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Research Article
Effect of Different Doses of Meloxicam on the Development of Chick Embryo

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Abstract

Background and Objective: Pregnancy is a special physiological condition. Administration of some drugs during pregnancy may cause harm to the embryos. Congenital defects are physical deformities that affect one or more organs. The aim of this study was to demonstrate the effect of different doses of meloxicam on different stages of chick embryo development. **Materials and Methods:** One hundred chicken eggs were incubated at 37.8±0.1°C and 65-75% humidity. The eggs were divided into 8 groups (each set contained 12 egg): Six groups were administered meloxicam in increasing doses (0.001 mg/0.1 mL, 0.01 mg/0.1 mL and 0.02 mg/0.1 mL) post 2 days and 6 days of incubation respectively and the two rest groups represented the control groups. All of the embryos were extracted from the eggs after 48 hrs of injection for morphological and quantitative studies. **Results:** The results of the current study on different stages of chicken embryo development demonstrated that application of meloxicam in high doses caused weakly or absence of vitelline vascularization. The high doses of meloxicam caused embryonic malformations such as subcutaneous haemorrhage, absorbed embryos, reduced fore and hind limb, very thin skin and exencephaly. The percentage of dead embryos of groups was injected with meloxicam post 2 and 6 days of incubation was 53.13 and 42.43, respectively. A significant decrease was noted in the length and weights of treated groups post 6 days of incubation. **Conclusion:** The results revealed that the administration of meloxicam in high doses caused weak or absence of vitelline vascularization, growth retardation to embryos and many congenital malformations. Further studies needed to determine the safety of meloxicam in therapeutic doses.

Key words: Chick embryo, meloxicam, pregnancy, congenital malformation, therapeutic dose

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Drug therapy is a source of concern because the physiology of pregnancy affects the pharmacokinetics of the drugs used and some drugs may cause harm to the fetus but at the same time, the mother cannot completely avoid drug therapy during pregnancy, because some women enter pregnancy with medical conditions that require continuous and occasional treatment such as high blood pressure, asthma and epilepsy[1].

Meloxicam is one of the non-steroidal anti-inflammatory drugs (NSAIDs) which considered cornerstones of the treatment of painful and inflammatory conditions in animals and humans: However, prolonged administration of NSAIDs can cause adverse effects for some patients by long-term administration of these[2]. Meloxicam is a drug commonly used in the treatment of osteoarthritis and rheumatoid arthritis[3,4]. It is one of the only certain drugs on the pharmaceutical market that are considered cyclooxygenase-2 (COX 2) inhibitors[5]. Each MOBIC tablet contains 7.5 or 15 mg meloxicam for oral administration. It is chemically designated as 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide. The molecular weight is 318.13. Its chemical formula is C14H13N3O4S2 and it has the following structural formula[6].

Birth defects are a diverse group of disorders of prenatal source that can be occurred by defects in a single gene, chromosomal syndromes, multifactorial inheritance, environmental teratogens and micronutrient lacks[7-9]. They are one of the causes, which lead to death universal. About 60% of the causes of congenital malformation in humans are still unknown. However, in about 25% of cases, the causes seem to be "multifactorial", indicating a complex interface between genetic and ecological risk factors. The association of several risk factors with the occurrence of birth defects with maternal age, parity, consanguinity, poor nutrition, lifestyle factors, low socioeconomic condition and many more[8].

The drug can cause the fetus to have abnormal growth, damage, birth defects or death. It can alter the function of the placenta by reducing the blood supply with oxygen, narrowing the blood vessels and reducing the supply of nutrients from the mother to the fetus, resulting in a child suffering from a lack of growth and weight. It can also cause the muscles of the uterus to contract severely, injuring the fetus indirectly by stimulating labor and premature labor[10].

NSAIDs cross the placenta and cause serious harm to the fetus depending on the type, dose, period of pregnancy, duration of treatment and time elapsed between the time the mother takes NSAIDs and the period of delivery[2]. Some women use NSAIDs to self-medicate because some of these medicines are available over the counter[11]. The present study aimed to investigate the effect of alternative doses of meloxicam on the chick embryo in different stages of development.

