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Effect of *Ferula asafetida* extract on the development of chick embryo

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Industrialized societies health care systems rely heavily on herbal plants. Asafetida is a common herbal plant, has various health benefits. The study aimed to evaluate the effects of *Ferula asafetida* extract on the development of chick embryo. One hundred sixty-two eggs were incubated at 37.8 ± 0.1 °C and 65% - 75% humidity. The eggs were separated into 9 sets; each enclosed 18 eggs. The first group (G1) characterized control of G2 and G3 groups which received 50 mg /20-egg and 100 mg /20-egg of *Ferula asafetida* resin- extract respectively one day post incubation, the fourth set (G4) characterizes control for G5 and G6 groups which received 50 mg /20-egg and 100 mg /20-egg of *Ferula asafetida* resin-extract respectively 8 days post incubation. The seventh set (G7) represented the control for G8 and G9, which received 50, 100mg /20-egg respectively post 12 days of incubation. After *Ferula asafetida* (50 mg/kg and100mg/kg form eggs) resin-extract injection one day post incubation, noted a weak, absence of vitelline vascularization in the treated groups. After *Ferula asafetida* resin- extract injection in the eggs post 8 days and post 12 days of incubation, weak and absence of vitelline vascularization with high dose. Subcutaneous hemorrhage and excencephaly were noted in the embryos of the treated groups. The high doses of *Ferula asafetida* caused various malformations in the chick embryo.

Keywords: *Ferula asafetida,* vascular toxicity, chick embryo, development

INTRODUCTION

In industrialized countries, herbal plants play an important role in the health care system. Ferula is a carrier, Asa means resin, and foetidus is a smell that can be described as fetid. Essential oil of Asafoetida contains sulfur compounds, which create a strong odor (Mahdavi et al. 2016). *Ferula asafoetida* is herbaceous plant of the umbelliferae family. It is a creamy-colored oleo gum that has a strong odor and a nauseating taste. In the middle ages, people were known to wear a small piece of the gum around their neck to ward off colds and fevers. In Persia, the *Ferula asafetida* was used as a spice. This herb is commonly used in a wide range of dishes, and it is also known to be beneficial in Europe, the Far East, and the Near East. When it is used to prepare meat, it is often applied to hot plates (Mahendra et al. 2012).

Asafoetida can be found in various Central Asian countries, such as Afghanistan and Iran. It is mainly farmed in these regions, and it is typically shipped around the world (Sood , 2020). The various names given to asafoetida include Anghuzeh (Farsi); asafétida (Spanish); awei (Chinese); aza(Greek); devil's dung; férule persique or merde dudiable (French); haltit (Arabic); hing (Hindi). Asafoetida is used in various food and medical products, such as in dressings and in the treatment of various diseases, such as asthma and whooping cough. It can also be used in the treatment of other conditions, such as bronchitis and anxiety. Asafoetida gum resin can be used in multiple forms of medicine, such as sedatives, analgesics and laxatives (Paparozzi , 2005; Mahendra et al. 2012).

The large roots of Ferula plants are used to extract asafoetida. The taproot is then exposed by cutting the stem of the shrubby plant close to the ground. A small amount of latex is collected every day (Mahdavi et al. 2016).

Asafetida has a total of 68% carbohydrates, 16% moisture, and 1% of protein. It also has 7% fat, 4% minerals, and 7% of fiber (Mahendra et al. 2012).The main components of asafoetida are resin, gum, and essential oil. It has been used in traditional medicine in Malaysia. Due to the various properties of asafoetida, it has been

widely used as an antihelminthic and antispasmodic agent.

There is also a correlation between the traditional and modern uses of asafoetida. For instance, studies conducted on the effects of umbelliprenin on the lipoxygenase have revealed that this component of the plant has a powerful inhibitory effect (Iranshahi et al. 2008).

Large doses of asafoetida can cause headache and various digestive issues, such as bloating, nausea (Amalraj and Gopi, 2017).

In a 5-week-old infant, a case of methemoglobinemia was reported after asafoetida was used to treat colic (Heck et al. 2000). The present study aimed to evaluate the effect of *Ferula asafetida* resin-extract on the development of chick embryo.

