Kg egg-weight of *Berberis vulgaris* fruit-extract. The embryos are normal with no gross abnormality, (c and d) the chicken embryos treated with 100 mg/Kg egg-weight of *Berberis vulgaris* fruit-extract. (c) The embryo is stunted, (d) The embryo is characterized by defect in feather formation and the general congestion of the body surface.

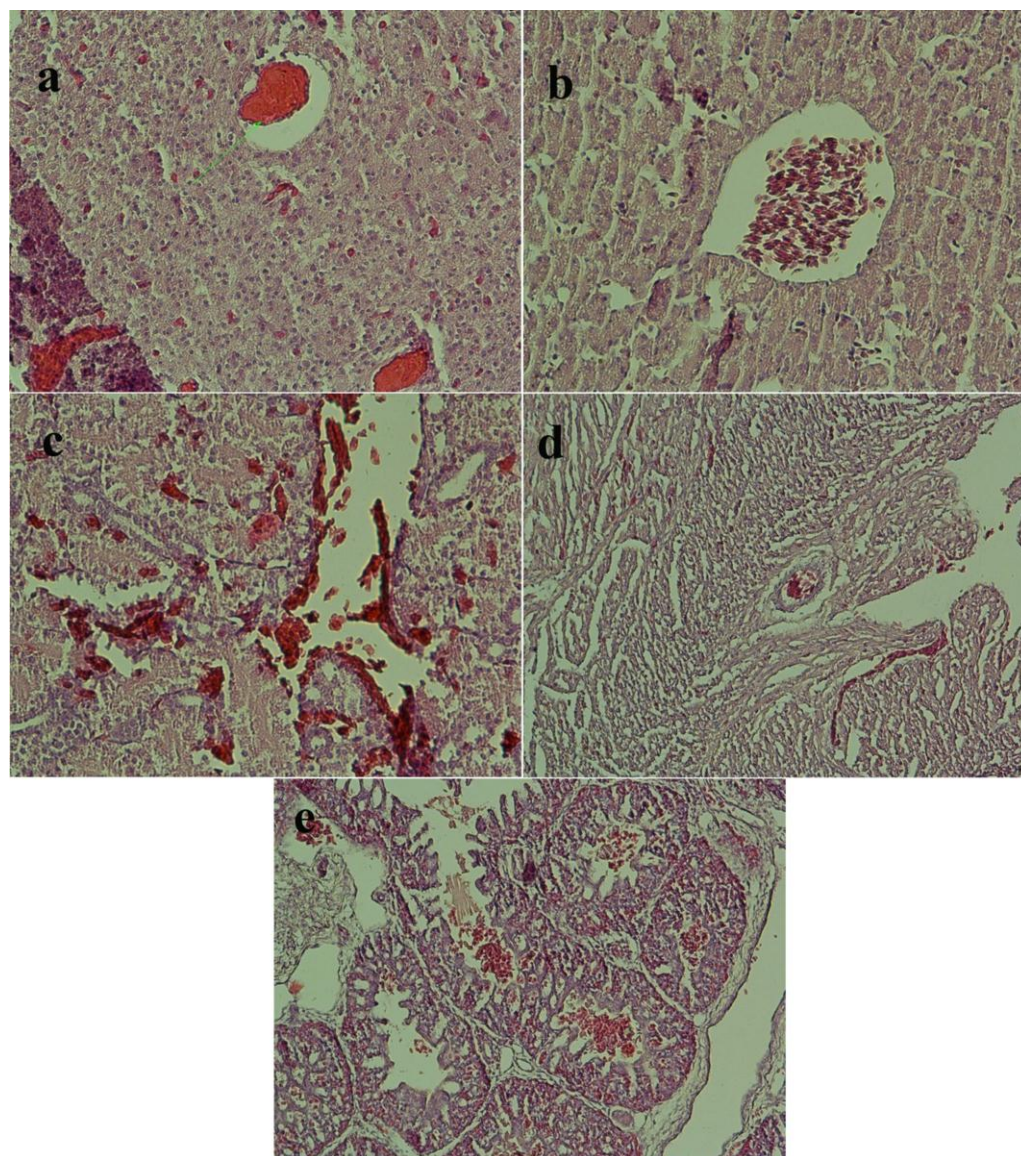


Figure 2. Photomicrograph of the chicken embryo treated with 10 mg/Kg egg-weight of *Berberis vulgaris* fruit-extract. (a) Congestion, edema and micro-thrombus (arrow) are seen in the brain ($\times 200$, H&E), (b) Congestion is seen in the liver ($\times 200$, H&E), (c) Renal congestion is seen ($\times 200$, H&E), (d) Congestion is seen in the heart ($\times 100$, H&E), (e) Pulmonary congestion is seen ($\times 100$, H&E).

congestion, edema and micro-thrombi were obvious (Figure 2 a-e). In the embryos of group 3 (treated with 50 mg *Berberis vulgaris* fruit-extract/Kg egg-weight), pulmonary congestion and hemorrhage, pulmonary interstitial edema and formation of fibrinous exudates as fibrin casts were seen. Kidney, liver and heart were congested. In the brain,

congestion, edema and micro-thrombi were diagnosed (Figure 3 a-f). In the embryos of group 4 (treated with 100 mg *Berberis vulgaris* fruit-extract/Kg egg-weight), severe congestion and, to a lesser extent hemorrhage, was seen in the liver. Heart was congested severely and in the kidney besides congestion, tubular necrosis was diagnosed. The lesions of