MATERIALS AND METHODS

Study area: The study was conducted at Jouf University, Biology Department Laboratories from September-November, 2020.

Incubation: One hundred-fifty egg were weighed, the mean of weight (56±2 g) and incubated at 37.8±0.1°C for 48 hrs at 65-75% humidity. The incubator rotated the eggs automatically every 2 hrs. After 24 hrs of incubation, two randomly selected eggs were sacrificed by window procedure to identify the embryonic discs in Fig. 1a, b.

Method of injection: The eggs were washed with 70% alcohol and labelled on the outer shell. A hole was made on the blunt pole of the egg with a sharp needle under sterile condition. Then the hole created in the egg is covered. By using a sterile syringe, the eggs were injected with a single dose of meloxicam dissolved in 0.1 mL of saline. The gap was created in the eggs will be closed.

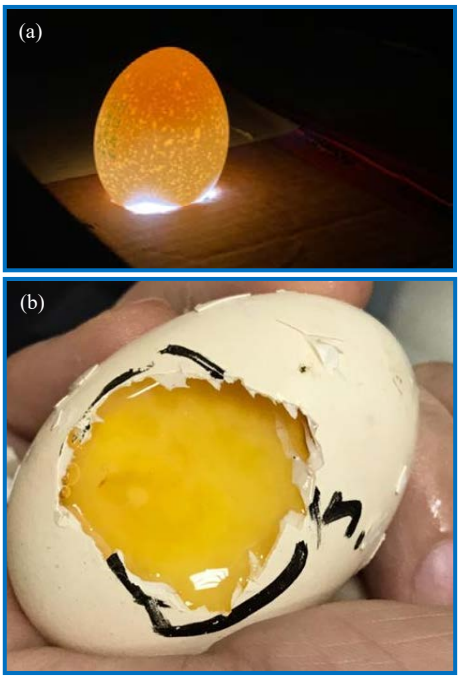

Fig. 1(a-b): Window procedure

Preparation of drug: Meloxicam was purchased from one of the pharmacies in Sakaka city and it was crushed. Meloxicam's powder with different doses was dissolved in saline, the first dose was 0.001 mg/0.1 mL, the second dose was 0.01 mg/0.1 mL and the third dose was 0.02 mg/0.1 mL. These doses were injected into 9 groups of eggs at different times of incubation. All sets were injected with 0.1 mL of solution.

Groups of study: The eggs were divided into 8 groups, each group contained 12 egg. The first group (C1) represented the control for G2, G3 and G4 groups. The second group (G2) received 0.001 mg/0.1 mL of meloxicam, the third group (G3) received 0.01 mg/0.1 mL of meloxicam, the fourth group (G4) received 0.02 mg/0.1 mL of meloxicam after 48 hrs of incubation. The fifth group (C5) represents the control for G6, G7 and G8 group. The sixth, seventh and eighth group received 0.001 mg /0.1 mL, 0.01 mg /0.1 mL and 0.02 mg/0.1 mL of meloxicam respectively after 6 days of incubation. All groups of eggs were sacrificed after 48 hrs of injection.

Morphological studies: After 48 hrs of injection meloxicam, by window procedure, the eggs of all groups were opened and photographed for morphological examination and the embryos were transferred to a petri dish for morphological examination and photography. The viability of the embryos was evaluated by the heartbeat in the young embryos and by the locomotion in the old embryos.

Quantitative observations: The number of embryos which normal development (Live (and the number of embryos which no development (dead) were determined in all groups. In addition, the lengths and the weights of embryos were determined from the fifth group to the eighth group.

Statistical analysis: The data were analyzed by SPSS version 21 and the difference between groups were determined by one way ANOVA test with a value of $p<0.05$ indicating statistical significance.