MATERIALS AND METHODS

The study was conducted in the laboratories of Biology department, Jouf University from September to October 2021 after ethical approval.

Eggs

Fertilized chicken eggs were acquired from one of the farms in Sakaka city, Al-jouf region, Saudi Arabia, which the eggs were produced under normal environments.

Incubation

One hundred sixty-two eggs with average weight (47.52 ± 2 g) were weighed in the lab and incubated at 37.8 ± 0.1 °C and 65% - 75% humidity .The incubator automatically rotates eggs every two hours. Post twenty-four hour of incubation, 2 eggs were opened for the purpose of identifying the embryonic disc by window procedure (Kwan *et al.* 2017).

Injection method

The outer shell of the eggs was sterilized with 70% alcohol. A puncture was made at the pointed side of the egg by using an acute needle. Then, the eggs were injected with varying doses of *Ferula asafetida* extract. The hole created in the egg was covered (Sachdeva *et al.* 2009; Li *et al.* 2018; AbdRabou, 2021).

Preparation of *Ferula asafetida* resin- extract

Ferula asafetida- resin was got from one of the condiment trader shops in Sakaka city, Al-Jouf region, and was recognized by Department of pharmacological resin with 200 ml of solvent. The extracts were filtered and steal in the refrigerator until use (AbdRabou, 2021).

Study groups

The eggs were separated into 9 sets; each set contained 18 eggs .The first set (G1) considered control for G2 and G3 sets. Group G2 received 50 mg /kg of eggs of *Ferula asafetida* resin- extract post 24 hour of incubation, the third group (G3) received 100 mg /kg-eggs of *Ferula asafetida* resin- extract. The fourth group (G4) represents the control for G5 and G6 groups. The fifth and sixth groups received 50 mg /kg-eggs and 100 mg /kg-eggs of *Ferula asafetida* resin- extract respectively 8 days post incubation. Seven set (G7) represented control for G8 and G9, which received 50 mg /kg-eggs and 100 mg /kg-eggs of *Ferula asafetida* resin- extract respectively 12 days after incubation. All groups of eggs were re-inoculated 48 hour then sacrificed. The extract was injected inside the egg as described previously (Oosterbaan et al. 2012; Tavakkoli et al. 2020; AbdRabou, 2021).

Morphological studies

After 48 hour from injection of *Ferula asafetida* resin-extract via the window method, the eggs of both control and treated groups were examined and photographed for morphological study; also the vasculature was also observed. Then, the chick embryos were removed in a petri dish for examination and photographing. The live embryos were determined by the heartbeat and locomotion.

Quantitative studies

The numeral of embryos that have normal development were recorded, also the number of malformed embryos were determined.

Ethical consent:

An approval of the study was obtained from Ethical Committee in Jouf University.

Statistical analyses

The results of the present study were analyzed using the SPSS program version 22. The one-way Anova test was used to calculate the significance of the differences between the treated and control groups (p value ≤ 0.05 was determined as significant)

RESULTS

Morphological studies

Normal vitelline vascularization was noted in G1 set, but a weak and absence of vitelline vascularization were determined in G2 and G3 groups respectively after *Ferula asafetida* resin- extract injection one day post incubation (Figure 1).

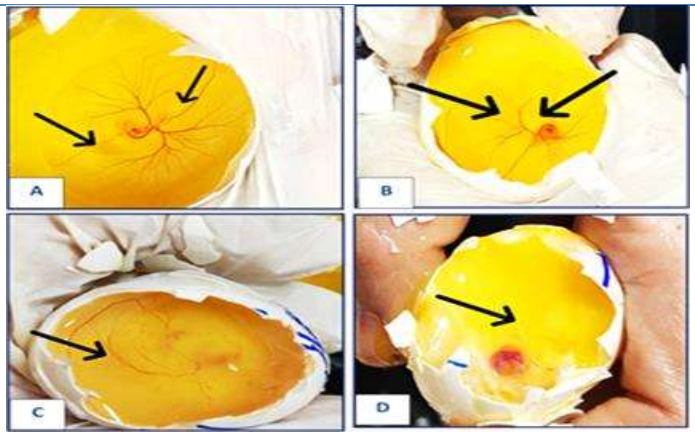

Figure 1: Shows the vitelline vascularisation in the egg after *Ferula asafetida* resin- extract injection one day post incubation; a (G1); normal vitelline vascularization (↑), b & c (G2); weak vitelline vascularization (↑), d (G3); absence of vitelline vascularization.