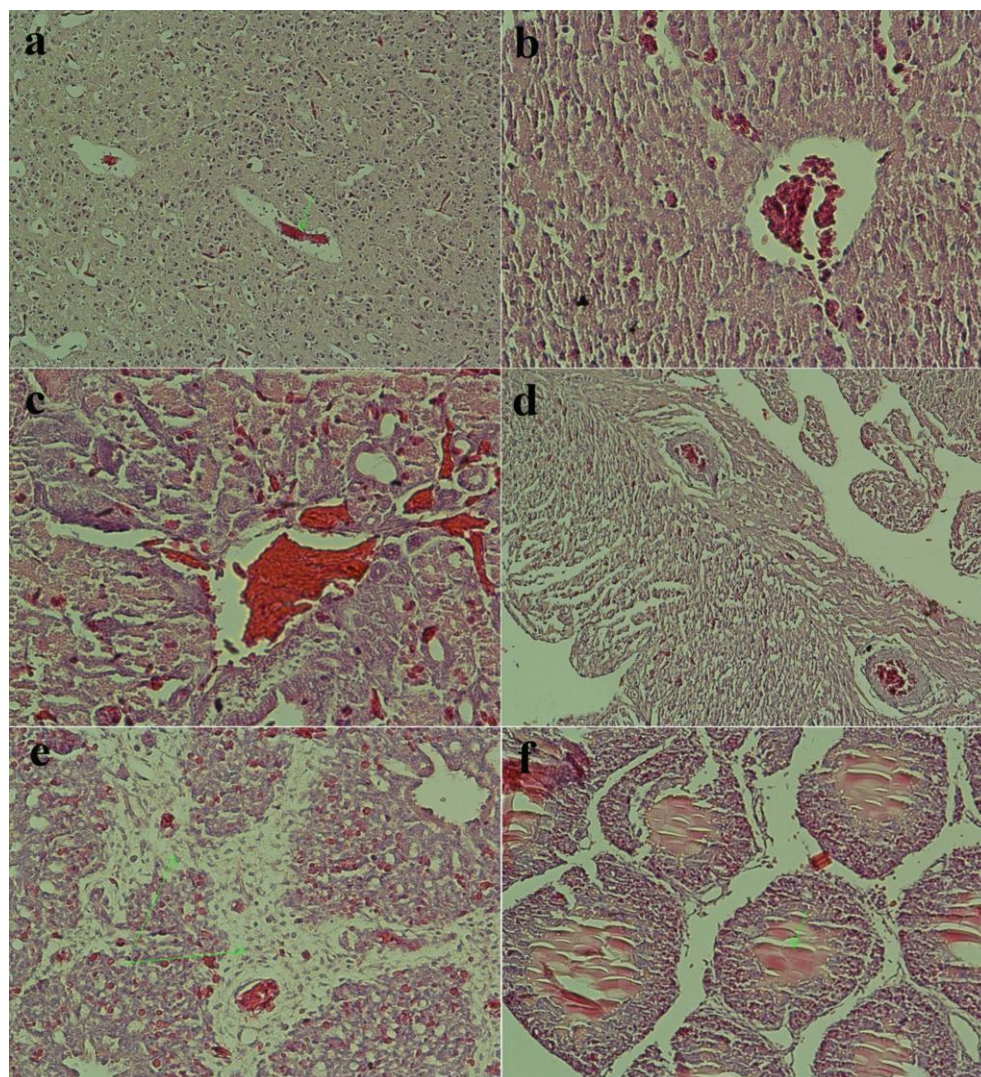


Figure 3. Photomicrograph of the chicken embryo treated with 50 mg/Kg egg-weight of *Berberis vulgaris* fruit-extract. (a) Congestion, edema and micro-thrombus (arrow) are seen in the brain ($\times 100$, H&E), (b) Congestion is seen in the liver ($\times 200$, H&E), (c) Renal congestion is seen ($\times 200$, H&E), (d) Congestion is seen in the heart ($\times 100$, H&E), (e) Pulmonary congestion, hemorrhage and interstitial edema (arrows) are seen ($\times 200$, H&E), (f) Formation of hyaline casts,(fibrinous exudates) (arrow) is seen in the lung ($\times 100$, H&E).

the brain and lung were the same as the embryo of group 3 (Figure 4 a-d). No microscopic lesion was seen in the embryos of the control group that were injected with phosphate buffered saline solution.

3.3. Embryo Weight and Body Length Evaluation

A marked depression in the embryo-weight/egg-weight and the embryos body length of group 4, which were treated with 100 mg *Berberis vulgaris* fruit-extract/Kg egg-weight, occurred during 18 days of growth period ($P < 0.05$). Table 1 shows the data yield

Table 1. Effect of in-ovo injection of *Berberis vulgaris* fruit-extract on embryo-weight/egg-weight and the body length of the chicken embryo.

	Control group	<i>Berberis Vulgaris</i> injected group (mg/Kg egg-weight)		
		10	50	100
Embryos weight/egg-weight (g)	0.75±0.10 ^a	0.74±0.13 ^a	0.73±0.18 ^a	0.57±0.15 ^b
Body length (mm)	94.68±5 ^a	94.53±4 ^a	93.64±6 ^a	67.85±8 ^b

*Values are Mean ± SD. Values in a row followed by different superscripts differ significantly (p<0.05).