RESULTS

Morphological studies: Figure 2 shows normal distribution of vitelline vascularization in C1 group in Fig. 2a, weak and unequal distribution of vitelline vascularization shown in groups G2 in Fig. 2b and G3 in Fig. 2c, respectively, decrease and absence of vitelline vascularization shown in group G4 in Fig. 2d and e.

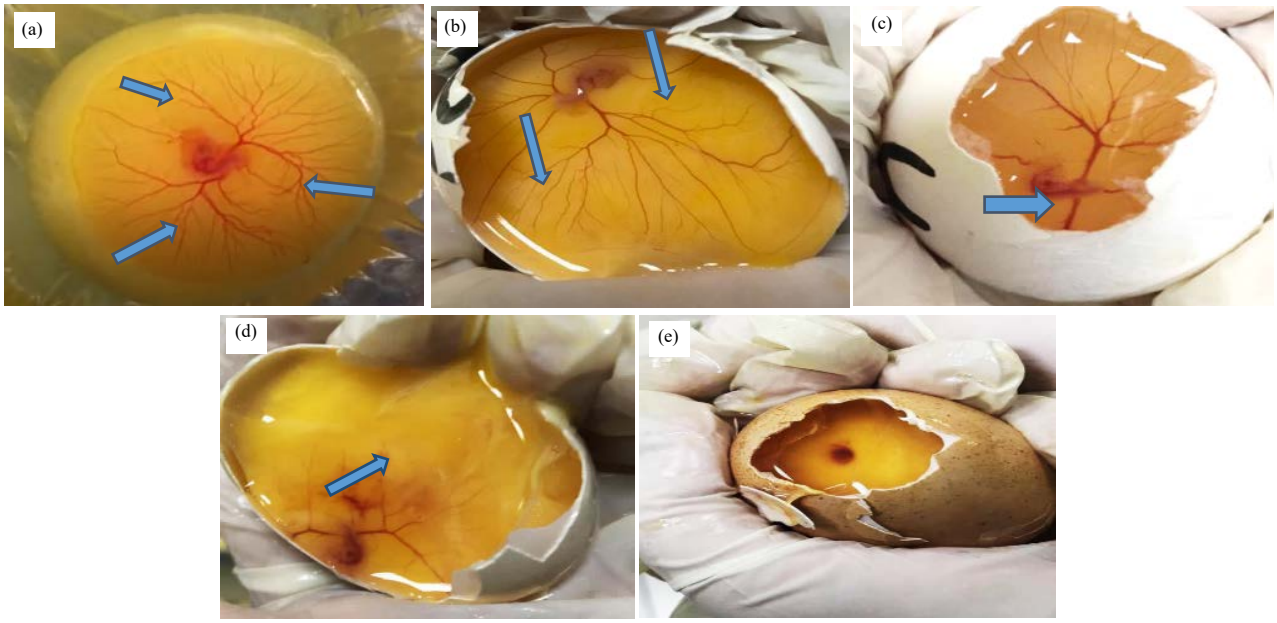

Fig. 2(a-e): Photographs of the vitelline vascularization of the embryos inside the egg 48 hrs after the meloxicam injection in the eggs after 2 day of incubation

(a): (C1) Normal distribution of vitelline vascularization (↑), (b): (G2) weak vitelline vascularization (↑) , (c): (G3) unequal distribution of vitelline vascularization (↑) (d and (e): (G4) decrease and absence of vitelline vascularization (↑)