In figure 2: normal embryo (G1), subcutaneous hemorrhage and excencephaly (G2) and embryo with undifferentiated anterior region and subcutaneous hemorrhage (G3) were determined. In figure 3, normal (G4), weak (G5), absence (G6) of vitelline vascularization after *Ferula asafetida* resin- extract injection eight day post incubation.

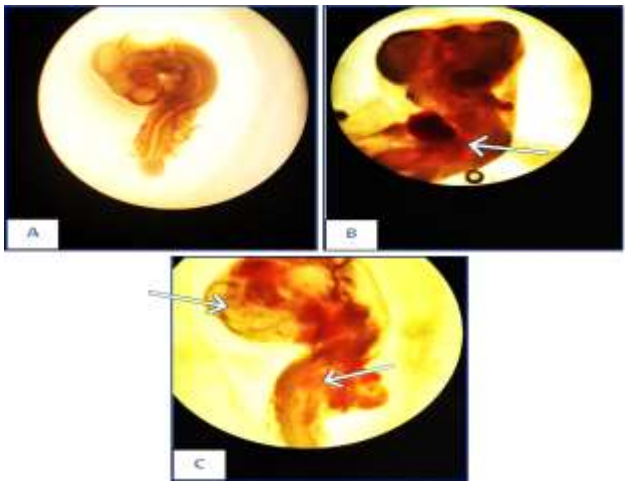

Figure 2: Shows photographs for embryos after *Ferula asafetida* resin- extract injection one day post incubation. a (G1); normal embryo, b (G2); subcutaneous hemorrhage (↑) and Excencephaly (↑), c (G3); undifferentiated anterior region (↑)and subcutaneous hemorrhage (↑).

Normal embryo (G4), thin skin and absence feathers (G5), embryo with subcutaneous hemorrhage in neck region (G6) were showed in figure 4. Figure 5 shows normal embryo beak in G4, shortened beak in G5, but absence of beak in G6.Figure 6 shows photographs of normal embryo head in G4&G5, but, excencephaly was noted in G6.

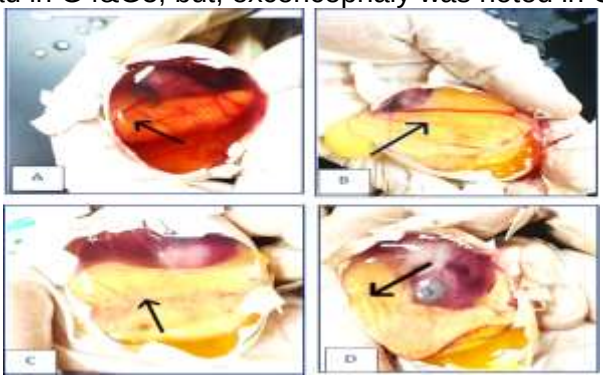

Figure 3: Shows photographs of vitelline vascularisation in the egg after *Ferula asafetida* resin-extract injection 8 days post incubation; a (G4); normal vitelline vascularization (↑), b&c (G5); weak vitelline vascularization, d (G6); absence of vitelline vascularization (↑).

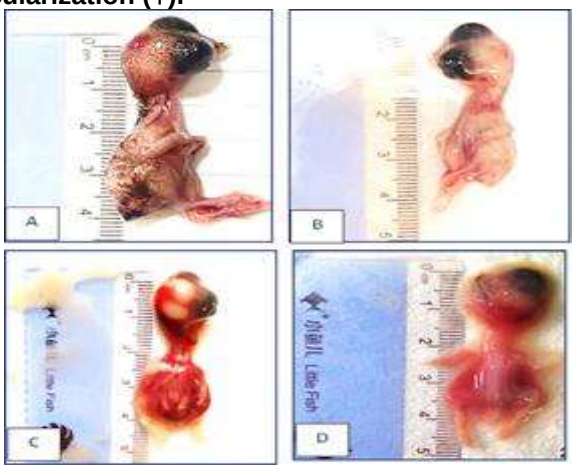

Figure 4: Shows photographs of the embryos after *Ferula asafetida* resin- extract injection 8 days post incubation. a (G4); normal embryo, b (G5); absence of

feathers, c&d (G6); malformed embryos with subcutaneous hemorrhage in neck region and also absence of feathers.