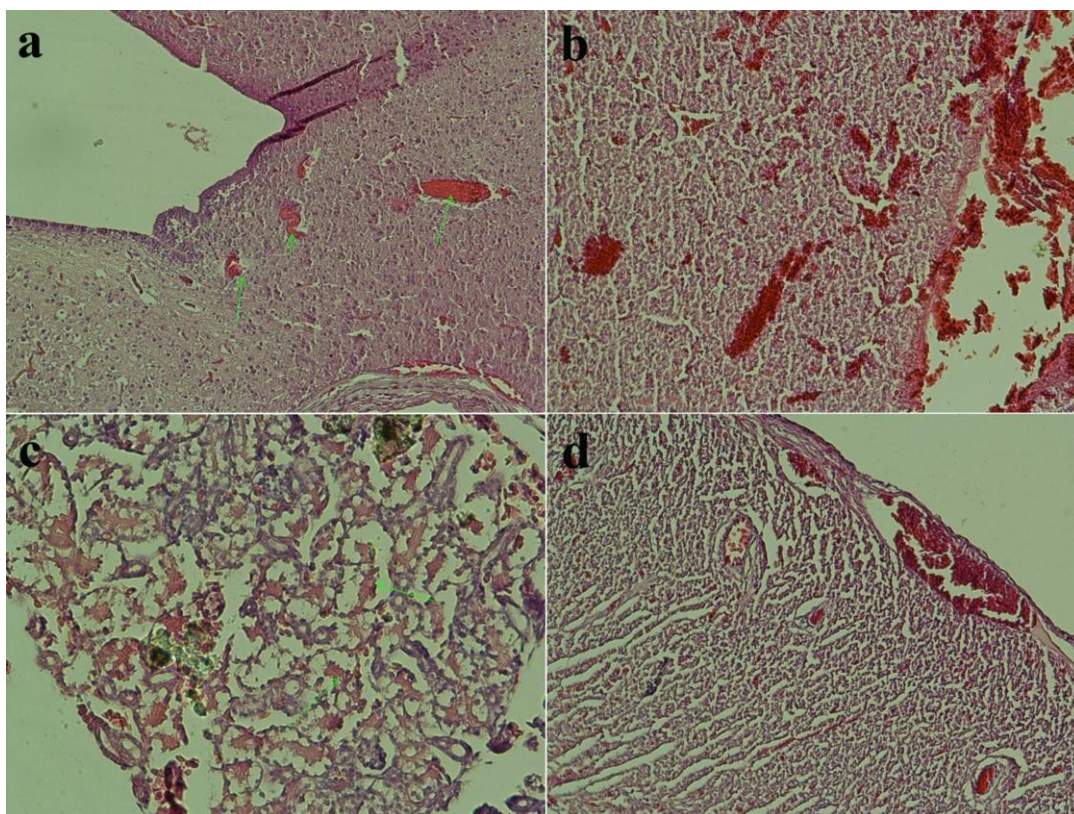


Figure 4. Photomicrograph of the chicken embryo treated with 100 mg/Kg egg-weight of *Berberis vulgaris* fruit-extract. (a) Congestion, edema and micro-thrombus (arrows) are seen in the brain (×100, H&E), (b) Sever congestion and to lesser extent hemorrhage are seen in the liver (×100, H&E), (c) Tubular necrosis (arrows) is seen in the kidney (×200, H&E), (d) Sever congestion is seen in the heart (×100, H&E).

from the injected and control embryos. The embryos in groups 2 and 3, which were treated with a *Berberis vulgaris* fruit-extract at a

dosage of 10 and 50 mg per Kg egg-weight respectively, were normal as embryos in the control group.

In recent years, there is a trend towards the use of traditional medical systems that involves the use of herbal drugs and remedies. In these societies, herbal remedies have become more popular in the treatment of various ailments. They are also considered in primary health care programs. Herbal plants are an integral part of ethno-veterinary medicine, too. In spite of extensive applications and numerous properties of herbs, considering their adverse effects on animal and human health still needs to be justified.

The present investigation indicates that systemic alterations can be induced by in-ovo inoculation of chicken embryo by the fruit-extraction of the *Berberis vulgaris*. The first indicator of these alterations was an obvious depression in the embryos weight and body length. Monitoring of the embryos weight and body length revealed that they significantly reduced during 18 days of growth period. The lowest values were seen in embryos that were treated with 100 mg *Berberis vulgaris* fruit-extract/Kg egg-weight. Based on this result, it is concluded that the *Berberis vulgaris*, especially with a dosage equal/higher than 100 mg/Kg, can cause adverse effect on embryonic growth throughout the total period of development.

According to most studies, the majority of the medical properties of the *Berberis vulgaris* is due to the various alkaloids present in different parts of the plant. The fruit contains dihydropalmitinium hydroxide, which is an anti-estrogen agent that causes endometrial atrophy and crumple of gastric glands [19]. Anti-estrogenic activity of the barberry

compounds is also able to inhibit the protein synthesis, reduce the serum protein and decrease the sialic acid and glycogen contents of the genital tract. These changes result in nutrition disorders in the fetus and its growth [20]. It is also shown that hypoglycemic herbs can decrease body weight during administration [21]. Some barberry species, such as *Berberis vulgaris* and *Berberis aristata*, are reported to possess hypoglycemic properties and are effective in lowering the blood sugar level [21-23]. Therefore, they can affect the embryonic weight during development. Further investigations are required to assess the association between administration of anti-estrogen/hypoglycemic herbs and weight loss in embryo.