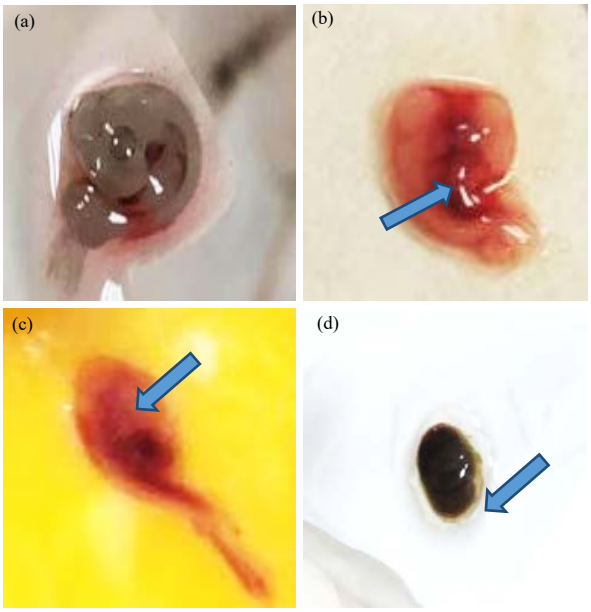

Fig. 3(a-d): Photographs of the embryos after 48 hrs of meloxicam injection in the eggs post 2 day of incubation
(a): (C1) Normal embryo, (b): (G2) Subcutaneous haemorrhage (↑), (c): (G3) Malformed and dead embryo (↑) and (d): (G4) Absorbed embryo (↑)

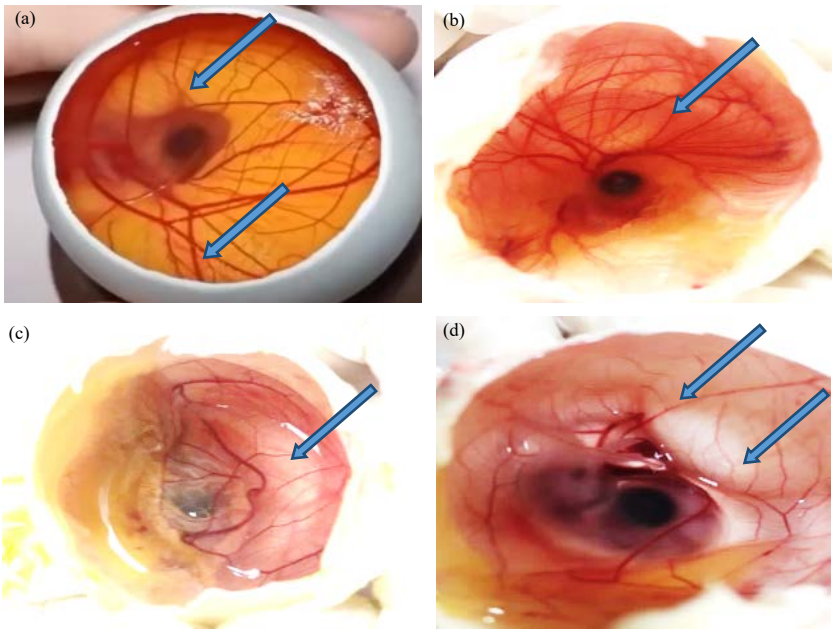

Fig. 4(a-d): Photographs of the vitelline vascularization of the embryos inside the egg 48 hrs after the meloxicam injection in the eggs after 6 day of incubation
(a and b) (C5 and G6): Normal distribution of vitelline vascularization (↑), (c and d) (G7 and G8): Weak vitelline vascularization (↑)

Figure 3 showed photographically embryos after 48 hrs of meloxicam injection in the eggs post 2 days of incubation, normal embryo in C1 in Fig. 3a, subcutaneous haemorrhage in G2 in Fig. 3b, malformed and dead embryo in G3 in Fig. 3c, absorbed embryo in G4 in Fig. 3d.

Figure 4 showed photographs of the vitelline vascularization of the embryos inside the egg 48 hrs after the meloxicam injection in the eggs after 6 days of incubation, normal distribution of vitelline vascularization in groups C5 in Fig. 4a and G6 in Fig. 4b, weak vitelline vascularization in groups C7 in Fig. 4c and G8 in Fig. 4d.