Figure 5: Shows photographs of the embryo beak region after *Ferula asafetida* resin- extract injection 8 days post incubation. a (G1); normal beak (arrow) and eyes, b (G2); shortened beak (arrow) and normal eyes, c (G3); absence of beak (arrow) and malformed eyes .

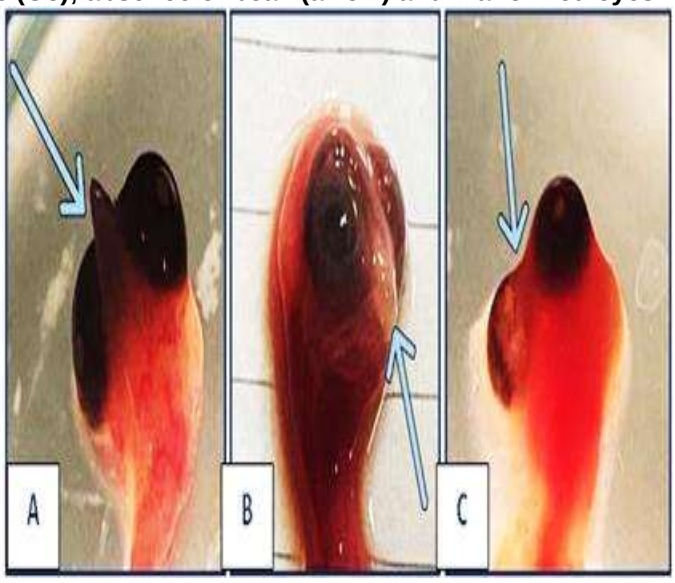

Figure 6: Shows photographs of embryo head region after *Ferula asafetida* resin- extract injection 8 days post incubation. a &b (G4&G5); normal head (arrow), c (G6); excencehaly (arrow.)

After Ferula asafetida resin- extract injection 12 day post incubation, normal vitelline vascularization was noted in G7& G8, but in G9 showed a weak vitelline vascularization (Figure 7). Normal embryo was noted in G7, protrusion in the brain region in G8 and subcutaneous hemorrhage in G9 (Figure 8). Normal posterior region of embryo head was noted in G7, hemorrhage in the posterior region of head in G8&G9 (Figure 9).

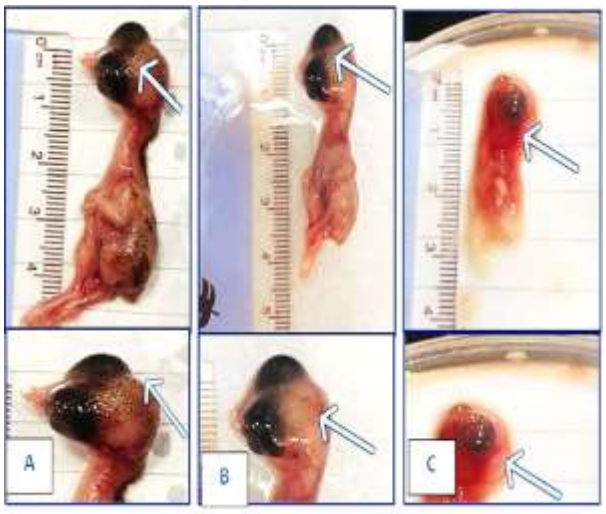

Figure 7: Shows photographs of vitelline vascularisation in the egg after *Ferula asafetida* resin- extract injection 12 day post incubation.A&B (G7& G8); normal vitelline vascularization (arrow), c & d (G9); weak vitelline vascularization (arrow).