The second indicator of the systemic alteration due to *Berberis vulgaris* in the embryo was the gross abnormalities of the body structure. These abnormalities were characterized by disorder in body color and feather growth, so that the embryos were congested and the feather formation, in some instances, was not normal. Vasodilator activity of the *Berberis vulgaris* is reported [24]. Furthermore, clinical studies suggest that the aqueous extract of barberry is able to efficiently increase cardiac contractility [19]. Thus, it may be suggested that the congestion mechanism by barberry in embryo is through its effect on the cardiovascular system.

The third indicator of the embryonic alteration induced by *Berberis vulgaris* was histopathological lesions. Various histopathological lesions were observed in multiple tissues including brain, liver, kidney,

heart and lung. The most severe lesions occurred in embryos which received the highest dosages. The lesions observed indicate that the embryo is very susceptible to the effects of in-ovo injection of *Berberis vulgaris* fruit-extract during the embryonic development. Among lesions diagnosed as histopathological lesions, alterations in the brain such as congestion, edema and micro-thrombi were noticed, even in low dosage and attract attention to the disorder of the central nervous system in embryo due to the *Berberis vulgaris* fruit-extract administration. Moreover, histopathological lesions were seen in some embryos without any gross abnormality, especially those treated with low dosage of *Berberis vulgaris* (10 and 50 mg extract/Kg egg-weight). So, it is suggested that the microscopic lesions can occur in embryo following consumption of barberry compounds without any obvious gross alteration.

It has been reported that pregnant women should not use the *Berberis vulgaris* compounds during pregnancy as it may cause uterine stimulation, can be toxic for embryo and cause jaundice in the newborn [14, 25]. Various alterations and adverse effects have been reported in human and animal models following administration of barberry compounds [24, 26]. In man, *Berberis vulgaris* has inotropic and chronotropic properties and there is evidence that it can cause arterial hypotension, dyspnea, flu-like symptoms, gastrointestinal discomfort, constipation, and cardiac damage [27, 28]. In female gerbils, following chronic administration of dihydropalmitinium hydroxide, an active

ingredient of *Berberis schitria*, reduction in the weights of ovary, uterus and vagina, atresia of the large ovarian follicles, vacuolization of follicular cells, shrinking of the luteal cells and endometrial glands, prolonged diestrous cycle and suppression of estrogenic activity were evident [20].

In our investigation, the alterations observed during in-ovo administration of *Berberis vulgaris* fruit-extract may be the result of the major or active chemical compounds present in the plant, especially anti-estrogen agents. Phytochemical analyses of different species of *Berberidaceae* family have detected various chemical compounds including alkaloids (mainly berberine and berbamine), tannins, phenolic compounds, sterols, triterpenes and anti-estrogen agents. Another hypothesis to account for the alteration induced by *Berberis vulgaris* fruit-extract would be due to the apoptotic effect. For example, Hanachi et al. [29] showed that the aqueous extract of barberry with the concentrations of 50, 100, and 250 mg/kg could promote apoptosis in rat with liver cancer.

In addition to the major/active chemical compounds in *Berberis vulgaris* fruit, there may be other chemicals/metabolites which correlate with adverse and histopathological effect. Furthermore, certain inherent properties of fruit-extract have been postulated to be associated with its effects. Now, new efforts are required to find new biochemicals and mechanisms that might be of great relevance in lesion occurrence associated to the *Berberis vulgaris* in embryo.

4. Conclusion

In conclusion, the diversity of gross and histopathological lesions that were observed suggests the high susceptibility of chicken embryo to the administration of *Berberis vulgaris* fruit-extract. These lesions occurred in a dose-related manner. These alterations may be due to different factors including the influence of active chemical compounds, apoptotic effect, metabolites and mechanisms which contribute to the inherent properties of the fruit. The current study also advises caution in the extended use of *Berberis vulgaris* compounds. Further studies are needed to clarify the toxic effects of this plant on the development of the human fetus.

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