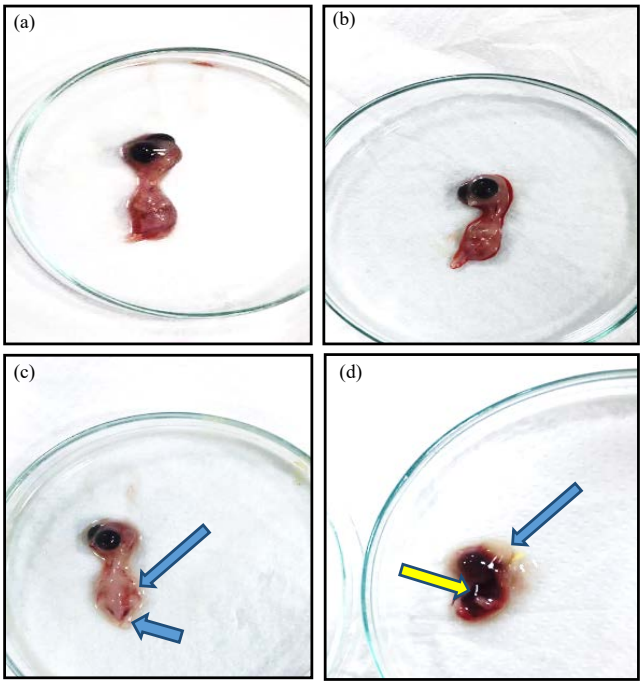

Fig. 5(a-d): Photographs of the embryos after 48 hrs of meloxicam injection in the eggs post 6 day of incubation
(a and b) C5 and G6: Normal embryo, (c) (G7): Reduced fore and hind limb (↑) and (d) (G8): Exencephaly(blue arrow) and internal bleeding (yellow arrow)

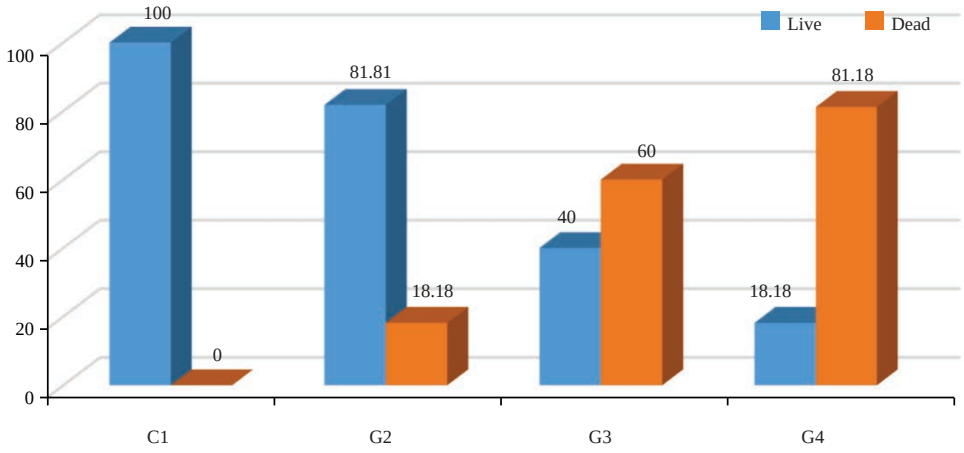

Fig. 6: Number and percentage of live and dead chick embryos after injection of meloxicam in eggs post 2 day of incubation
y-axis: (%)

Figure 5 shows photographs of the embryos after 48 hrs of meloxicam injection in the eggs post 6 days of incubation, normal embryo in G5 in Fig. 5a, G6 in Fig. 5b, reduced fore and hind limb in G7 in Fig. 5c and exencephaly in G8 in Fig. 5d.

Quantities measurement: Figure 6 showed the number and percentage of live and dead chick embryos after injection of meloxicam in eggs post 2 days of incubation. The high percentage of dead embryos was in G4 (81.81%). The percentage of dead embryos decreased in groups G3 (60%), G2 (18.18%) but there are no dead embryos in the control group C1 (0).