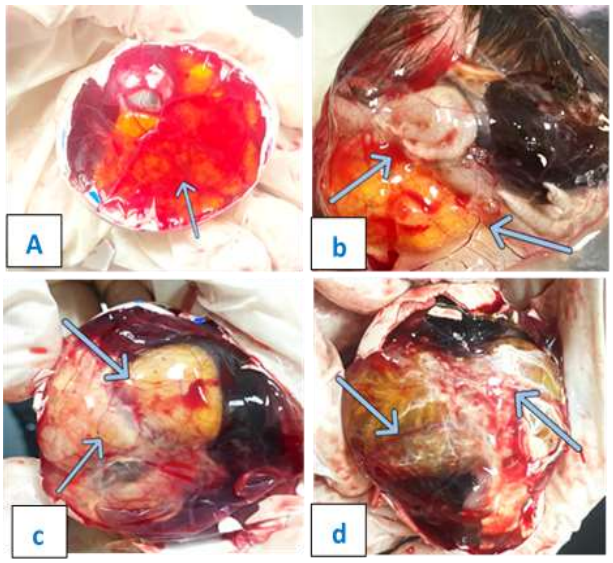

Figure 8: Shows photographs of the embryos after *Ferula asafetida* resin- extract injection 12 day post incubation. a (G7); normal embryo, b (G8); Protrusion in the brain region (arrow), c (G9), subcutaneous hemorrhage (arrow.)

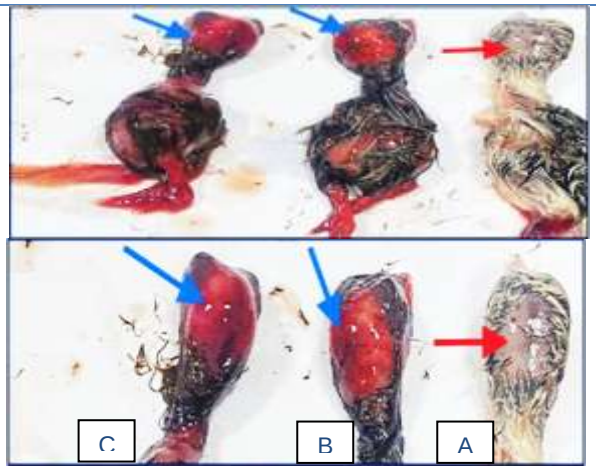

Figure 9: Shows photographs of the embryo head region after *Ferula asafetida* resin- extract injection 12 day post incubation. a (G7); normal posterior region of head (red arrow), b&c (G8&G9); hemorrhage in the posterior region of head (blue arrows).

Table 1 show the number and percentage of live and dead chick embryos after 48 hours of *Ferula asafetida* resin- extract injection in the eggs post one day post incubation. Ratio of dead embryos was 50.0% in G3, but it deceased to 38.89% in G2. There was no dead embryos in G1.

Table 1: The number and percentage of live and dead chick embryos after *Ferula asafetida* resin- extract injection one day post incubation.

Groups	Number of fertile eggs	Live N (%)	Dead N (%)
G1	18	18 (100%)	0 (0%)
G2	18	11 (61.11%)	7 (38.89%)
G3	18	9 (50.0%)	9 (50. 0%)

Table 2 show the number and percentage of live and dead chick embryos after *Ferula asafetida* resin- extract injection 8 days post incubation. The percentage of dead embryos was in 38.89% in G6, but it deceased to 27.78% in G5. There was no dead embryo in the G4.

Table 2: The number and percentage of live and dead chick embryos after *Ferula asafetida* resin- extract injection 8 days post incubation.

Groups	Number of fertile eggs (N)	Live N (%)	Dead N (%)
G4	18	18 (100%)	0 (0%)
G5	18	13 (72.22%)	5 (27.78%)
G6	18	11 (61.11%)	7(38.89%)

Table 3 show the number and percentage of live and dead chick embryos after *Ferula asafetida* resin- extract injection 12 days post incubation. The percentage of dead embryos was 22.22 % in G9, but it decreased to 11.11% in G8. There was no dead embryo in the G7.

Table 3: The number and percentage of live and dead chick embryos after *Ferula asafetida* resin- extract injection 12 days post incubation.