Figure 7 showed the number and percentage of live and dead chick embryos after injection of meloxicam in eggs post 6 days of incubation. The high percentage of dead embryos was in G8 (63.63%). The percentage of dead embryos decreased in groups G7 (50%), G6 (16.66%) but there are no dead embryos in the control group C5 (0).

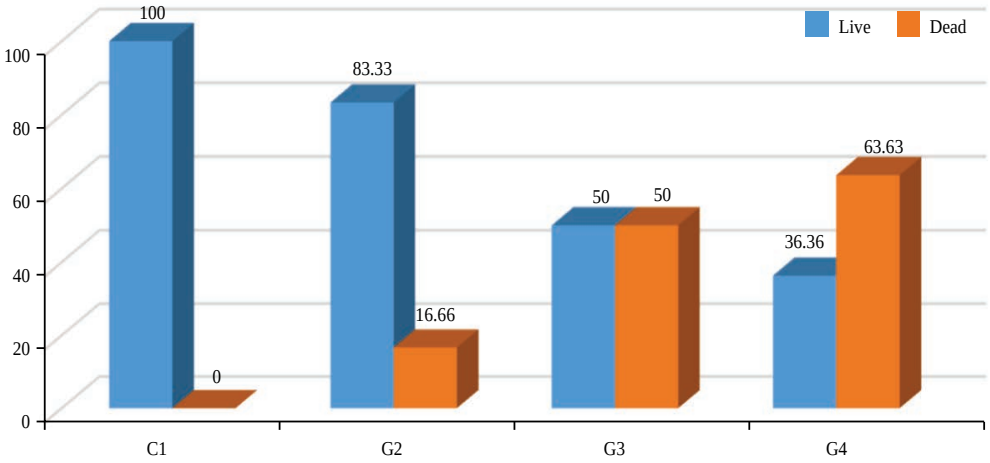

Fig. 7: Number and percentage of live and dead chick embryos after injection of meloxicam in eggs post 6 days of incubation
y-axis: (%)

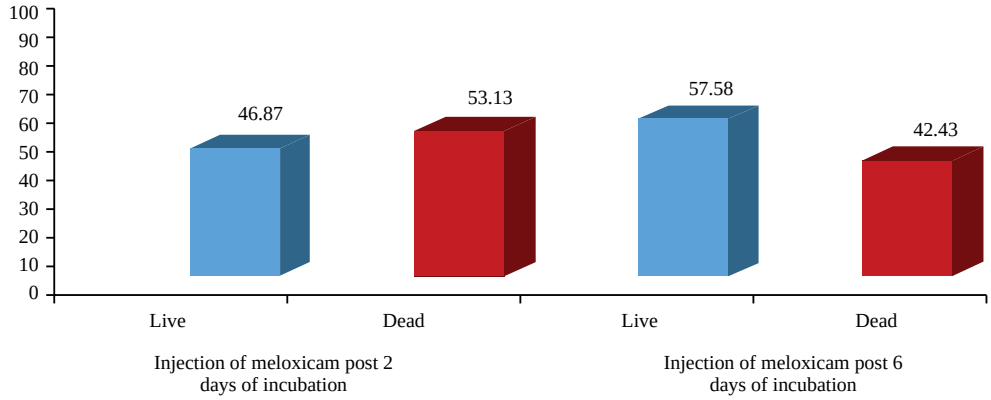

Fig. 8: Total number and percentage of live and dead chick embryos in all treated groups
y-axis: (%)

Table 1: Mean and SD of lengths (cm) and weights (cm) of chick embryos after injection of meloxicam in eggs post 6 days of incubation

Groups	Length (cm) (Mean±SD)	Sig.	Weight (g) (Mean±SD)	Sig.
C5	3.98±0.32	-	4.53±0.22	-
G6	3.82±0.41	0.22	4.25±0.51	0.253
G7	3.31±0.16	0.000**	3.38±0.45	0.000**
G8	3.14±0.36	0.000**	2.84±0.86	0.000**

**: Significant when $p \leq 0.05$, SD: Standard deviation

Figure 8 show the total number and percentage of live and dead chick embryos in all treated groups. The percentage of dead embryos of groups was injected with meloxicam post 2 and 6 days of incubation was 53.13 and 42.43.

Table 1 showed the presence of a significant decrease in the mean of embryos length of group G7 (3.31±0.16) and G8 (3.14±0.36) compared to the control group (3.98±0.32) but there is no significant difference in the length of the embryo of group G6 (3.82±0.41) compared to the control group. In addition, Table 1 showed the presence of a significant decrease in the mean of embryos weight of group G7 (3.38±0.45), G8 (52.84±0.86) but there is no significant difference in the weight of the embryo of group G6 (4.25±0.51) compared to the control group.

DISCUSSION

Many medicines have contraindications during pregnancy, some can be used in a limited way and some cannot be used because they are dangerous for the mother and the fetus. A pregnant woman should reduce medications during pregnancy but some women may have to take

medications in cases of treating mother's diseases, so the medications prescribed by the doctor must be used in specific doses[12].

Exposure to non-steroidal anti-inflammatory drugs after 30 weeks of pregnancy increases the risk of premature closure of the fetal arterial duct as well as oligohydramnios. It could cause dangerous effects on the fetus. If the mother is exposed to it before birth, it can affect the brain, kidneys, skeleton, lung, cardiovascular system and digestive system and the treatment with non-steroidal anti-inflammatory drugs are pregnancy: Fever, inflammation and pain[11].

In the present study, the administration of different doses of meloxicam in different stages of chick embryo development demonstrated that meloxicam caused weak or absence of vitelline vascularization compared to the control group. This result agrees with the study of Cetinkal *et al.*[13], they used meloxicam in different doses 30 hrs post-incubation of eggs.

The drug can alter the function of the placenta by reducing the blood supply with oxygen, narrowing the blood vessels and reducing the supply of nutrients from the mother to the fetus, resulting in a child suffering from a lack of growth and weight. It can also cause the muscles of the uterus to contract severely, injuring the fetus indirectly by stimulating labor and premature labor[10].

In the current work, supratherapeutic doses of meloxicam may be responsible for many embryonic malformations such as subcutaneous haemorrhage, absorbed embryos, reduced fore and hind limb, very thin skin and exencephaly. Meloxicam caused cerebral dilatation, microcephaly and haemorrhage around the internal organs[14]. Taking non-steroidal anti-inflammatory drugs such as meloxicam during early pregnancy, increased the risk of having embryos with birth defects, especially heart septal defects[15].

Meloxicam in the present study caused fetal growth retardation due to low body weight and height. Meloxicam caused significant retardation of the weight, size and height of the mother-treated newborns[16].

Previous studies indicated similar results as intrauterine growth retardation was found in rats treated with the highest dose of piroxicam[14]. Some medications such as meloxicam, celecoxib and leflunomide caused a significant decrease in the ossified lengths of some axial and appendicular bones[17]. Meloxicam caused non-closure of the neural tube as embryonic malformations[18]. Due to the risks of the drugs to the fetus from the results of the malformation, it may be appropriate for the prescription drugs but they may be more dangerous compared to the non-prescription drugs[18].

CONCLUSION

The results of the current study conclude that on different stages of chicken embryo development, application of high doses of meloxicam causes weak or absence of vitelline vascularization and unfortunately, leads to embryonic death. Moreover, it also affects growth retardation in embryos (lengths and weight). Supratherapeutic meloxicam doses may be responsible for embryonic malformations such as absorbed embryos, subcutaneous haemorrhage, reduced fore and hind limb, very thin skin and exencephaly.

SIGNIFICANCE STATEMENT

This study discovered the hazardous effect of meloxicam on the different stages of chick embryo development that can be beneficial for knowledge the effect of meloxicam during the gestation period. This study will help the researchers to uncover the critical areas of using meloxicam during pregnancy that many researchers were not able to explore. Thus, a new theory on avoiding meloxicam during pregnancy may be arrived at.

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