Groups	Number of fertile eggs	Live N (%)	Dead N (%)
G7	18	18 (100%)	0 (0%)
G8	18	16 (88.89%)	2 (11.11%)
G9	18	14 (77.78%)	4 (22.22%)

Table 4 show the total number and percentage of live and dead chick embryos in all groups which treated with *Ferula asafetida* resin. The highest percentage of dead embryos (44.44%) was in the groups which injected post 1 day of incubation, but it decreased 33.33% post 8 days. The lowest percentage was recorded post 12 days of incubation (16.67%).

Table 4: The total number and percentage of live and dead chick embryos in all treated groups.

Treated groups		N (%)	Total (N)
Groups treated with *Ferula asafetida* resin- extract post 1 day of incubation (G2&G3)	Live	20 (55.56%)	36
	Dead	16 (44.44%)	
Groups treated with *Ferula asafetida* resin- extract post 8 days of incubation (G5&G6)	Live	24 (66.67%)	36
	Dead	12 (33.33%)	
Groups treated with *Ferula asafetida* resin- extract post 12 days of incubation (G8&G9)	Live	30 (83.33%)	36
	Dead	6 (16.67%)	

DISCUSSION

The normal development of the fetus is a major concern due to the presence of compounds that can affect the vascular network and genes expression (Popova *et al.* 2016). Ingesting certain herbs during pregnancy can increase the risk of fetal defects, also it can also cause vascular injury and affect the growth of the embryo (Feng and Yang, 2017).

In several studies, the effects of certain herbs on the development of the fetus were shown. One of the compounds that can be toxic to the embryo is the plant Ferula asafetida. Despite the increasing consumption of this plant, its toxic effects were still observed (Kwan et al. 2016).

The present study showed normal vitelline vascularization in the control groups, but weak or absence of vitelline vascularization in the treated sets after Ferula asafetida resin- extract admission one day post incubation. After Ferula asafetida resin- extract injection in the eggs post 8 days and post 12 days of incubation, the present study showed normal vitelline vascularization in the control group, reduction and lack of vitelline vascularization with high dose. The results of the study support the previous studies that showed the harmful effects of herbs on the vascular system of fetuses. These results of our study agree with Tavakkoli et al. (2020), the researchers found that the effects of the herbs on the

development of the fetus were significant. They noted that the effects of the Dorema ammoniacum on the vascular system resulted in a reduction in the vessels area and a decrease in the total vessels length.

The study was conducted using the model of an extra embryonic membrane in chick, which was used by others researchers to assess the belongings of numerous agents on the development of the fetus (Araghi et al. 2016; Gheorghescu et al.2015; Paradkar et al.2017).

After *Ferula asafetida* resin- extract injection 8 days after incubation, the present study showed normal embryo in control group, subcutaneous hemorrhage and excencephaly, embryo with undifferentiated anterior region and subcutaneous hemorrhage in the treated groups. Normal embryo in control group, thin skin and absence feathers, embryo with subcutaneous hemorrhage in neck region of treated groups.Normal beak in control group but shortened beak and absence of beak in the treated groups. After 48 hours of Ferula asafetida resin-extract injection 12 days after incubation, protrusion in the brain region and subcutaneous hemorrhage were noted in the treated groups.

A 5-week-old infant developed a type of blood disorder known as methemoglobinemia after being treated with asafoetida gum for treatment the alleviate colic (Heck et al. 2000).Vascular alteration might be due to the cytotoxic activity of this herb (Yousefzadi et al. 2011). Besides its negative effects on the body, asafoetida gum also has various inherent properties that could be used against it. One of these is its genotoxic activity (Eskandani et al. 2014).

The present study revealed that the highest percentage of dead embryos was in the groups, which injected post 1 day of incubation, but the lowest percentage was recorded post 12 days of incubation. This result may be due to after one day of incubation, the fetus was not fully developed, but after 12 days of incubation, all organs of fetus was fully developed, so the effect was less.

CONCLUSION

In conclusion, *Ferula asafetida* applied to investigate the effect of it on the development of chick embryo. The high doses of *Ferula asafetida* caused many embryonic malformations and growth retardation. The study recommended that *Ferula asafetida* may be not satiable during pregnancy.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Authors contributed equally in the study.

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